

PH.D. SCHOOL  
OF SEMMELWEIS UNIVERSITY

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**ALMANAC**



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# INTRODUCTION

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Our third almanac (this time in English language) introduces the Ph.D. School of Semmelweis University with news and events of the last two years. Additionally, we include some information about recent developments in the structure of the school, including new programs and topics. These two years include a period of time without dramatic changes. The turbulence following the merger of the universities and the introduction of departmental doctoral schools covering broader branches of sciences is over. As the distinguished readers of our booklet should all know, the formal training programs were transferred to (or, better say, gathered under the umbrella of) departmental doctoral schools (TDIs, as abbreviated in Hungarian). Although the implementation of these changes proceeded without difficulties, the new structure created artificial divisions within the Ph.D. programs; therefore the present departmental doctoral schools do not cover all aspects of the biomedical sciences and the doctoral training is in need of a more flexible structure. Still, on behalf of the Doctoral Council the President may declare that the transition has improved the output of the Doctoral School. This must be stated even though during the editing of this book new clouds gather on the sky of the higher education system. The process of disintegration has been started for a time, and the new Higher Education Act is about to impose further changes on the relatively young establishment of our higher education institution.

Since 1993 two goals have been adopted in the Ph.D. training programs and later in the departmental doctoral schools of Semmelweis University. One goal is to create the best possible conditions for the Ph.D. students' research and for maintaining training programs and courses of a high quality. The other one is to control standards and professional levels of current and future doctoral theses. Although the evaluation of scientific work has its difficulties, the past few years have allowed us to reveal and, at least partly, overcome these difficulties. Decisions made at Ph.D. defenses were based on agreed standards. Naturally, exceptions and debatable cases can still occur, but generally we are able to consider and evaluate the academic work of our Ph.D. students with a great deal of pride. It is to be applauded that - due to the tutors' professional contacts - most Ph.D. students are able to attend laboratories abroad but only a few of them abandon our doctoral school forever without defending their theses. Traveling abroad is promoted by financial support provided by the pre-doctoral competitions (This financial support might be transformed into a scholarship at a future date).

The high standards of the Doctoral School of Semmelweis University are characterized by an increasing number of performance-based grants allocated to us by the state each academic year. The number of Ph.D. students who pay tuition fees has remained constant. But we would prefer to have more Ph.D. students studying at our doctoral school from abroad. There are almost 600 Ph.D. students either in the educational or the qualification phase (i.e. examination, writing up and defending a thesis) within the doctoral programs of the university. In the past few years more than one hundred Ph.D. candidates were yearly conferred the degree of doctor (on the traditional *Dies Academicus*), a number that indicates that the Doctoral School has become a key institution of the university and increasingly contributes to the enhancement of its academic reputation.

The value of academic work in the Doctoral School is acknowledged not only by the leadership of the university but also it manifests itself in a number of aspects of Ph.D. training and professional activities; i.e. university teachers show a high degree of interest in joining the doctoral staff and many graduates at other universities apply for admission to our doctoral school. Furthermore, institutions and professionals from Hungary and abroad have a keen interest in collaborating with us.

It has to be highlighted that the Ph.D. students' high performance and the successful functioning of departmental doctoral schools are facilitated by the expertise of the overwhelming majority of our tutors. Tutors provide professional guidance and financial support to the research of Ph.D. students. The Doctoral Council - which was assisted by other key committees - introduced the theoretical and practical principles of the Doctoral School and made essential decisions concerning its operation. These decisions were followed by a great deal of administrative work which has been managed commendably partly by the heads of departmental doctoral schools and partly by the staff of the Doctoral Secretariat. Our website is increasingly relied upon and contains much practical information. The overture of opening the new English language website is close ahead! Although the electronic media is able to eliminate a great deal of paperwork, the traditional information channels are also necessary.

The Almanac is one of the latter, and records essential events which took place in the Doctoral School of Semmelweis University. Making use of this opportunity I thank everybody who contributed to our performance and current results. I owe special thanks to some of my colleagues and friends. At first I have to pay tribute to **Professor Kopper László** who both as the former President of the Doctoral School and as one of the Heads of Departmental Ph.D. Schools plays a decisive role in the life of the Doctoral Council and School. **Gábor Makara, a member of the Hungarian Academy of Sciences**, Deputy President of the Council, and the renowned figure of many other scientific bodies is the key person of setting the stage for the present Hungarian doctoral training. Without his contribution the Doctoral School would be far less successful and productive. I am especially thankful to Doctor **János Rigó**, the President of the Evaluation Committee, who keeps his fingers constantly on the freshly prepared theses produced by our candidates of doctoral degree, thereby ensuring the high standards of the dissertations. Neither this book, nor the progress of the School of Ph.D. studies could have been imagined without the everlasting help and expertise of the brilliant work of the Doctoral Secretary. Last but not least, I have to mention that the engine of this board is **Emőke Márton, the director of the Secretary**. It is largely due to her devotion to the doctoral education that we can navigate on rough waters. Her team includes **Anikó Marádi, Anita Lengyel and Tímea Rab**, who are always ready to serve students and tutors and whose practice and patience are a key to the smooth and perfect management of the School. Herewith I wish much success for the continuation of this noble activity exerted both by my friends in organizing the training and by those who endeavor to acquire the doctoral degree at our University.

Ágoston Szél  
President of the Doctoral Council

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# PH.D. SCHOOL OF SEMMELWEIS UNIVERSITY

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The Doctoral School is an autonomous educational body of the University; it functions as subject to the decisions made by Doctoral Council (In Hungarian: EDT) that meets regularly every second month of the academic year. The work of the Doctoral Council is supported by the Doctoral Secretary. The Doctoral School of Semmelweis University includes eight departmental doctoral schools representing a branch of science each, and manage the educational programs, sub-programs and offer a wide selection of research topics that are of highest importance for Ph.D. students.

## ADMISSION, EDUCATION AND QUALIFICATION

---

Ph.D. applicants must be university graduates (with a Master's degree) or students registered for the last semester of their studies at the university. The Doctoral Council of the University might accept the application of other students in exceptional cases. Applicants are required to indicate on the application form the training program and research topic selected within a Departmental Doctoral School. The Doctoral council determines the content of the Ph.D. programs, the admission procedures and the admission fee. The Doctoral Secretariat Office provides detailed information about these to the applicants.

Applicants can be admitted to the educational phase (with state grants, private scholarships and fee based education) or to the qualification phase (Ph.D. examination, writing and defending a thesis) of the doctoral training. The admission procedure is based on evaluating.

- 1) the candidates' level of general knowledge and personal ability,
- 2) his/her special (topic related) knowledge and academic competence.

Candidates must have a university (Ms.C.) degree with the result at least 3.51 (cum laude) in order to gain admission. The result of their diploma is taken into consideration at the oral entrance examination only if it was acquired within the three years prior to application. A further condition is that applicants must possess at least a certified C type (oral and written) state language exam or an equivalent certificate.

The admission board of each departmental doctoral school creates a ranked list of applicants which is submitted to the Doctoral Council of the University. This committee makes decisions about the final admissions by considering the number of (state) grants available and the positions candidates have on the ranked lists.

The activity of the School of Ph.D. is divided into two parts:

Part I: educational phase – consisting of program courses and research activity (for Ph.D. students - doctoranduses).

Part II: qualification phase – consisting of examination, writing and defending dissertations (for Ph.D. candidates).

Although the educational and qualification phases can be continuous, each contains features that provide greater flexibility in obtaining a degree. Basically, anybody holding a university diploma with a Master's degree (not necessarily a medical one) can join either Phase I or II. The aim of Phase I is to train students to become scientists by providing them courses. Credit points can be accumulated upon completion of a course. The selected scientific topic will become the core of the thesis. Research is usually conducted in laboratories in collaboration with faculty members. A qualified tutor supervises each student.

Phase II provides an opportunity to evaluate the results of the experiments and to publish them in acknowledged scientific journals. Obviously, this is, or can be an on-going activity in Phase I as well. The student is required to pass a comprehensive examination and to write and defend a dissertation. Phase II follows Phase I, but one may join Phase II without completing Phase I, providing the necessary prerequisites have been fulfilled. However, if one joins the School of Ph.D. Studies directly in Phase II it is necessary to be accepted by a tutor within a program.

The School of Ph.D. Studies offers three forms of education. Full-time (ft) for scholarship holders (scholarship obtained from state, agency, foundation etc.) entering Phase I as students - Part-time (pt)

students, entering Phase I as students. Non-affiliated (n/a, individual) students, entering Phase II as candidates.

Both full-time and part-time students must meet the same requirements. The main difference between those with and without scholarship is that the latter have jobs and are combining studies and work. Individuals who join only Phase II will not be students with record-book and student identity card, but they will be candidates for the doctoral degree. The total number of candidates at present is over 400. Certain costs of education, scientific training and official procedures are covered for students and candidates. Most of the fees are equal to or close to what is ordinarily paid by undergraduate students.

The table summarizes information about the applicants and students of the School of Ph.D. Studies at Semmelweis University.

## MEMBERS OF THE DOCTORAL COUNCIL

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**President:** Dr. Ágoston Szél

<b>Dr. Gábor Makara</b>	Vice-President, President of the Educational Board
<b>Dr. Veronika Ádám</b>	Vice-Rector of Scientific and International Affairs
<b>Dr. Sándor Juhász-Nagy</b>	Ph.D. School of Basic Medical Sciences
<b>Dr. Zsolt Tulassay</b>	Ph.D. School for Clinical Science in Medicine
<b>Dr. Károly Rác</b>	Ph.D. School for Clinical Science in Medicine
<b>Dr. Éva Szőke</b>	Ph.D. School of Pharmaceutical and Pharmacological Sciences
<b>Dr. István Bitter</b>	Ph.D. School Mental Health Sciences
<b>Dr. György Nagy</b>	János Szentágothai Neurosciences Ph.D. School
<b>Dr. József Mandl</b>	Ph.D. School of Molecular Medical Sciences
<b>Dr. László Kopper</b>	Ph.D. School of Pathology (former President)
<b>Dr. József Tihanyi</b>	Ph.D. School of Sport Sciences
<b>Dr. Pál Magyar</b>	Representative of Faculty of Medicine
<b>Dr. Gábor Varga</b>	Representative of Faculty of Dentistry
<b>Dr. Kálmán Magyar</b>	Representative of Faculty of Pharmacy
<b>Dr. János Rigó</b>	President of the Quality Control and Evaluation Board

## PERMANENT COMMITTEES OF THE DOCTORAL COUNCIL

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### Educational Board (EB)

The Educational Board (EB) meets at least once in a half year. It expresses opinion on course proposals and requests for financial support for courses. If needed, the EB can alter courses. It can make proposals in the following matters: (1) the structure of teaching, (2) the co-ordination of courses, (3) the nature of the method of course registration and registration deadlines, (4) the establishment of credit points which can be given to each course and (5) the recognition of credit points.

Students receiving tuition are advised to choose those courses which are promoted by the Doctoral Council of Semmelweis University. Theoretical knowledge and skills necessary for research are obtained from the compulsory course modules. Throughout the year the Ph.D. schools organize optional courses. Some of them organize courses which are obligatory for all students who are registered in that particular school.



## Ph.D. School of Pharmacological and Pharmaceutical Sciences (3.)

Program	1993 - 2002		2002/03		2003/04		2004/05		2005/06		Total		Ph.D. Candidates			Ph.D. Graduates			Full time		All pers.		
	ft	pt	ft	pt	ft	pt	ft	pt	ft	pt	ft	pt	ft	pt	n/a	ft	pt	n/a	%	%			
3/1. Rácz I.	44	76	5	14	10	8	8	6	12	11	79	115	9	3	4	21	24	8	38	36			
3/2. Magyar K.	11	10	3	2	2	4	3	3	1	2	20	21	2	1	1	5	6	6	35	51			
<b>Total</b>	<b>55</b>	<b>86</b>	<b>8</b>	<b>16</b>	<b>12</b>	<b>12</b>	<b>11</b>	<b>9</b>	<b>13</b>	<b>13</b>	<b>99</b>	<b>136</b>	<b>11</b>	<b>4</b>	<b>5</b>	<b>26</b>	<b>30</b>	<b>14</b>	<b>37</b>	<b>38</b>			
											<b>Sum</b>	<b>235</b>								<b>20</b>			<b>70</b>

## Ph.D. School of Mental Health Sciences (4.)

Program	1993 - 2002		2002/03		2003/04		2004/05		2005/06		Total		Ph.D. Candidates			Ph.D. Graduates			Full time		All pers.		
	ft	pt	ft	pt	ft	pt	ft	pt	ft	pt	ft	pt	ft	pt	n/a	ft	pt	n/a	%	%			
4/1. Tringer L.	24	113	5	5	2	2	2	2	1	2	37	124	2	3	1	6	14	5	22	19			
4/2. Kopp M.	*	*	*	*	2	6	5	5	5	8	12	19	3	8	0	1	5	1	short	short			
<b>Total</b>	<b>24</b>	<b>113</b>	<b>5</b>	<b>5</b>	<b>7</b>	<b>8</b>	<b>7</b>	<b>7</b>	<b>6</b>	<b>10</b>	<b>49</b>	<b>143</b>	<b>5</b>	<b>11</b>	<b>1</b>	<b>7</b>	<b>19</b>	<b>6</b>	<b>24</b>	<b>26</b>			
											<b>Sum</b>	<b>192</b>								<b>17</b>			<b>32</b>

## Ph.D. School of Sport Sciences (5.)

Program	1993 - 2002		2002/03		2003/04		2004/05		2005/06		Total		Ph.D. Candidates			Ph.D. Graduates			Full time		All pers.		
	ft	pt	ft	pt	ft	pt	ft	pt	ft	pt	ft	pt	ft	pt	n/a	ft	pt	n/a	%	%			
5/1. Pavlík G.	*	*	*	*	2	3	0	0	0	2	2	5	1	5	0	0	0	4	2	short	short		
5/2. Frenkl R.	21	78	6	15	2	3	2	3	3	4	34	103	6	5	2	5	19	7	32	32			
5/3. Földesiné Sz. Gy	*	*	*	*	3	10	4	17	3	10	10	37	7	13	3	1	11	2	short	short			
<b>Total</b>	<b>21</b>	<b>78</b>	<b>6</b>	<b>15</b>	<b>7</b>	<b>16</b>	<b>6</b>	<b>20</b>	<b>6</b>	<b>16</b>	<b>46</b>	<b>142</b>	<b>13</b>	<b>18</b>	<b>5</b>	<b>6</b>	<b>30</b>	<b>9</b>	<b>41</b>	<b>43</b>			
											<b>Sum</b>	<b>188</b>								<b>36</b>			<b>45</b>

## János Szentágotthai Ph.D. School of Neurosciences (6.)

Program	1993 - 2002		2002/03		2003/04		2004/05		2005/06		Total		Ph.D. Candidates			Ph.D. Graduates			Full time		All pers.		
	ft	pt	ft	pt	ft	pt	ft	pt	ft	pt	ft	pt	ft	pt	n/a	ft	pt	n/a	%	%			
6/1. Palkovits M.	44	70	9	3	2	1	5	2	3	3	63	79	7	7	0	26	22	5	52	47			
6/2. Liposits Zs.	*	*	*	*	3	0	2	1	2	0	7	1	1	1	0	0	0	1	14	38			
6/3. Vizi E. Sz.	*	*	*	*	0	2	2	2	3	4	4	7	0	1	2	0	1	2	0	55			
6/4. Nagy Z.	19	41	1	5	3	2	1	1	2	4	26	53	2	3	0	8	6	9	38	35			
6/5. Szirmai I.	2	0	1	0	2	2	0	0	0	0	5	2	0	0	0	0	1	3	0	57			
6/6. Faludi G.	*	*	*	*	0	0	0	1	0	0	0	1	0	0	2	0	0	1	short	short			
<b>Total</b>	<b>44</b>	<b>70</b>	<b>9</b>	<b>3</b>	<b>10</b>	<b>7</b>	<b>10</b>	<b>7</b>	<b>9</b>	<b>10</b>	<b>105</b>	<b>143</b>	<b>10</b>	<b>12</b>	<b>4</b>	<b>34</b>	<b>30</b>	<b>21</b>	<b>42</b>	<b>45</b>			
											<b>Sum</b>	<b>248</b>								<b>26</b>			<b>35</b>

## Ph.D. School of Molecular Medical Sciences (7.)

Program	1993 - 2002		2002/03		2003/04		2004/05		2005/06		Total		Ph.D. Candidates			Ph.D. Graduates			Full time		All pers.	
	ft	pt	ft	pt	ft	pt	ft	pt	ft	pt	ft	pt	ft	pt	n/a	ft	pt	n/a	ft	%	ft	%
7/1. Spät A.	25	13	1	1	2	2	0	2	3	0	31	18	4	0	0	14	11	1	58	61		
7/2. Mandl J.	23	32	2	3	2	1	4	0	4	4	35	40	3	4	2	17	17	0	57	57		
7/3. Oláh I.	10	3	1	0	3	1	1	1	1	0	16	5	2	0	0	4	0	0	38	29		
7/4. Falus A.	17	38	2	7	3	1	4	3	3	1	29	50	3	1	1	8	17	5	38	44		
7/5. Gergely P.	14	18	4	3	1	2	1	1	1	1	21	25	2	2	3	5	12	5	33	63		
Total	89	104	10	14	11	7	10	7	12	6	132	138	14	7	6	48	57	11	47	53		
	Sum											270	27			116						

## Ph.D. School of Pathology (8.)

Program	1993 - 2002		2002/03		2003/04		2004/05		2005/06		Total		Ph.D. Candidates			Ph.D. Graduates			Full time		All pers.	
	ft	pt	ft	pt	ft	pt	ft	pt	ft	pt	ft	pt	ft	pt	n/a	ft	pt	n/a	ft	%	ft	%
8/1. Kopper L.	27	61	4	1	5	4	5	1	3	2	44	69	7	4	2	14	18	11	48	50		
8/2. Schaff Zs.	6	13	0	1	2	0	0	0	2	1	10	15	0	1	0	4	5	4	40	56		
8/3. Rozgonyi F.	1	10	1	1	1	4	0	3	2	2	5	20	0	0	1	1	3	1	20	24		
8/4. Sótónyi P.	11	42	3	4	1	8	1	3	1	9	17	66	2	8	0	6	7	6	47	35		
Total	45	126	8	7	9	16	6	7	8	14	76	170	9	13	3	25	33	22	45	43		
	Sum											246	25			80						

Data processing closed in February 2006.

## Students of the Cooperation Research Centre:

	01/02	02/03	03/04	04/05	05/06	Tot.	Candidates	Graduated
7. Mandl J.	11	4	1	2	3	21	1	0
3. Szőke É.				3	3	6	4*	0
8. Kopper L.				1		1	0	0

\*Primarily, they have started their studies in Ph.D. School No. 7

Keys: \*

The program was not initiated in that year

Ratio of candidates and graduates as compared to all students

Including exclusively full time students

Including all persons (full time, part time, non-affiliated)

short: recently started program

ft full time

pt part time

n/a non-affiliated

% All persons: %

## MEMBERS OF THE EDUCATIONAL BOARD

---

**President:** Dr. Gábor Makara

<b>Dr. László Rosivall</b>	1. Ph.D. School of Basic Medical Sciences
<b>Dr. Béla Molnár</b>	2. Ph.D. School for Clinical Science in Medicine
<b>Dr. Sylvia Marton</b>	3. Ph.D. School of Pharmaceutical and Pharmacological Sciences
<b>Dr. László Tringer</b>	4. Ph.D. School Mental Health Sciences
<b>Dr. Gábor Pavlik</b>	5. Ph.D. School of Sport Sciences
<b>Dr. Emília Madarász</b>	6. János Szentágothai Neurosciences Ph.D. School
<b>Dr. Ágota Vér</b>	7. Ph.D. School of Molecular Medical Sciences
<b>Dr. Ferenc Rozgonyi</b>	8. Ph.D. School of Pathology
<b>Miklós Antal</b>	Representative of Doctoral Students' Union

The Educational Board (In Hungarian: OB) used to fulfill the functions of the MD-Ph.D. entrance examination committee. Members of this body debate and decide whether a candidate has a thorough grounding in academic work by examining his/her previous performance in education and research and the elected topic for research. The board decides whether the candidate will or will not be able to produce a thesis three or four years after his/her entrance into the system. The number of applicants for the MD-Ph.D. training courses was six in 2001; twelve were in 2002 and seven in 2003. According to the new legislation, no student may start the Ph.D. studies until the diploma of a higher education institution (Master) is not obtained.

## Quality Control and Evaluation Board (QCEB)

The efficiency of the doctoral training is shown in the frequency of completed doctoral theses and academic publications. The standards of these works are judged by scientometric indicators. This process evaluates both the academic competence of individual Ph.D. candidates and also the academic standards of the Doctoral School.

One of the most important acts of quality control is that everybody involved in the process complies with the instructions given in the qualification phase by the Doctoral Council (DC). The importance of this issue led to the establishment of The Quality Control and Evaluation Board (In Hungarian: VMB). This first evaluating forum controls whether the submitted work fulfils the conceptual, structural and formal requirements of a doctoral thesis. Consequently, a formal opinion is released stating whether the Ph.D. candidate acquired the scientometric indicators prescribed in the Book of Regulations of the Doctoral School or not.

## MEMBERS OF THE QUALITY CONTROL AND EVALUATION BOARD

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**President :** Dr. János Rigó

<b>Dr. Sándor Juhász-Nagy</b>	1. Ph.D. School of Basic Medical Sciences
<b>Dr. László Herszényi</b>	2. Ph.D. School for Clinical Science in Medicine
<b>Dr. István Antal</b>	3. Ph.D. School of Pharmaceutical and Pharmacological Sciences
<b>Dr. Katalin Hegedűs</b>	4. Ph.D. School Mental Health Sciences
<b>Dr. Gyöngyi Szabó (Földesiné)</b>	5. Ph.D. School of Sport Sciences
<b>Dr. András Csillag</b>	6. János Szentágothai Neurosciences Ph.D. School
<b>Dr. László Hunyady</b>	7. Ph.D. School of Molecular Medical Sciences
<b>Dr. Janina Kulka</b>	8. Ph.D. School of Pathology
<b>László Harmat</b>	Representative of Doctoral Students' Union

Considering the proposals given by the QCRB (VMB in Hungarian) the Doctoral Council decides whether the doctoral thesis can be sent to the opponents. At a first glance this process might seem unnecessary because the control of requirements looks like a simple administrative issue. However, the process of evaluation described above - which applies the general rules (sometimes with a great deal of empathy) - is highly desirable for a number of reasons: there is a great variety of research topics, the issues discussed range from molecular biology to behavioral sciences which must be able to win academic recognition in Hungary and abroad.

Apart from works which meet all the standards there are doctoral theses which are inadequate in their content and structure and are therefore unacceptable. Candidates who do not fulfill the main requirements are advised to withdraw their dissertation prior to a detailed, expert review. We are proud that only thoroughly controlled doctoral theses are given in the hands of official opponents and reviewers. It is worth mentioning some of these requirements which doctoral candidates must take into consideration.

- (a) Only those candidates deserve scientific degrees who are able to publish their results intelligibly and based on a coherent analysis. Summarizing the content of two or three excellent publications is not equal to a doctoral dissertation.
- (b) Some articles which were published in high-rank international journals with a sophisticated title and a high impact factor are not sufficient to create the basis for Ph.D. qualification. Only those articles are potential items for recognition which are the result of real academic work. Therefore, articles summarizing the academic literature of a particular topic or commenting on the academic work of others as “letters to the editor” are not acceptable. By contrast, articles published in a journal under the latter heading could include original scientific results. But this must become clear from the presentation of scientific methods and/or from the editor’s professional opinion.
- (c) The QCRB during its evaluation takes into consideration the grammatical correctness and style of the English or Hungarian language, the length as well as the external appearance of the thesis and the quality of illustrations.

These examples illustrate that the board has a high level of responsibility in defining the academic quality of Ph.D. qualifications. At the same time the board is meticulously tactful in giving criticisms. Therefore, written judgments also include constructive suggestions for correcting the deficiencies discovered. This professional opinion is sent to both the candidate and the head of the training program. This process creates grounds for the necessary corrections and gives the possibility of legal remedies.

When this evaluation process is applied in practice, for instance, nine doctoral theses out of ten are found acceptable. The other one is rejected usually because basic requirements are not fulfilled, i.e. the shortage of sufficient acceptable publications. Furthermore, it occurs that two or three theses are rejected in form (i.e. the outward appearance and/or some of the essential requirements are missing from the package which was submitted to the QCRB.) In these cases the professional review of the doctoral theses will be delayed until the corrections are completed and necessary supplements are submitted.

One of the major duties of the QCRB is to report its experience to the Doctoral Council and to suggest proposals concerning the alterations in the Rules of the Doctoral School if necessary. Apart from the duties described above the QCRB has authority over any stage of the doctoral procedure in scientific matters. Furthermore, it passes judgments on applications, looks into the matters of complaints and makes decisions concerning naturalizations (e.g. the recognition of qualifications).

Since 2000 the Quality Control and Evaluation Board has had this role of quality evaluation within the Doctoral School.

## COMMITTEE OF DISCIPLINARY PROCEDURES

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<b>Dr. Árpád Fazekas, President</b>	Ph.D. School for Clinical Science in Medicine
<b>Tivadar Tamás Dankó</b>	Ph.D. School of Basic Medical Sciences
<b>Dr. István Rácz</b> <b>(at present Dr. István Antal)</b>	Ph.D. School of Pharmaceutical and Pharmacological Sciences
<b>Anna Salamon</b>	Ph.D. School of Molecular Medical Sciences
<b>Bálint Szuromi</b>	Ph.D. School of Mental Health Sciences

It is the pride that the President of the Ph.D. School declares that this is the least employed and loaded body. Its activity is needed only exceptional cases, of which plagiarism and disharmony between student and tutor have given some work to the committee in the last few years.

## DOCTORAL SECRETARIAL OFFICE

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<b>Emőke Márton</b>	Head
<b>Anna Marádi (Pintérné)</b>	Financial adviser
<b>Anita Lengyel</b>	Adviser
<b>Tímea Rab</b>	Adviser

The administrative duties of the Doctoral School are managed by the Doctoral Secretarial Office which creates a link between the Doctoral Council, the Ph.D. training programs and the Ph.D. students. It maintains permanent contact with the educational bodies outside of the university (e.g. Ministry of Education, Hungarian Accreditation Board, etc.). The Secretary is available for Ph.D. students on an office-hours basis, however, they are ready to help anytime in urgent cases. In one little room practically every major step of the degree obtaining process is handled, starting from the first inquiries and paper work of the entrance examination, all the way up to the preparation of the diplomas. (Address: H-1085 Budapest, Üllői út 26. ground floor Nr. 14.)

## The Doctoral Students' Union (DSU)

The DSU (In Hungarian: DHÖK) is a body elected by the doctoral students in order to legally represent their interests both inside and outside the university; e.g. in the Doctoral Council and the National Association of Doctoral Students. Members of the union participate in the *ad hoc* commissions dealing with matters in their interest and are responsible for organizing the Scientific Ph.D. forums. The head office of the DSU is: The Students' Office of Semmelweis University (H-1089 Budapest, Nagyváradi tér 4. ground floor Nr. 18.)

Members of the Union of the Doctoral Students are also accessible through the Doctoral Secretarial Office.

## MEMBERS OF THE DOCTORAL STUDENTS' UNION

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<b>Tivadar Tamás Dankó</b>	1. Ph.D. School of Basic Medical Sciences
<b>Sándor Spisák</b>	2. Ph.D. School for Clinical Science in Medicine
<b>Szabolcs Szarka</b>	3. Ph.D. School of Pharmaceutical and Pharmacological Sciences
<b>Bálint Szuromi</b> <b>(President)</b>	4. Ph.D. School Mental Health Sciences, Institute of Psychiatry and Psychotherapy
<b>László Harmat</b>	

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László Révész	5. Ph.D. School of Sport Sciences
Miklós Antal	6. János Szentágothai Neurosciences Ph.D. School
Éva Margittai	7. Ph.D. School of Molecular Medical Sciences
Beáta Lizák	
Anna Salamon	
Gyöngyi Rábai	
Csaba Bődör	8. Ph.D. School of Pathology

## INTRANET

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**Website:** <http://www.phd.sote.hu>

The website of the Doctoral School at Semmelweis University is an essential means for organizing doctoral education. Hundreds of Ph.D. students take the courses as part of more than thirty training programs of eight doctoral schools under the supervision of hundreds of tutors in different locations. Every half year the school organizes 60 - 120 courses; the lectures and seminars are given in more than 50 locations within the university.

There are great advantages stemming from the intensity and variety of our education system. Therefore, it is a high priority to publish information which is clear-cut and accessible by everybody. The gradual augmentation of the website leads to the accumulation of information about every training program and sub-program and of every teacher and doctoral defense since 2000. Importantly, the website provides information about on-going courses and application possibilities. All application forms and documents, which are necessary for the administration of doctoral matters and the resolutions of the Doctoral Council, are also accessible on the website. Other detailed information and curricula are also available electronically. The website provides addresses, telephone numbers and e-mail addresses.

The website was completed in the end of 2002, and it is this year that we start sending out information in English language. The regulations, the forms, the course and program data, the decisions of the Doctoral Council and the invitations to all defense ceremonies are accessible via the Internet. News on important conferences, university events, calls for proposals are also not missing from the repertoire.

In 2000-2001 – after the internet server of the Doctoral School had been installed - the system was entirely renewed. The previous Windows NT server was cracked several times by unknown hackers, so the Linux-Apache system based on new technology has been introduced. Additionally, the informative database of the website was modernized.

The new database connected with the website opens a possibility for course leaders to put the information about their courses directly to the website. The Doctoral Secretarial staff manages and publishes all the relevant information: e. g. general news, advertisements and Ph.D. defenses without the assistance of a web supervisor.

The website has considerable web traffic according to statistical figures. The average number of visitors a day was 2000 between 1 March and 30 June 2003, in May it went up to 3500 daily visits. The number of downloaded files was 800-1000 every day and in May it reached a daily 2000. Since then this figure then has increased due to the high demands for electronic admission information. (These figures represent only the general turnover not the actual numbers of visitors. Repeated visits by the same person are registered each time, therefore, the real numbers of inquirers are unknown.)

The database system of presenting doctoral theses on the internet was set up. One hundred and fifty doctoral theses and synopses there of are accessible and their number increases on a day to day basis. It is required that doctoral theses be available to the public before the defense so they may be accessed electronically in full through the internet simultaneously with the announcement of the Ph.D. defense.

Two years ago we have introduced a new exhibition channel for our dissertations. The “Dissertation Abstracts”, a trademark of ProQuest, allows for the availability of each recently defended thesis all

over the world. Older works are not available yet, however we try to put out as many of our precious creations as possible.

### 'Veritas et Virtus' Foundation in memory of the young Dr. Zsolt Farkas

After the tragic death of Dr. Zsolt Farkas, a Ph.D. student of the Doctoral School, his parents, Dr and Mrs Zsolt Farkas established a foundation in memory of their late son. The aim of the foundation is to support financially research by Ph.D. candidates.

Some of the aspects of the charter are discussed below:

"The aims of the Foundation are to subsidize the work of Ph.D. students under thirty-five years who are concerned primarily with physiological research in the Doctoral School of Semmelweis University. Additionally, it contributes to the realization of the aims of the Doctoral School, i.e. to improve the quality of doctoral education, to facilitate the acquisition of widely recognized scientific degrees, to provide financial support for the expansion of accredited doctoral research programs, to establish pre-doctoral scholarships and to improve scientific communication. ..."

"... Those Ph.D. students are able to benefit from the payments of the foundation whose submitted work wins a public competition advertised by the trustees of the Foundation.

The type of work submitted can be in the process of publication but the candidate must be its first author. Other details are defined by the committee of trustees who are responsible for both advertising and reviewing the submitted work. ..."

"The awards must be transferred ceremonially to the winners every year on the Dies Academicus (first Saturday of November)."

The Office of the Foundation is: H-1085 Budapest VIII., Üllői út 26.

The trustee committee is the Advisory Board of the Foundation.

The president of the Advisory Board of the Foundation is always the current head of the Doctoral Council at the Semmelweis University, at present, university professor, Dr. Ágoston Szél.

## RECIPIENTS OF 'VERITAS AND VIRTUS' AWARDS

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2003	<b>Andrea Fekete</b> (I. Prize)	Ph.D. School for Clinical Science in Medicine
	<b>Tamás Tábi</b> (II. Prize)	Ph.D. School of Pharmaceutical and Pharmacological Sciences
	<b>Géza Vass</b> (II. Prize)	Ph.D. School of Sport Sciences
2004	<b>Zsuzsanna Lénárd</b> (I. Prize)	Ph.D. School of Basic Medical Sciences
	<b>Balázs Rada</b> (I. Prize)	Ph.D. School of Molecular Medical Sciences
	<b>Brigitta Balogh</b> (II. Prize)	János Szentágothai Neurosciences Ph.D. School
	<b>Csongor Csekő</b> (II. Prize)	Ph.D. School of Basic Medical Sciences
2005	<b>Zoltán Jakus</b> (I. Prize)	Ph.D. School of Molecular Medical Sciences
	<b>Gábor Széplaki</b> (I. Prize)	Ph.D. School of Molecular Medical Sciences
	<b>Szabolcs Török</b> (II. Prize)	Ph.D. School of Sport Sciences

## PH.D. COURSES

Every semester there are there a number of courses (60-120) announced on the website. Previously, the Educational Board of the Doctoral school filtered down the number of courses to between sixty and seventy. The Doctoral Council of the Semmelweis University took into consideration the views the Educational Board and restricted the number of courses which can be run by each departmental doctoral school in one semester to between five and seven.

Since then the following procedure has been established concerning Ph.D. courses, i.e. the Doctoral Committee of each Departmental Doctoral School proposes between five and seven courses at the beginning of each semester which are entered the database of current courses. However, the Educational Board can recognize and award credit points for participation in Ph.D. courses of the appropriate standard at other universities. In this case the Educational Board needs the recommendation of the tutor and the head of the departmental doctoral school of the student in question.

The list of courses, which have to be taken during the Ph.D. training period, is finalized. In 2002 the Departmental Doctoral School published a plan which restricted the number of courses for the entire three years long training period. The database of available courses is accessible at the website of the Doctoral School. Consequently, students are able to access the database and construct their own individual study plan.

	<b>Compulsory Courses</b>	<b>Course Leader</b>	<b>Semester</b>
KV	Elements of molecular biology	László Buday, Mária Sasvári, András Váradi	2003/2004/1, 2004/2005/1
KV	Isotopic methods and radiation protection	István Voszka	2004/2005/2
KV	Evidence based medicine, planning and organization of clinical studies	István Mucsi	2003/2004/1
KV	Methods and ethics of scientific research	Péter Csermely	2003/2004/1, 2004/2005/1
KV	Good Clinical Practice	Zsolt Tulassay	2003/2004/2
KV	Introduction to biometry	Elek Dinya, Gábor Makara	2003/2004/1, 2004/2005/1
KV	Rhetoric course	Miklós Benczúr	2003/2004/2
KV	Planning and evaluation of experiments	Gábor Makara	2003/2004/2
KV	Animal in research - animal experimentation	Piroska Anderlik	2003/2004/1, 2004/2005/1
KV	Methods in searching the scientific literature	Lívia Vasas	2003/2004/1, 2003/2004/2, 2004/2005/1, 2004/2005/2
KV	General methods of scientific research	Pál Tomcsányi	2003/2004/2
KV	Systems Biology: Complex methods in medicine and drug research - genomic considerations	András Guttmann, Mária Sasvári	2004/2005/2
KV	Clinical biometry	György Füst	2004/2005/2
KV	Theoretical and practical questions in research ethics	Ferenc Oberfrank	2004/2005/2
KV	Good Clinical Practice	Zsolt Tulassay	2003/2004/2, 2004/2005/2
KV	Genetic basis of human molecular biology	András Váradi	2003/2004/2

	<b>Compulsory Courses</b>	<b>Course Leader</b>	<b>Semester</b>
	Problem-based learning (PBL) in a postgraduate environment	David B. Ferguson, Gábor Varga	2003/2004/2
	Regulation of circulation, heart and myocardial blood supply. Part I. Concepts in circulation and their originators	Sándor Juhász-Nagy	2003/2004/1
	Regulation of circulation, heart and myocardial blood supply. Part II. Regulation of heart function and myocardial blood supply	Sándor Juhász-Nagy	2003/2004/2
	Experimental and clinical methods of studying cerebral circulation	Péter Sándor	2003/2004/1
	Treatment of cerebrovascular diseases. Surgical techniques	László Entz	2004/2005/1
	Medical physiology	Márk Kollai, Emil Monos	2003/2004/2, 2004/2005/1
	Clinical cardiovascular physiology	Márk Kollai, Emil Monos	2003/2004/2, 2004/2005/2
	Phototherapy, photochemotherapy: from physical foundations to applications	Gabriella Csik, Györgyi Rontó	2003/2004/1
	Phototherapy, photochemotherapy: from physical foundations to the clinics	Gabriella Csik, Györgyi Rontó	2003/2004/2
	Medical physics and statistics for medical students	Judit Fidy	2003/2004/2
	Study of molecular interactions and molecular movements of biological systems, as studied by optical and spectroscopical methods	Pál Gróf	2004/2005/1
	Methods in structure determination of macromolecules	Judit Fidy	2003/2004/1, 2003/2004/2, 2004/2005/2
	Methods and phenomena in folding-unfolding-misfolding of proteins	Judit Fidy	2003/2004/2
	XI. Budapest Nephrology School (nephrology, hypertension, dialysis, transplantation)	László Rosivall	2003/2004/2
	The 12th Budapest Nephrology School (nephrology, hypertension, dialysis, transplantation)	László Rosivall	2004/2005/2
	Medical and pharmaceutical applications of liposomes, and model membranes	Judit Fidy	2004/2005/2
	The heart and coronary circulation in health and disease	Ákos Koller	2003/2004/1, 2004/2005/1, 2004/2005/2
	Practical and theoretical basis of successful PhD studies	Anna Blázovics	2003/2004/1, 2004/2005/1
	Artificial feeding	Katalin Darvas	2003/2004/1

	<b>Compulsory Courses</b>	<b>Course Leader</b>	<b>Semester</b>
	Treatment of pain	Katalin Darvas	2003/2004/1
	Cell analytical conference	Béla Molnár	2003/2004/2
	Methods in cell analysis	Béla Molnár	2004/2005/1
	Preoperative exploration and coincident diseases in preparation for surgery	Katalin Darvas	2003/2004/2, 2004/2005/2
	Problems in perioperative treatment of old patients	Katalin Darvas	2003/2004/2
	Surgical anesthesia	Katalin Darvas	2004/2005/1
	Inhalation anesthesia	Katalin Darvas	2004/2005/2
	Diabetes mellitus and arteriosclerosis	Anikó Somogyi	2003/2004/1
	Free radicals in biological systems	Anna Blázovics	2003/2004/2
	Hepatology	János Fehér	2003/2004/2, 2004/2005/2
	Ultrasound in gynecology and pregnancy	Zoltán Papp	2003/2004/1
	New diagnostic and therapeutic possibilities in pediatric diseases of the airways and allergy	Endre Cserháti	2003/2004/1
	Problems in pediatric neurology	Viktor Farkas	2004/2005/1
	Gastroenterology	Zsolt Tulassay	2004/2005/1
	V. Gastroenterology course for advanced learners	Zsolt Tulassay	2004/2005/2
	Clinical immunology and allergology	Miklós Benczúr	2003/2004/1
	A model of bone fractures	János Hamar, Tibor Mózes	2004/2005/1
	Orthopedics	Miklós Szendrői	2003/2004/1
	Bronchial asthma	Pál Magyar	2003/2004/1
	Systems of drug therapy	Sylvia Marton	2003/2004/2
	Pharmaceutical biotechnology	Éva Szőke	2004/2005/2
	Quality control in pharmacy	Romána Zelko	2003/2004/2, 2004/2005/2
	Phytotherapy	Gizella Petri	2003/2004/1, 2004/2005/1
	Formulation of medicines	István Rácz	2003/2004/1
	Interactions, side effects and simultaneous actions in phytotherapy	Ágnes Kéry	2004/2005/1
	Pharmacodynamics	Kornélia Tekes, Zsuzsanna Füst	2003/2004/1
	Rational use and clinical pharmacological study of analgesics	András Telekes	2003/2004/2
	The fate of drugs in the body: drug metabolism, pharmacokinetics	Imre Klebovich	2003/2004/2
	Chromatographic methods and their use in pharmacology	Huba Kalász	2003/2004/1, 2004/2005/1
	Bioanalytical methods in pharmacokinetics	Imre Klebovich	2004/2005/1

	<b>Compulsory Courses</b>	<b>Course Leader</b>	<b>Semester</b>
	Clinical pharmacology and rational use of anticancer drugs	András Telekes	2003/2004/1
	Introduction in drug research	Béla Noszál	2003/2004/1, 2004/2005/2
	Protein dynamics, folding and aggregation. Structural basis of the phenomena, methods and physiological importance	Judit Fidy	2003/2004/1
	Molecular recognition and supramolecular chemistry	Lajos Barcza	2003/2004/2
	Drug design	Péter Mátyus	2003/2004/2
	Toxic plants, plant poisoning	Gabriella Marczal	2003/2004/2, 2004/2005/2
	NIR spectroscopy in pharmaceutical sciences	István Antal	2004/2005/1
	Behavior of macromolecules at interfaces	Ferenc Csempesz	2004/2005/2
	Foundations of studying calcium and bone metabolism	Péter Lakatos	2004/2005/2
	Nuclear magnetic spectroscopy	Ádám Demeter	2004/2005/2
	Biopharmacy	Sylvia Marton	2004/2005/2
	Plant fermentation in producing active substances of medicinal plants	Miklós László	2004/2005/2
	The role of family in causing, maintaining and treating disease	János Füredi	2003/2004/2, 2004/2005/1
	Theory of communication in practice of psychotherapeutics and psychiatry	János Füredi	2003/2004/1
	Basis of behavioral medicine I	Mária Kopp	2003/2004/1
	Basis of behavioral medicine II	Mária Kopp	2004/2005/1
	Basis of behavioral medicine III	Mária Kopp	2003/2004/2
	Basis of behavioral medicine IV	Mária Kopp	2004/2005/2
	Methods in health sciences	Mária Kopp	2004/2005/2
	Introduction in the clinical practice of family therapy	Tamás Kurimay	2003/2004/1
	Research methods for studying family functioning	Tamás Kurimay	2003/2004/2, 2004/2005/2
	Evidence based medicine in behavioral medicine. I. Methods and approach. Clinical epidemiology	István Mucsi	2003/2004/1, 2004/2005/2
	Evidence based medicine in behavioral medicine. II. Evidence based methods in behavioral medicine	István Mucsi	2003/2004/2, 2004/2005/2
	Introduction to methods in epidemiological research	Sándor Rózsa, Mária Kopp	2003/2004/1
	Introduction to methods in epidemiological research	Mária Kopp	2004/2005/1
	Clinical neuropsychology	Ilona Pataky	2003/2004/2, 2004/2005/2

	<b>Compulsory Courses</b>	<b>Course Leader</b>	<b>Semester</b>
	Application of modern methods in cognitive psychology	Dóra Perczel-Forintos	2003/2004/2, 2004/2005/2
	Neuropsychiatry of cognitive disturbances of organic origin: clinical EEG studies	Péter Rajna	2003/2004/2, 2004/2005/2
	Complaints, symptoms, disease, patient, ill person? The basic dilemma of medical treatment, the trap of superspecialization	Péter Rajna	2003/2004/1, 2004/2005/1
	Expression, perception and measurement of emotions, based on universality	Lajos Simon	2003/2004/2, 2004/2005/2
	Psychotherapy in medical practice	László Tringer	2003/2004/2, 2004/2005/2
	Physiology and pathophysiology of the aging brain	Csaba Nyakas	2004/2005/2
	Psychopharmacology	István Bitter	2004/2005/2
	Introduction in the methodology of epidemiological studies: Multivariate statistical methods in behavioral sciences	Sándor Rózsa, Mária Kopp	2003/2004/2
	Introduction in the methodology of epidemiological studies. II.	Mária Kopp	2004/2005/2
	Physiology of loading	Tamás Szabó	2003/2004/2, 2004/2005/2
	Pedagogical aspects of general theory and methodology of training	Endre Rigler	2004/2005/1, 2004/2005/2
	Pedagogical roles in the sports movement	János Gombocz	2004/2005/2
	Theoretical and methodological basis of curriculum in physical education	Pál Hamar	2004/2005/2
	Theory of effectiveness in acting (fighting sports, sports games)	Csaba Nagykáldi	2004/2005/1
	Learning and coordination of movements	Péter Molnár	2004/2005/1, 2004/2005/2
	Recreation	László Jakabházy	2003/2004/1
	Introduction in sports cardiology	Gábor Pavlik	2004/2005/1
	Endocrinology and metabolism	Róbert Frenkl	2004/2005/2
	Medical physiology	Róbert Frenkl	2003/2004/1
	Research methods (basics of multimedia)	János Mészáros	2003/2004/2
	Free radicals, physical exercise and aging	Zsolt Radák	2004/2005/2
	Dietetics	Róbert Frenkl	2004/2005/1
	Biomechanics of movements	József Tihanyi	2003/2004/2, 2004/2005/1
	Medical physiology	Róbert Frenkl	2003/2004/2, 2004/2005/1, 2004/2005/2
	Recreation	László Jakabházy	2003/2004/2, 2004/2005/2

	<b>Compulsory Courses</b>	<b>Course Leader</b>	<b>Semester</b>
	Theory and practice of recreation	László Jakabházy	2004/2005/1
	History of philosophy	Ferenc Takács	2003/2004/2, 2004/2005/1, 2004/2005/2
	Sports pedagogy. II.	Edit Nagy (Bíróné)	2003/2004/2, 2004/2005/2
	Research methods in social sciences	Edit Nagy (Bíróné)	2003/2004/2
	Sports pedagogy. IV.	Edit Nagy (Bíróné)	2003/2004/2
	Sport psychology in enhancing performance	Csaba Nagykáldi	2003/2004/2, 2004/2005/2
	Game theory – theory of sports games	Endre Rigler	2004/2005/1, 2004/2005/2
	Authority, aggression, prejudice	Roger Csáky-Pallavicini, András Ittész, Teodóra Tomcsányi	2004/2005/2
	Methods in data processing	Károly Ozsváth	2004/2005/2
	Modern history of sports	Katalin Szikora	2004/2005/2
	Structure and function of brain cortex	Tamás Freund	2003/2004/2
	Neuroendocrinology	Béla Halász, Gábor Makara	2003/2004/1
	Function of glial cells	Zsuzsanna Huszti, Mihály Kálmán	2003/2004/1
	Electrophysiology of integrative functions in the brain	György Karmos	2004/2005/2
	Neuronal cell differentiation	Emilia Madarász	2003/2004/1
	In vitro cell technology	Emilia Madarász	2003/2004/1
	Human neuroanatomy. III. Functional neuroanatomy	Miklós Palkovits	2003/2004/1
	Gene technology in the neurosciences	Gábor Balázs Szabó	2003/2004/2
	Neuropharmacology	E. Szilveszter Vizi	2003/2004/1
	Introduction to molecular neurobiology	Dénes Ágoston	2004/2005/2
	Lab course: In vitro cell technology	Emilia Madarász	2003/2004/2
	In vitro cell technology, theory and practice	Emilia Madarász	2004/2005/2
	Human neuroanatomy. I. Pathways	Miklós Palkovits	2004/2005/2
	Oxidative stress	Veronika Ádám	2004/2005/2
	Modelling, neurochemistry and pharmacology of behavior	György Bagdy	2004/2005/2
	Neurobiology and clinical aspects of anxiety and mood disorders	Gábor Faludi	2003/2004/2, 2004/2005/2
	Neurobiological foundations of stroke	Zoltán Nagy (OPNI)	2003/2004/1, 2004/2005/1
	Electrophysiological methods in studying disturbances in movements and thinking	Anita Kamondi	2004/2005/1

	<b>Compulsory Courses</b>	<b>Course Leader</b>	<b>Semester</b>
	Parkinson's disease and Parkinson's syndrome	Anita Kamondi, Annamária Takáts	2004/2005/1
	Methods in clinical electrophysiology	Anita Kamondi, Imre Szirmai	2004/2005/1
	Signaling in the nucleus	Péter Csermely	2003/2004/2
	Isotopic methods in cell physiology	István Voszka	2003/2004/1
	Structure and function of cell membrane receptors	László Hunyady	2004/2005/2
	Functional cytology	Edit Oláh	2004/2005/1
	Light and electron microscopy in research at the cell and molecular levels	Pál Röhlich	2004/2005/2
	Receptors and signal transduction in the immune system	Gabriella Sármay	2004/2005/2
	Role of the phagocytes in natural immune defense	Erzsébet Ligeti	2003/2004/2
	Reversible protein phosphorylation in the regulation of cell function and proliferation	Anna Faragó	2003/2004/1
	Stress proteins	Péter Csermely	2003/2004/2
	Structure and function of biological membranes	Balázs Sarkadi	2003/2004/2
	Rational planning of drugs. Usage of high resolution separation methods	Miklós Idei, György Kéri	2003/2004/1
	Rational drug design and signal transduction therapy	György Kéri	2004/2005/1
	Developmental biology. Embryos and genes	Imre Oláh	2003/2004/2, 2004/2005/1, 2004/2005/2
	Medical genomics	András Falus	2003/2004/1
	Histamine biology	Zsuzsa Darvas	2003/2004/2, 2004/2005/2
	Molecular genetic background of inherited skin diseases	Sarolta Kárpáti	2003/2004/1
	Clinical and biological importance of chemotaxis	László Kőhidai	2003/2004/2, 2004/2005/2
	Tumorbiology	Zoltán Marcsek	2003/2004/2, 2004/2005/2
	The genetics of sex. From no to yes	Sára Tóth	2003/2004/2, 2004/2005/1, 2004/2005/2
	Genomic background of multifactorial diseases	Csaba Szalai	2003/2004/2, 2004/2005/2
	Clinical immunology and allergology. II. (practical)	Péter Gergely	2003/2004/1, 2003/2004/2, 2004/2005/2
	Clinical immunology and allergology I (theoretical)	Péter Gergely	2004/2005/1
	Clinical oncology	László Kopper	2003/2004/1, 2003/2004/2, 2004/2005/2
	Tissue function disturbances in tumors	Ilona Kovalszky	2003/2004/2

	<b>Compulsory Courses</b>	<b>Course Leader</b>	<b>Semester</b>
	Molecular oncology	Ilona Kovalszky	2004/2005/2
	Molecular novelties in virology, virus diagnostics and molecular epidemiology	György Berencsi	2004/2005/2
	Changes in vocation in nursing with the appearance of highly trained nursing experts	Sándor Holló	2004/2005/2
	Options for health education of people in marginal situations	Mária Zám	2004/2005/2
	Disturbances of social adaptation	József Rácz	2003/2004/2
	Foundations of social medicine	Iván Forgács	2003/2004/2, 2004/2005/2
	Molecular novelties in virology, virus diagnostics and molecular epidemiology	György Berencsi	2003/2004/2
	Health economics and pharmaceutical economics, technology evaluation, measurement of life quality, quality control in infectious diseases	László Gulácsi	2003/2004/2
	Health economics and pharmaceutical economics	László Gulácsi	2004/2005/1
	Role of vocation in practicing health-related professions	Péter Balázs	2003/2004/2, 2004/2005/2
	Scientific evaluation of public health and technology in health, with special emphasis on prevention and treatment of infections	László Gulácsi	2004/2005/1
	Public health in prevention of chronic non-contagious diseases	Magda Antal	2004/2005/2
	Dietotherapy	Mária Barna	2004/2005/2
	Health education in infant and child care in hospital and outpatient practice	György Harmat	2004/2005/2
	Hormonal regulatory mechanisms. Functional morphology, pathophysiology and clinics	Károly Rácz	2003/2004/1, 2004/2005/1
	Clinical use of homologous transplantation of vessels and clinical conservation of arteries and veins	Attila Nemes	2003/2004/2
	Role of endovascular graft implantation in treatment of aneurysms	Kálmán Hüttl	2003/2004/2
	Clinical laboratory diagnosis in origin of aneurysm formation	István Karádi	2004/2005/1
	Clinical instrumental diagnosis and therapy	Attila Nemes	2004/2005/1
	Intraoperative biochemical monitoring of cerebrospinal fluid in surgery of thoracic and thoracoabdominal aorta aneurysms	György Acsády, Csaba Dzsínich	2003/2004/1

	<b>Compulsory Courses</b>	<b>Course Leader</b>	<b>Semester</b>
	Clinical study of therapeutic angiogenesis	György Acsády	2003/2004/1
	Pharmacodynamics. II.	Zsuzsa Fürst, Kornélia Tekes	
	Neurochemistry, pharmacology and models of behavior. (In English)	György Bagdy	2003/2004/2
	Trauma and recovery. I.	Jancis Long	2004/2005/1
	Methods in research	János Mészáros	2003/2004/1
	Research methods in social sciences	János Mészáros	2003/2004/1, 2004/2005/1
	Sport in contemporary society	Gyöngyi Szabó (Földesiné)	2004/2005/1, 2004/2005/2
	Physiology of fitness	Gábor Pavlik	2003/2004/1
	Physiology of training	Gábor Pavlik	2004/2005/2
	Sports pedagogy IV	Edit Nagy (Bíróné)	2004/2005/2
	History of philosophy	Ferenc Takács	2003/2004/1
	Research methods in social sciences. II.	Edit Nagy (Bíróné)	2004/2005/2
	Biometry	János Mészáros	2004/2005/2
	Research methods in pe. and sport	Gyöngyi Szabó (Földesiné)	2004/2005/2
	Sport and the European Union	Gyöngyi Szabó (Földesiné)	2003/2004/1, 2003/2004/2
	Sports pedagogy. I.	Edit Nagy (Bíróné)	2003/2004/1
	Sports pedagogy. I.	Edit Nagy (Bíróné)	2004/2005/1
	Sports pedagogy. III.	Edit Nagy (Bíróné)	2003/2004/1
	Sports pedagogy. III.	Edit Nagy (Bíróné)	2004/2005/1
	Sociology (Sport sociology)	Gyöngyi Szabó (Földesiné)	2004/2005/1
	Social values, social norms	Gyöngyi Szabó (Földesiné)	2004/2005/2
	Experimental pathology	Tibor Kerényi	2003/2004/1
	Bioinformatics and the study of anti-biotic resistance	Ferenc Rozgonyi	2003/2004/2, 2004/2005/2

## **PH.D. SCIENTIFIC DAYS**

The Ph.D. training program provides opportunities for every candidate to acquire practical knowledge of the methodology of presenting results gained in scientific research. Ph.D. students therefore are required to present their work regularly both among fellow workers and in a wider professional environment. The need for an overall Ph.D. conference of the Doctoral School was promoted even though the departmental doctoral schools organize scientific forums for their own Ph.D. students. The primary objective was that participants would be able to familiarize themselves with the scientific work of each program.

In 2000, for example, seventy-eight lectures and fifty-two poster presentations were given while in 2001 the figures were fifty-six and forty-eight respectively. In 2005 the students gave over 100 lectures and poster presentations. On these occasions Ph.D. students and candidates had the opportunity to present their work in twelve sections with a jury. Candidates with works of a high standard

gained awards in each section. The conferences were highly successful. Ph.D. students participated from partner universities in Budapest and in the provinces as well. Scientific results were presented in high standard lectures and presentations of a high standard. The relatively low turn out, however, not only of students but by their tutors as well, did not create a good impression. But conference feedback supports the idea of organizing further Ph.D. Scientific Days. Every academic year two highly regarded professionals, normally holders of the distinction “Excellent Ph.D. Supervisors” have been invited to give plenary lecture with great success.

## Plenary Lecturers

- 2003**      **Dr. Lajos Szollár (Ph.D. School of Basic Medical Sciences)**  
 “New” risk factors of atherosclerosis in the reflection of the “Hungarian paradoxon”  
**Dr. Imre Szirmai (János Szentágothai Neurosciences Ph.D. School)**  
 Recent progress in the research of cognitive and emotional disorders
- 2004**      **Dr. János Gombocz (Ph.D. School of Sport Sciences)**  
 Sport as reflected in the science of education  
**Dr. Mária Sasvári (Ph.D. School of Molecular Medical Sciences)**  
 Research on the psychogenetic association of dopamin D1 receptor
- 2005**      **Dr. Erzsébet Ligeti (Ph.D. School of Molecular Medical Sciences)**  
 Time switches in the cell: physiologic and pathogenetic role of GTP binding proteins  
**Dr. András Jeney (Ph.D. School of Pathology)**  
 Oncopharmacological investigations with training of tumor biologic aspects the doctoral studies

The plenary speakers are carefully selected from among those who have been awarded with the distinction of the “Excellent Ph.D. Supervisors”.

## ‘EXCELLENT PH.D. SUPERVISOR’ AWARD

Each year 5 to 8 outstanding personalities of the Ph.D. School receive the Excellent Ph.D. Supervisor Award. The medal goes not only to those who are most productive in training many Ph.D. students. The most efficient leaders of the Ph.D. Schools, retired presidents with outstanding merits are also awarded. The nominations are made by the Heads of the individual Ph.D. Schools, and it the voting of the University Doctoral Council that decides on the final list of awardees. The number of awardees is limited, and each year less and less people obtain the Award. The reason for the narrowing of the list is the aim that some years after the foundation of the Award, only two excellent persons should be awarded, thereby increasing the value of the distinction.

- |             |                             |   |
|-------------|-----------------------------|---|
| <b>2003</b> | <b>Dr. János GOMBOCZ</b>    | Ph.D. School of Sport Sciences                              |
|             | <b>Dr. Miklós PALKOVITS</b> | János Szentágothai Neurosciences Ph.D. School               |
|             | <b>Dr. István RÁCZ</b>      | Ph.D. School of Pharmaceutical and Pharmacological Sciences |
|             | <b>Dr. László ROSIVALL</b>  | Ph.D. School of Basic Medical Sciences                      |
|             | <b>Dr. Mária SASVÁRI</b>    | Ph.D. School of Molecular Medical Sciences                  |
|             | <b>Dr. Ferenc SZALAY</b>    | Ph.D. School for Clinical Science in Medicine               |
|             | <b>Dr. József TÍMÁR</b>     | Ph.D. School of Pathology                                   |

	<b>Dr. László TRINGER</b>	Ph.D. School Mental Health Sciences
<b>2004</b>	<b>Dr. Róbert FRENKL</b>	Ph.D. School of Sport Sciences
	<b>Dr. Béla HALÁSZ</b>	János Szentágotthai Neurosciences Ph.D. School
	<b>Dr. András JENEY</b>	Ph.D. School of Pathology
	<b>Dr. Mária KOPP</b>	Ph.D. School Mental Health Sciences
	<b>Dr. Erzsébet LIGETI</b>	Ph.D. School of Molecular Medical Sciences
	<b>Dr. Kálmán MAGYAR</b>	Ph.D. School of Pharmaceutical and Pharmacological Sciences
<b>2005</b>	<b>Dr. Anna KÁDÁR</b>	Ph.D. School of Pathology
	<b>Dr. László KOPPER</b>	Former President of University Doctoral Council
	<b>Dr. Emil MONOS</b>	Ph.D. School of Basic Medical Sciences
	<b>Dr. Péter RAJNA</b>	János Szentágotthai Neurosciences Ph.D. School
	<b>Dr. Zoltán VINCZE</b>	Ph.D. School of Pharmaceutical and Pharmacological Sciences

Besides the yearly organized Ph.D. Scientific Days, another possibility is given for students who are fluent in English and successful in results. Sir George Radda, a *honoris causa* doctor of Semmelweis University, initiated the interactive communication between students and outstanding teachers in 2005. Under the umbrella of the Doctoral Council, an unforgettable event has been organized with the contribution of the British Council to pick the best communicators among our Ph.D. Students.

## ESSAY COMPETITION (ORGANIZED IN 2005)

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### Introduction of the President of the Doctoral Council

*„There are more things in heaven and earth, Horatio,  
Than are dreamt of in your philosophy.”  
(Shakespeare: Hamlet)*

The **Semmelweis University** is once again the scene of an unprecedented event. The most outstanding students of our Ph.D. School compete for attractive and challenging awards sponsored by the **British Council** and the Doctoral School. The winner of the Science Essay and Communication Competition will spend one week in a British research laboratory on the expenses of the Council. The second and third places are worth two and one hundred thousand Hungarian Forints, respectively. All one had to do in order to catch this opportunity was to use 800 English words for an essay and to prepare for giving an eight-minute lecture based upon his or her research activity. The only restraint is that the text was to be made clear for lay persons. What is it if not the offer of the year? The applicants have submitted their essays, and expert reviewers have been working on the evaluation of the collected material. We share a secret with you; ten of twenty-three submitted reports have been selected for the second run, the oral presentation. Herewith we publish the works of all competing Ph.D. students and candidates, and you may bet on the prize-winner scientific masterpieces. If you show up and listen to the promising presentations, you may even witness and enjoy the method of the jury when selecting the top readers of our 23 prides. The engine of this venture is **Sir George Radda at Oxford University** who is the *honoris causa* doctor of Semmelweis. He presides the jury recruited from renowned journalists, the representatives of British Council and of our professors. The organizer is greatly indebted to professor Radda for the idea of this unique meeting as well as to those who contribute to the success of this event with either presenting their interesting research achievements or reviewing and judging them.

Have fun, enjoy the essays and don't miss out the 10 excellent talks!

## The Winners

<b>I. Prize:</b>	<b>Krisztina Katalin Sallai</b>
<b>II. Prizes:</b>	<b>Péter Mandl and Zsuzsanna Horváth</b>
	Szilárd Szabó I
	Gábor Sirokmány
	Zsuzsanna Lénárd
	Gábor Németh
	Ágota Bíró A
	Viktória Kovács
	Gábor Márk Somfai

Below we publish the three winning essays in detail.

## The Sticky Blood of Lupus Patients

**Krisztina Katalin Sallai**, tutor: Dr. Péter Gergely  
Program: Theoretical and Clinical Immunology

Do you remember the case of ailing old Bishop Stewart treated by Dr Kovaè until his death in three episodes of the popular TV series ER? Hardly anyone does. Well, that was the first time I heard of lupus, or systemic lupus erythematosus, usually called its short name, SLE. The second time I encountered lupus was when I was looking for a Ph.D. position. Probably every young molecular biologist longs to discover the cure for cancer or AIDS. So did I. But somehow I was attracted to a different field, perhaps by the sheer amount of mystery in autoimmunity which also causes SLE. Autoimmunity is a failure of the immune system. The cells and molecules of our immune system are responsible for protecting against threatening bacteria, viruses, and anything harmful, or non-self, all marked with the name: antigen. The antibodies – proteins secreted by a subpopulation of immune cells – are widely known to be essential contributors to our immunity. They bind to, and label the antigens' surface as targets for destruction and elimination by the immune system.

Autoimmune diseases, such as lupus, occur when our guard dog bites its own master, and an immune response develops to normal tissues. At the front-line of this “self-aggression” you will find antibodies directed against DNA, proteins, or cell organelles inducing a boisterous inflammation in many organ systems of the body. This manifests as skin rash, joint aches and kidney problems among others. My research focuses on a special group of such self-threatening antibodies (designated autoantibodies), namely the family of antiphospholipid antibodies. These monsters bind to the cell membrane's main building blocks, the phospholipids. What will go wrong if the phospholipid surface is covered by autoantibodies? To understand the answer, we have to look at the mechanism of blood clotting.

Platelets are a type of blood cells that help us stop bleeding. The phospholipid surface of the platelet membrane presents an ideal environment for clotting. The antiphospholipid antibodies can shift the subtle balance between clotting and bleeding towards too much clotting, resulting in the formation of a clot inside blood vessels. This is called a thrombus. The growing thrombi may obstruct the circulation, which can be fatal if vital vessels in the lung, brain, or heart are affected, or can lead to miscarriage if vessels inside the placenta are blocked. Antiphospholipid antibodies were first described by Professor Graham Hughes at Hammersmith Hospital in London many years ago. Since then, our knowledge about this phenomenon of “sticky blood” has grown considerably, but many questions remain unanswered. How big is the risk? Do lupus patients need to receive anticoagulant therapy, and live with the danger of potential side effects? These were the main questions of a study in our laboratory last year. More than hundred patients with SLE were involved in the investigation. They were all tested for antiphospholipid antibodies, as well as for other thrombotic risk factors. This work included testing for genetic mutations, measurements of plasma levels and activities of a dozen proteins, and a profound statistical analysis. The results showed that the incidence (i.e. chances each year) of thrombosis is more than 10-fold higher among patients with SLE, than among healthy persons.

Not every lupus patient has antibodies against phospholipids, only a third of them do. But the thrombotic effect of antiphospholipid antibodies became obvious, as half of the patients with antiphos-

pholipid antibodies already had had thrombosis. On the other hand, almost every lupus patient with a history of thrombosis proved to be positive for these antibodies. To our surprise, the presence of antiphospholipid antibodies was found to be the single most important risk factor for thrombosis among lupus patients, while the other, genetically determined risk factors known to cause thrombosis in the general population, were shown to occur no more frequently than among non-SLE people. Last week, I had the opportunity to discuss these results with colleagues from other parts of Europe at a meeting in Glasgow. The most debated issue was drug treatment of lupus patients predisposed to thrombosis. To appreciate the heat of the debate, we have to realize that needless anticoagulant therapy is not only costly and an inconvenience for the patient, but may also be risky. If anticoagulation is overdone, it is out of the frying pan into the fire. Life threatening bleeding can occur. Considering all issues, I share the opinion of most experts not to treat patients who have never had a thrombo-embolic episode, except in high-risk situations such as a pregnancy. Certainly, Bishop Stewart was not the last lupus patient visiting a hospital's Emergency Room. But, as our knowledge of the vicious anti-phospholipid antibodies advances, perhaps fewer-and-fewer of them need to show up in the ER due to a thrombotic or bleeding event.

## Its depression Jim, but not as we know it

**Péter Mandl**, tutor: Dr. Szilveszter Vizi E.  
Program: Functional Neuroscience

„Never mind Jim.” While borrowing a phrase from Star Trek's Dr. McCoy may draw a few frowns from the scientific community, it also reveals an increasing trend of public indifference to depression. People have grown accustomed to the fact that at any given time, multiple friends and family members carry the psychological and often physical burdens of depression. And just like the lay public, the scientific community has struck its own truce with the disease.

For almost fifty years, the so-called monoamine theory has been the driving force behind both pharmacology-driven and industrial drug research in this field. Nerve cells communicate with each other via specific molecules, called neurotransmitters. The synchronised activation and inhibition of nerve cells via neurotransmitters is the basis for higher processes, such as memory, cognition or perception. Serotonin and norepinephrine belong to a group of neurotransmitters, the monoamines. The first antidepressants were discovered by chance almost fifty years ago, when drugs that have been developed for other disorders were found to elevate the mood of psychiatric patients. These drugs were shown to increase the concentration of serotonin and norepinephrine. Based on this finding, the monoamine hypothesis proposes that depression and mood disorders are caused by a deficiency of monoamines at functionally important sites in the brain. It soon became apparent, however, that this hypothesis fails to explain several key properties of antidepressant therapy: the two-three week lag between treatment initiation and symptom alleviation, the fact that patients suffering from depression often have normal serotonin and norepinephrine levels and that several verified antidepressants have no impact whatsoever, on monoamine levels. Therefore, recent research focuses on other neurotransmitter systems - drugs, receptors and signal mechanisms - that may be affected by antidepressants and thus might contribute to both beneficial and potentially adverse effects of these drugs.

Acetylcholine, is yet another neurotransmitter that is directly involved in cognition, learning and memory. Released from a nerve cell in either the brain or in the body's peripheral nervous system, it binds to specific acetylcholine receptors situated on other nerve cells, forming functional networks. Usually, nerve cells have a preference for certain neurotransmitters and can be grouped accordingly. A selective loss of specific acetylcholine utilizing nerve cells for instance can lead to Alzheimer's disease. Our work group has found some previous evidence that antidepressants block a certain type of acetylcholine receptor. Nerve cell networks can be thought of as local communities, collectives with common goals, rather than a bunch of individuals with separate needs. In this sense, depression can be thought of as overheated bickering within certain neighborhoods in the brain. In real life, arguments often hinder the flow of information. Not surprisingly, depression is characterized by flawed information processing. In such a setting, antidepressants might act as relaxants - tempering and correcting communication within malfunctioning cellular networks. This function may contribute to the antidepressant effect of these drugs. Part of my current work deals with measuring the brain levels of acetylcholine in vivo, that is in live, freely-moving rats through a sampling device embedded in

their brain. Via this device I can also introduce drugs, such as antidepressants into specific brain areas, thereby enabling me to study how the drugs affect key areas responsible for mood disturbances. Another part of my work focuses on finding a possible explanation for a common adverse drug effect reported by many patients on antidepressant therapy: constipation. 6-10% of patients on Prozac suffer from constipation. Sold to an estimated 40 million people worldwide since hitting the market, Prozac was one of the drugs we tested on the network of nerves responsible for motility, the muscular contractions of the intestines which help us digest our food and move it along the gut. Prozac works the same way in the gut, as it works in the brain: it blocks the acetylcholine receptor. In the brain, this may be the reason why it alleviates the well known symptoms of depression – hopelessness, guilt, depressed mood. The same action in the gut, however, leads to a serious adverse effect, constipation, because motility is fuelled by acetylcholine. By blocking the communication between acetylcholine-utilizing nerve cells, Prozac practically blocks motility, thus leading to constipation. History shows us, how long established truces can and must be broken by progress. Just as the lay public must learn to utilize the multitude of psychotherapeutic and pharmaceutical options available, so must the scientific community search for new answers to old questions. Understanding what antidepressant drugs really do in our body as a whole is a multi-layered challenge, that may one day lead us to comprehend depression, this most elusive and ubiquitous blight on our society. Now back to you Jim. Only this time, instead of: depression, same as usual, let's think depression, quite the unusual.

## HISTAMINE – A LITTLE GEM OF REGULATION?

**Zsuzsanna Horváth**, tutor: Dr. Edit Buzás

Program: Fundamentals of human genetics and gene diagnostics

April 26th 1986. Early morning, the small town of Pripjatj was shaken by two explosions coming from the nearby atomic power plant, Chernobyl. Overall casualties of the nuclear meltdown: ranging from 300 to 300,000. Although it has been 20 years from now and the exact number of victims are still under debate, what the world inevitably came aware of was the importance of radiation on mankind, focusing on the effects exerted on the human organism, i.e. our cells. Studying radiation effects on cells may help treat problems arising from accidental radiation exposure. And what does it all have to do with histamine? Well, that is my real challenge: to prove that histamine, this small molecule indeed influences cells in their reaction to radiation effects.

What do we usually think about when we hear the word histamine? Allergy, asthma, everlasting runny nose, hayfever, antihistamines used for therapy... But that is only the peak of the iceberg! Histamine is proved to play essential roles in the lives of various cells, for example in the brain and in the stomach (that is why we use antihistamines in the treatment of ulcer). Nevertheless, a recently found and quite stunning idea is that histamine is produced by almost every cell of our body! So what is then the exact role of this particle which can be found everywhere in our body? We think that it regulates basic life supporting processes within our cells, and this regulation can be tested under unnatural circumstances, for instance by irradiating cells. For this purpose we irradiate mice by a dose of radiation that does not kill them. However, due to the effect of radiation the most sensitive cells in our body, i.e. cells of the bone marrow, are depleted. Since the normal function of these cells is to produce cells of the blood, slowly the bone marrow regenerates. During regeneration we can examine important processes going on within the proliferating cells.

A great help for my research came from the field of genetics. With the ability to manipulate the genes of experimental animals, a special histamine free mouse trait was developed by disrupting the enzyme (so-called HDC) that synthesizes histamine. These mice are called HDC-knockout mice. Doing experiments on them, we can deduce the roles of histamine. Irradiating these HDC-knockout mice, we can see that the regeneration of their bone marrow is delayed compared to normal mice. The early stem cell populations responsible for repopulating the bone marrow are affected by irradiation to a greater extent than the normal cells and they are incapable of sufficiently proliferate. So we concluded that histamine must be an important factor in regulating normal regenerating processes. Also, irradiation depletes the bone marrow by killing cells or inducing them to commit suicide by the so called 'apoptosis' meaning programmed cell death. We found that in HDC-knockout mice cells die at a greater rate after irradiation, indicating that their protective factor, histamine is absent.

From all these results we see that there must be some way by which histamine keeps cells alive, but

the exact mechanisms are yet to be investigated. 'All right, but what is it all good for?' you could ask. These basic investigations helps us better understand the normal functioning of our cells, and by this we get more and more information about the factors that affect our lives. One step closer to get answers is the recent discovery of the fourth histamine receptor (H4R). Until now it was believed that there are 3 receptors receiving messages from histamine. Antihistamines used in therapy act on these receptors by blocking them. With focusing ont he presence of H4R on bone marrow cells in my research, the regulating functions of histamine can be unveiled even more, and this opens new ways to develop sophisticated new drugs to be used in therapy for various diseases.

Moreover, there is growing evidence stating that histamine has effects not only on normal cells but on tumor cells as well. For instance, histamine is proved to make leukemic cells proliferate more, and that these malignant cells produce more histamine anyways. If we can provide more information on how histamine influences tumor growth, we may contribute to help development of treatment. This is true especially when therapy itself depletes bone marrow and transplantation is needed to regenerate normal bone marrow functions. If it is shown that histamine indeed has essential functions within normal cells and perhaps tumor cells also, then we may be able to influence our lives, and also therapy can be more effective. The same is true for radiation accidents: they may not be prevented by biological experiments, but the scientific results can contribute to effectively solve their aftermath. But of course, until then, there is still a long way to go...

## **Awarding of the Doctoral Degree with Distinction**

The President of the Republic consented to the awarding of the doctoral degree to **Dr. Peter Studinger** with the distinction 'Promotio sub auspiciis praesidentis Rei Publicae'. It was **Dr. László Sólyom, the President of the Republic** in person who handed over the Diploma and the traditional Golden Ring to the inaugurated Dr. Studinger. This ceremonial program was part of the Dies Academicus of Semmelweis University.

## **The spectrum of the scientific interest of our Doctoral School**

In the following chapters we would like to introduce the individual Ph.D. (Doctoral) Schools with all participating students and tutors. Even the abstracts (summaries) of successful recipients of the doctoral degree are included – in order to show the depth and colorfulness of our Ph.D. training.

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# 1. PH.D. SCHOOL OF BASIC MEDICAL SCIENCES

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## *Head of School*

**László ROSIVALL**

Department of Pathophysiology  
Nagyvárad tér 4, 1089 Budapest  
Tel: (361)-210-4409, Tel/fax: (361)-210-4409  
E-mail: rosivall@net.sote.hu

The School of Basic Medical Sciences at Semmelweis University consists of five multi-disciplinary research and training Ph.D. programs. These programs are closely related to physiological/pathophysiological sciences, and are chaired by internationally recognized professors. Accredited individually in 1994, all the Ph.D. programs were integrated into a Ph.D. School in 2002. So far, more than 200 postgraduate students have received education in Hungarian and English; above 40% have already graduated.

The Ph.D. programs are mainly focused on investigating the mechanisms of diseases with high morbidity and mortality (e.g. cardiovascular and renal diseases, hypertension, obesity), and to study hazardous environmental effects (UV and X-Radiation) on humans. These issues are addressed employing a complex approach from molecular levels to bedside. Investigating the molecular-cellular background of physiological and pathophysiological processes and integration of the knowledge at organ and organism levels leads us to new scientific results and discoveries which may promote the development of up-to-day methods for health prevention, diagnostics, and therapy. We offer several basic and clinical studies to those who wish to join our research activity as Ph.D. students.

## ***1/1. PROGRAM***

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### **PHYSIOLOGY AND CLINICS OF CARDIOVASCULAR DISEASES**

#### *Coordinator*

**Sándor JUHÁSZ-NAGY**

Department of Cardiovascular Surgery  
Városmajor u. 68. 1122 Budapest  
Tel/fax: (361)-355-4684  
E-mail: jns@erseb.sote.hu

The complex Program is directed to well-qualified students who are interested in cardiovascular research. The spotlight is on regulatory aspects, and according to the scientific interest of most of coordinators, on problems connected to pathophysiology (clinical physiology) of myocardial and coronary regulation. (However, as the list of sub-programs shows, other circulatory topics are included, too.)

The Program prepares students for careers in either clinical science (especially cardiovascular surgery, invasive cardiology, and anesthesiology) or basic sciences. Preference is given to those who are ready to study overlapping territories of these sciences. Although the individual postgraduate sub-programs have an overall general similarity in their logistic aspects, the main characteristic of the entire educational process is the flexibility. Consequently, that research work can be tailored to the tutor's mutual interest within the territory covered by the general aims.

*Sub-programs*

Studies on vascular surgery. Surgical efficiency and surgical techniques in the invasive therapy of cerebrovascular disorders  
 Pathophysiology of the visceral circulation  
 Vasoactive peptides in the surgical heart diseases and in their experimental models  
 Pathophysiology of the heart and coronary circulation  
 Electrophysiological investigations of the mechanisms of ventricular arrhythmias. New aspects in the non-pharmacological therapy of arrhythmias

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**ANDRÁS CSÓKAY (2003)**

## **New surgical methods in the treatment of ischaemic brain damages caused by aggressive traumatic brain swelling**

*Supervisor: Dr. János Hamar*

One and a half million people die per year after severe head injury as WHO reports. In Hungary the number of deaths is about 2000. The occurrence of treatment-refractory, raised ICP, due to cerebral edema following severe craniocerebral trauma is a difficult management problem, often with poor outcome. Approximately 50% of all patients who die after severe head injury do so as a result of a treatment resistant increase of ICP. Decompressive craniectomy, as a last resort therapy has a role in the treatment of severe traumatic brain swelling according to the literature. The method was described at the dawn of the last century, however its effectivity has not been proved until now. The insufficiency is most likely due to the compression of the cortical veins and arteries supplying the herniated brain, a situation provoked by shearing and pressure forces between the dural edge and brain tissue. Furthermore, a venous congestion may induce edema in the protruding parts of the brain, thus further compromising the viability of the neurons. The new surgical method is based on a stellate type durotomy and creation of a vascular tunnel, around the main cortical veins and arteries, with the aim that the vessels do not become compressed by the dural or bone edges. The effect of the novel vascular tunnel technique has been proven by measuring the blood flow of the protected and non-protected veins with Doppler ultrasound, intra- as well as postoperatively in humans and rats. This operation method was performed on 30 patients. All of them were in severe GCS < 5 status with or more than 30 mmHg ICP. In comparison with the traditional surgical and non-surgical treatment, in which the reported mortality rates are around 80% in severe cases, the mortality rate of our technique was reduced to 40% and the recovery (GOS 4,5) rate also increased significantly. Further space winning methods were improved by skin enlargement and open skin procedure.

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- Csókay A, Pataki G, Nagy L, Belán K (2002) *Vascular tunnel construction in the treatment of brain swelling caused by trauma and SAH (Evidence based on intraoperative blood flow measure). Neurol Res 24:157-160.*

**LÁSZLÓ GELLÉR (2003)**

## **Electrophysiological changes induced by endothelin-1, ischemia and reperfusion in experimental model**

*Supervisor: Dr. Sándor Juhász-Nagy*

Endo- or epicardial MAP reproduces the shape and time course of repolarization of myocardial cells, and it is applicable for examining the local myocardial repolarization process even in clinical settings. Moreover it is one of the most sensitive method for the in-vivo detection of local myocardial ischemia.

In experimental models we compared ET-1 induced presumably direct and coronary occlusion induced "clear" ischemic arrhythmogenesis by multichannel monophasic action potential recording and other electrophysiologic methods. In the same model effective inhibition of ET-1 induced arrhythmias was done by applying mixed ET A/B receptor antagonist. 60 pmol/perc intracoronary (ic.) ET-1 induced ventricular arrhythmias (VES, nsVT, sVT and VF in 6 cases) before the appearance of ischemic ECG and hemodynamic signs in 7 mongrel dogs, and in 4 instances concomitant EAD-s and significantly increased MAP dispersion were also noticed in some specimens. Increasing doses

(0,125-1,000 nmol/min) of intrapericardial (ip.) ET-1 infusion was found to exert dose-dependent arrhythmogenic action in 15 dogs. The 0,250 nmol/perc dose induced severe ventricular arrhythmias which were preceded by significant MAP lengthening and only moderate ischemic ECG and hemodynamic changes. Presumably as a result of the roughly uniform dispersion of ET-1 in the pericardial sac MAP dispersion did not change significantly in these experiments. The mixed ET A/B receptor antagonist bosentan was tested under similar experimental conditions. Similar arrhythmias and electrophysiological changes were observed in the control (ip. ET-1 group, n=6). Severe arrhythmias were not observed by ET-1 when bosentan bolus was given before the ET-1 infusion, an increase of MAPD90 was not seen and ischemic ECG changes were also significantly less pronounced even in case of applying higher dose (33 pmol/min/kg) ET-1 infusion.

In order to differentiate in between coronary occlusion induced and ET-1 induced arrhythmias a comparative study was done using ic. ET-1 and coronary blood flow measurements. In 8 dogs occlusion of left anterior descending (LAD) artery resulted in prompt and significant decrease of MAPD90 and UV during the period of ischemia and significant increase of both parameters was observed in the reperfusion period. In ET-1 groups (30 pmol/min n=8 and 60 pmol/min n=10, ET-1 was infused into the LAD) UV did not change significantly whereas significant increases were noticed in MAPD90 and in MAP dispersion. The fundamentally opposite characteristics of MAPD90, UV and MAP dispersion changes further support the postulated direct arrhythmogenic effect of ET-1.

In conclusion, multiple MAP recording seems to be suitable method for studying the extent and precise localization of myocardial ischemia, rendering the determination of arrhythmia pathomechanism possible. The major characteristics of ic. ET-1 induced direct arrhythmias are the significant lengthening of MAPD90 and unaltered UV values in the infused area. Increased MAP dispersion – which was described in the literature first by us – refers to the altered electrophysiological characteristics of the different areas of the heart which play an important role in the pathomechanism of intracoronary ET-1 induced ventricular arrhythmias, because it contributes to the development and maintenance of both triggered and reentry type arrhythmias. ET receptor antagonists seem to be promising agents, to prevent ET-1 induced direct arrhythmias and they might be used as antiarrhythmic drugs in the future.

By applying applying different investigational methods our research group concluded that the development and characteristics of ET-1 induced arrhythmias significantly differ from the coronary occlusion induced ischemic arrhythmogenesis, however ET-1 induced local ischemia can not be ruled out.

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- Geller L, Merkely B, Szokodi I, Szabo T, Vecsey T, Juhasz-Nagy A, Toth M, Horkay F (1998) *Electrophysiological effects of intrapericardial infusion of endothelin-1. Pacing Clin Electrophysiol* 21: 151-156.
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ORSOLYA KISS (2005)

## Experimental and clinical investigations of the arrhythmogenic and antiarrhythmic effects of agents lengthening the action potential

Supervisor: Dr. Violetta Kékesi

The remarkable role of repolarisation phase lengthening in the pathogenesis of triggered ventricular arrhythmias is supported by several literature data. However, prospective clinical studies proved that class III antiarrhythmic agents prolonging the repolarisation phase are among the most potent and reliable drugs. I examined the arrhythmogenic and antiarrhythmic effects of agents lengthening the action potential. I studied the effects of endothelin-1 (ET-1), an endogenous peptide having direct

arrhythmogenic properties based on repolarisation phase lengthening in dog. Moreover, I examined the effects of class III antiarrhythmic drugs on endocardial defibrillation threshold in a clinical study. 1. I investigated the role of triggered mechanism and myocardial ischemia in the arrhythmogenic effect of intracoronary (i.c.) bolus administration and infusion of ET-1 in the dog. The direct arrhythmogenic effect was dominant when low dose ET-1 was infused, while bolus ET-1 administration had mixed ischemic and direct arrhythmogenic properties. 2. I examined the receptor dependence of the direct arrhythmogenic action of low dose intrapericardial ET-1 infusion using the ETA-receptor antagonist LU 135.252. The LU 135.252 administration inhibited the electrophysiological and arrhythmogenic effects, but could not prevent the ischemic properties of ET-1. 3. I studied the electrophysiological and haemodynamic effects of the repolarisation phase lengthening i.c. ET-1 infusion given simultaneously with the repolarisation phase shortening intravenous isoproterenol (ISO) infusion. Isoproterenol showed antagonistic effect against ET-1 induced ventricular arrhythmia formation, while ET-1 proved to be protective against the development of ISO induced atrial fibrillation. An additive effect of ET-1 and ISO on left ventricular contractility was also observed. 4. We investigated the effects of class III antiarrhythmic treatment and defibrillation shock morphology on endocardial defibrillation efficacy in a randomized prospective clinical study. In patients treated with amiodaron or d, l-sotalol, markedly lower defibrillation threshold could be achieved by using biphasic pulses with shorter (2 ms) second phases. Our experimental and human studies demonstrate that the lengthening of the repolarisation phase can exert either arrhythmogenic or antiarrhythmic effects depending on the cumulative effect of numerous factors influencing cardiac function.

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- Kiss O, Gellér L, Merkely B, Szabó T, Raschack M, Seres L, Zima E, Juhász-Nagy A, Horkay F (2000) Endothelin-A-receptor antagonist LU 135.252 inhibits the formation of ventricular arrhythmias caused by intrapericardial infusion of endothelin-1. *J Cardiovasc Pharmacol* 36:S317-319.
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ZSUZSA RÉCSÁN (2003)

## New diagnostic and therapeutic approaches in ocular diseases with neovascularization

Supervisor: Dr. Kinga Karlinger

*Aim:* Our aim was to study the diagnostic value of MRI for the detection of scleral or extrascleral extension of uveal melanoma; to analyze the effectiveness of cryopexy (CP) and argon or diode laser photocoagulation (LC) for threshold stage 3 retinopathy of prematurity (ROP); to assess refractions, visual acuity (VA) in eyes underwent (LC) for threshold stage 3 ROP; and to compare the anatomical results of zone 1 prethreshold and stage 3 threshold retinopathy following argon LC. *Patients and Methods:* It is a non-randomized, observational case-series from 1991 to 2001. Using fat suppression technique, contrast enhancement, surface coil MRI was performed on 12 adult patients with clinically suspected large uveal melanoma. The clinical and histopathological findings were compared. CP was applied on 288 eyes of 146 premature infants, 222 eyes of 114 babies received LC. At the age of one year cycloplegic refraction was performed on 72 eyes of 39 babies underwent laser coagulation for stage 3 threshold retinopathy. VA could be measured on 25 eyes of 19 children at the age of 3 years. 71 eyes of 36 babies underwent argon LC at either prethreshold or threshold ROP in zone I. *Results:* MRI allowed detection of scleral infiltration with a sensitivity (SE) of 100% and a specificity (SP) of 50%. For extrascleral extension, MRI had a SE of 100% and SP of 89%. Following CP or LC, favorable outcome was achieved in 85% or 95%, respectively ( $p = 0,001$ ). Eyes of high-risk preterm infants (birth weight <800 gr, zone I disease, circular coagulation) treated with LC had significantly

better results compared with eyes treated with CP. The frequency of  $-4,0D \leq$  myopia was twice in zone I as was in zone II ( $p=0,01$ ). VA  $<0,8$  was found in 75% of eyes with myopia  $-4,0D \leq$  ( $p=0,028$ ). The posterior pole was complicated in 60% of eyes with VA  $<0,8$  ( $p=0,006$ ). As to the dragging of temporal vessels, the difference between the threshold and prethreshold groups was significant ( $p=0.0257$ ). *Conclusions:* Using fat suppression technique and contrast enhancement, MRI proved to be a valuable ancillary method for the assessment of scleral infiltration and extrascleral extension. The structural outcomes of laser coagulation were significantly better compared with cryopexy. The frequency of moderate or high myopia and visual problems may be increased when the posterior pole is even in mild degree complicated. Statistically significant improvement was found for posterior pole outcomes between zone I prethreshold and threshold ROP eyes.

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## BALÁZS TÓTH (2005)

### The role of gender following trauma and hemorrhagic shock

*Supervisor: Dr. János Hamar*

Despite significant advances in the management of trauma victims, sepsis and the ensuing multiple organ failure (MOF) continue to be the most common cause of mortality in surgical intensive care units. Several clinical and experimental studies suggest that gender affects the immune and organ functions under physiologic and pathophysiologic conditions. Although a huge amount of information is available on these gender dimorphic observations, the underlying mechanisms are less known. In our experiments we examined the effect of gender on neutrophil function, on heme oxygenase activity and on liver function following trauma (T) and hemorrhagic shock (H). To study this, proestrus female and male Sprague-Dawley rats underwent a 5 cm midline laparotomy (i.e., induction of a soft tissue trauma) and were bled to a mean arterial blood pressure of 35 mmHg for ~90 min, after which they were resuscitated with Ringer's lactate solution (4x the shed blood volume). Circulating polymorphonuclear granulocytes (PMNs) were assessed for superoxide ( $O_2^-$ ) and elastase production and different tissue samples were analyzed for myeloperoxidase (MPO) activity and TBARS (thiobarbituric acid reactive substances) as a marker of oxidative injury, at 2 and 24 hr after resuscitation. Liver samples were also collected for analysis of heme oxygenase expression and activity, while peripheral plasma samples were collected and alanine aminotransferase (ALT) levels were determined at 5 hr after resuscitation. In a different setting, the effects of heme oxygenase blockade on the liver function was studied.

Our results show that phorbol-13-myristate-12-acetate (PMA)-stimulated  $O_2^-$  production was not influenced by T-H or gender. In contrast, N-formyl-methionyl-leucyl phenylalanine (fMLP)-stimulated  $O_2^-$ , and lipopolysaccharide (LPS)-stimulated elastase release by PMNs from male T-H rats was greater than that of females. A significant increase in MPO activity and in TBARS levels was observed in different tissue samples of animals of both sexes following T-H, however, MPO activity and TBARS levels were higher in males than in female rats. Levels of the chemokine cytokine induced neutrophil chemoattractant (CINC-1) were elevated in the lungs of males, but not of proestrus females after T-H. Trauma-hemorrhage induced a two-fold increase in hepatic HO-1 expression in proestrus females compared to males. At the same time, hepatic expression of HO-2 was unaffected by gender or T-H. Blockade of HO with tin-protoporphyrin IX (SnPP) abolished the gender differences observed in HO-1 expression. This treatment also elevated the portal pressure, decreased bile production and increased ALT to similar levels in proestrus females and males following trauma-hemorrhage. As gender influenced the hepatic expression of HO-1 following trauma-hemor-

rhage, the enhanced induction of HO-1 expression and activity in females may act to attenuate hepatocellular dysfunction and injury probably by maintaining microcirculation. Moreover, the decreased PMN priming and activation in proestrus females, compared to males, occurs following trauma-hemorrhage resulting in decreased cellular injury and organ damage that is likely to contribute to improved outcome under those conditions.

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- Toth B, Yokoyama Y, Kuebler JF, Schwacha MG, Bland KI, Wang P, Chaudry IH (2003) Gender differences in hepatic heme oxygenase expression and activity following trauma-hemorrhagic shock. *Arch Surg* 138:1375-1382.
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## 1/2. PROGRAM

### MECHANISMS OF NORMAL AND PATHOLOGICAL FUNCTIONS OF THE CIRCULATORY SYSTEM

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The Program consists of 12 research sub-programs completed with appropriate theoretical courses for postgraduate students. Different aspects of normal and disturbed regulatory processes of the cardiovascular system are in the focus. Each Ph.D. student is working on his/her own individual research project under the guidance of a qualified scientific advisor. Successful completion of the Program including publications in recognized international journals provides an opportunity to summarize the results in a Ph.D. thesis.

#### *Sub-programs*

#### *Supervisors*

Pathophysiology of the cerebral circulation	Zoltán BENYÓ
Adaptation of cerebral circulation to NO deficient circumstances	Zoltán BENYÓ
The role of endogenous CO in the cerebral circulation	Zoltán BENYÓ
The role of bradykinin receptors in the adaptation of circulation under normal and pathophysiological conditions. Interactions with mechanisms regulating blood pressure	László DÉZSI
Mechanisms of circulatory adaptation in brain cortex and cardiac muscle	László DÉZSI
Fractal and chaos in physiological systems	András EKE
Spatial and time-related reactions during cognitive functions in the hemodynamics and oxygenations of the human brain	András EKE
Spatial and time-related reactions after migraine, cerebral vasospasm, stroke in the hemodynamics and oxygenations of the human brain	András EKE

Spatial and time-related reactions of the dynamic and static auto-regulation in the hemodynamics and oxygenations of the human brain. Human and experimental models	András EKE
1868174 The effect of artificial blood preparations on the micro-circulation of the brain under normal circumstances and hypoperfusion	András EKE
Interrelation between metabolic parameters, vascular compliance and neuropathy in metabolic disorders	László GERŐ
Interrelation between metabolic parameters, vascular compliance and neuropathy in patients with kidney transplantation	László GERŐ
Intracellular calcium homeostasis in developing cardiomyopathy	Tamás IVANICS
Relations between standard cardiovascular reflex tests determined by blood pressure measurements and blood pressure parameters	Péter KEMPLER
Clinical assessment of cardiovascular adaptation mechanisms among physiological and pathophysiological circumstances	Péter KEMPLER, László GERŐ
Interrelation between autonom function, hypertonia and cardiovascular risk factors	Péter KEMPLER
The effects of autonom neuropathy and diabetes on the cardiovascular system	Péter KEMPLER
Comparison of different clinical and experimental methods to analyze autonom and peripheral sensory neuropathy in the cardiovascular system	Péter KEMPLER
Interrelation between autonom cardiovascular regulation, ion transport processes and hemodynamic parameters	Márk KOLLAI
Investigation of mechanisms determining autonomic nervous system tonic and reflex activity – role of vascular properties	Márk KOLLAI
The role of mitochondria in ischemic and degenerative diseases	Zsombor LACZA
Regulation of intracellular calcium homeostasis in different types of muscles	László LIGETI
Regulation of intracellular calcium homeostasis in myocardial tissue	László LIGETI
Mechanism of adaption of vascular biomechanics and network properties to normal and pathological stress effects	Emil MONOS
Role of endogenous peptides and endothelium derived vasoactive substances in the regulation of the cerebral blood flow in physiological and pathological conditions	Péter SÁNDOR
Ischemia induced molecular biology changes in the function of the blood-brain barrier	Péter SÁNDOR
The role of female sexual hormones in the regulation of regional cerebral circulation	Péter SÁNDOR
The role of neural and endothelial factors in the regulation of cerebral circulation	Péter SÁNDOR
Role of oxidative stress in the pathogenesis of cardiovascular dysfunction	Csaba SZABÓ
Role of sexual steroids in the age- and hypertension-related vessel wall changes. The effects of postmenopausal sexual steroid therapy on circulation and on humoral factors regulating circulation	Béla SZÉKÁCS
Gender and hormonal changes in the cardiovascular system	Béla SZÉKÁCS
Investigation of the relative circulatory protection in the elderly	Béla SZÉKÁCS

Cerebral white matter-blood pressure-blood plasma components in the elderly

Béla SZÉKÁCS

Congenital heart diseases in adults

András TEMESVÁRI

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Péter Sándor
Márk Kollai
Péter Sándor
Márk Kollai

a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

SÁNDOR BÁTKEI (2003)

## The dynamics of intracellular calcium and tissue metabolism in intact slow- and fast-twitch skeletal muscle fiber

Supervisor: Dr. László Ligeti

The understanding of the fiber-specific differences under physiological and pathophysiological conditions, e.g. ischemia-reperfusion, in skeletal muscle is hampered by the lack of data gained from intact muscles. We describe an *in vivo* model for studying the dynamics of tissue metabolism and free  $[Ca^{2+}]_i$  in intact fast-twitch extensor digitorum longus (EDL) and the slow-twitch soleus (SOL) muscles of anesthetized rat. We developed a system based on an intravital microscopy using incident light combined with the Indo-1 ratiometric method in order to measure the microcirculation, metabolic state by tissue fluorescence and free  $[Ca^{2+}]_i$  combined. Fluorescence images were collected by means of a digital camera. We compared the effect of infrarenal ischemia and reperfusion on the macro- and microcirculation as well as tissue metabolism in fibers of different types. We also assessed the dynamics of free  $[Ca^{2+}]_i$  changes in mammalian slow- and fast-twitch muscles *in vivo* using caffeine superfusion. We assumed that differences in sensitivity between the two muscle types for this substance reflect differences in intracellular  $Ca^{2+}$  handling in the fibers of which these muscles consist. Caffeine induced a concentration-dependent increase in free  $[Ca^{2+}]_i$  and revealed differences in caffeine sensitivity between the muscle types, with the SOL being more sensitive. We may conclude that the dynamics of free  $[Ca^{2+}]_i$  can be assessed reliably in intact mammalian muscle *in vivo*.

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- *Ivanics T, Miklos Z, Ruttner Z, Batkai S, Slaaf DW, Reneman RS, Toth A, Ligeti L (2000) Ischemia/reperfusion-induced changes in intracellular free  $Ca^{2+}$  levels in rat skeletal muscle fibers—an in vivo study. Pflugers Arch 440:302-308.*

ERDŐS BENEDEK (2005)

## Cerebrovascular dysfunction in insulin-resistance

Supervisor: Dr. Péter Sándor

Obesity is a major and growing health problem in the world, and obese subjects are at high risk for developing insulin-resistance (IR) and cardiovascular diseases. It has been shown previously that IR impairs vascular function in the peripheral and coronary circulations, but its effects on cerebral arteries are virtually unexplored, despite the fact that IR increases the risk of stroke, and chronic hypoperfusion of the brain due to IR-induced cerebrovascular dysfunction might be related to increased prevalence of neurodegenerative disorders, particularly Alzheimer's disease.

We examined the effects of IR on the cerebral arteries using a dietary (fructose-fed rats) and a genetic (Zucker obese rats) model of IR. Vascular responses of isolated pressurized middle cerebral arteries (MCA) were studied *in vitro*, while reactivity of the basilar artery (BA) and its side branches was examined *in vivo* using a cranial window technique.

The results of our studies demonstrated that fructose-diet-induced IR impairs the function of the vascular smooth muscle (VSMC) KATP and BKCa-channels, and the dysfunction of these ion channels affects endothelium-dependent, cyclo-oxygenase- and prostacyclin-mediated relaxation, too. In contrast, endothelial nitric-oxide (NO)-dependent relaxation, Kir and Kv-channel function as well as endothelin-1-mediated vasoconstriction remain intact. Examination of cerebrovascular function in the Zucker obese rat revealed that these animals are in a more progressed state of IR, and beside the impairment of VSMC KATP and BKCa-channels, Kir-channel-mediated relaxation as well as NO-dependent dilation are also reduced compared to the control Zucker lean rats. However, vasocons-

trictor mechanisms mediated by endothelin-1, thromboxane A<sub>2</sub>, and Rho kinase remain unaltered. Further studies demonstrated that the adverse effects of IR are mediated by increased protein kinase C activity and NAD(P)H-oxidase-dependent superoxide anion production in the vascular wall. The revealed impairment of NO- and K<sup>+</sup>-channel-dependent dilator responses may be responsible for the increased risk of cerebrovascular events and neurodegenerative disorders in IR.

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## ZSOMBOR LACZA (2003)

### Interactions between prostanoids and other vasoactive systems in the cerebral circulation

Supervisor: Dr. Péter Sándor

The following studies investigate the interaction between the nitric oxide (NO) and the prostanoid systems in the cerebral circulation. It has been shown previously that the vasoconstrictor effect of NO synthase (NOS) blockade is mediated by the activation of thromboxane receptors. The present doctoral work further dealt with this question. First, we investigated the interaction between NO, uridine triphosphate (UTP) and thromboxane receptors in the vasomotion of isolated rat middle cerebral arteries (MCAs). Second, we tested whether thromboxane receptors contribute to the UTP induced vasoconstriction in the MCA. Third, we tested the suitability of the laser Doppler (LD) method to investigate the effects of NOS blockade in vivo. And finally, we investigated the interaction between NO and prostanoids in the cerebrocortical microcirculation.

Our in vitro studies in the isolated MCA showed that thromboxane receptors participate in the vasomotion-inducing effect of NOS blockade and UTP. Moreover, thromboxane receptors mediate the UTP induced vasoconstriction in this preparation. We have also showed that isolated MCAs produce more thromboxane A<sub>2</sub> (TXA<sub>2</sub>) and prostacyclin (PGI<sub>2</sub>) in the presence of UTP. The in vivo LD measurements revealed that the effect of NOS blockade has significant heterogeneity, which is related to the baseline LD flow. In contrast to the in vitro results, we showed that the in vivo vasoconstrictor effect of NOS blockade is partially compensated by the increased production of PGI<sub>2</sub>. Comparing the morphology of NOS blockade induced vasomotion in the MCA and in the microcirculation indicated that a similar mechanism may be responsible for this phenomenon in both preparations.

In conclusion, we revealed complex interactions between the two major vasoactive systems in the cerebral circulation. Disturbance of the NO and the purinergic systems is a common feature in cerebrovascular diseases like stroke or vasospasm. These newly described compensatory mechanisms by prostanoids may lead to novel therapeutic approaches in the treatment of cerebrovascular disorders.

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ZSUZSANNA LÉNÁRD (2005)

## The arterial baroreflex function from childhood to young adult age

*Supervisor: Dr. Márk Kollai*

The arterial baroreflex buffers abrupt transients of blood pressure. Distensibility of those arterial sites (carotid sinus and aortic arch), where baroreceptors are located significantly influences baroreflex sensitivity. Both baroreflex sensitivity and carotid distensibility shows great variability among healthy, young individuals. Since lower baroreflex sensitivity is a risk factor of several cardiovascular disease, the aim of this study was to determine the effect of age, family history of hypertension and regular physical activity, as possible causes of this variability, on baroreflex sensitivity in children and young adults aged 7-22.

We studied 235 sedentary and trained healthy subjects with a family history of normotension and hypertension between 7 and 22 years. Continuous radial pressure and carotid pulse pressure were measured by applanation tonometry. Baroreflex function was assessed by spontaneous sequence and frequency domain indices. Carotid diameter and pulsatile distension was measured by echo wall-tracking.

We found that 1. spontaneous baroreflex indices increased from early childhood to adolescence, in spite of gradual stiffening of the carotid artery; this indicates the maturation of neural mechanisms, attaining peak level at adolescence. 2. In sedentary subjects with a family history of hypertension baroreflex sensitivity was significantly lower than that in sedentary subjects with a family history of normotension. 3. in subjects with a family history of hypertension regular aerobic exercise training was associated with better baroreflex function as compared to the sedentary lifestyle.

The present results support the idea that regular, aerobic physical activity can attenuate the impairment of cardiovagal autonomic function and stiffening of the carotid artery in young subjects with family history of hypertension. As such, this may be an effective lifestyle intervention for minimising negative effects of a family history of hypertension on autonomic circulatory control.

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ZSOLT LOHINAI (2003)

## The biological significance of nitric oxide in the dental pulp and periodontium

*Supervisor: Dr. Péter Sándor*

Caries and periodontitis are widespread diseases in Hungary. Recent epidemiological and clinical studies have shown that the dental infections are not only local problems, but are risk factors of systemic diseases most particularly cardiovascular diseases. In addition, the increase of oral tumors has been more than five-fold since the sixties in our country. Consequently, the research of the biology of the oral cavity is very important, however, still has not received the required attention and support so far. In the past two decades there was an intensive interest in the research of various vasoactive agents of the endothelial cells aimed at the characterization of the endothelium derived relaxing factor (EDRF). One possible form of EDRF is nitric oxide (NO) free radical, which is produced from L-arginine by the isoenzymes of nitric oxide synthases (NOS). NO. is one of the smallest synthetic,

highly reactive, short lived intercellular messenger molecules of mammalian cells with important cardiovascular, neurological and immune functions.

Although the information on the effects of NO on the dental pulp and periodontal tissues are relatively scarce in the literature, it seems, that this free radical has substantial and versatile role in these tissues as well. In our anatomical studies in cat and dog we have demonstrated that the endothelial cells of both tissues are stained with NADPH-diaphorase, which is the histochemical marker of NOS. Furthermore, we observed a few NADPH-diaphorase positive and/or neuronal NOS immunoreactive perivascular and solitary axons in the dental pulp and abundant number of similar nerve fibres in the gingiva. These fibres travel within the inferior alveolar nerve to the pulp and the gingiva of the lower teeth and may participate in their sensory (i.e. pain) as well as in autonomic (i.e. regulation of blood flow) innervation.

In our functional studies we have found that the L-arginine/NO pathway plays an important role in the regulation of pulpal and gingivomucosal blood circulation measured by radioactive labeled microspheres in cat. Based on our data we suggest, that a NO dependent basal vasodilator tone exists in the dental pulp and the gingivomucosal tissue. In the pulp the resting tone of the vessels is probably influenced by NO derived from endothelial origin and not from the small number of NOS reactive perivascular axons, however, in the gingivomucosal tissue the basal NO may be derived from both sources. Furthermore, based on another study we presume, that the release of endogenous NO from various sources in response to stimulation may also be involved in the control of the dental pulp vascular tone since the dental pulp circulation is sensitive to exogenously administered NO

In a rat model of periodontitis, the ligature increases the local NO production through the expression of inducible NOS in the inflammatory cells of the connective tissue and in the epithelium of the gingiva. On the other hand, mercaptoethylguanidine, a selective inhibitor of inducible NOS and a peroxynitrite scavenger treatment in the same model protects against the periodontitis associated extravasation and bone destruction. Based on these data, we suggest that enhanced formation of NO and peroxynitrite play a significant role in the pathogenesis of periodontitis. We propose, that although the locally generated NO and peroxynitrite protect the gingiva against the invading microorganisms, the overproduction of these agents in periodontitis may lead to the host tissue damage as well. Therefore, selective inhibition of inducible NOS may be of therapeutic utility in periodontitis.

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## PÉTER STUDINGER (2005)

### Investigation of cardiovascular autonomic tone and reflex activity - the role of vascular properties

Supervisor: Dr. Márk Kollai

Arterial baroreflex sensitivity (BRS) is partly determined by the elastic properties of barosensory vessel wall. Thus, examination of central arterial elastic parameters plays an important role in studying autonomic cardiovascular function. Our aims were to determine 1.) how carotid artery wall tension and carotid pressure- diameter ratio (PDR) change during and after strenuous exercise; 2.) whether treatment with the antioxidant vitamin E influences carotid artery elasticity and BRS. We also tested whether automatic wall track (WTS) system that was used previously to assess the distensibility of superficial arteries is an eligible device to measure aortic arch distensibility.

We investigated healthy volunteers in our studies. Carotid and aortic diameter and their changes with pressure pulse were measured by the WTS system. Pulse wave analysis was used to calculate central arterial pressure from the radial pressure waveform recorded by applanation tonometry. BRS was determined based upon spontaneous changes in blood pressure and RR-intervals.

During aerobic exercise mean carotid artery diameter increased with mean arterial pressure, and

PDR decreased. Immediately after exercise carotid artery constricted and PDR remained reduced as compared to control condition. One hour post-exercise carotid artery dilated and PDR increased above control level. Post-exercise increase in BRS was closely correlated with that in PDR. Vitamin E increased both PDR and BRS, and increases in PDR and BRS were correlated with those in plasma vitamin E levels. It was possible to record aortic arch distension waveform with WTS, and the reproducibility of the measurements was within acceptable limits. Aerobic exercise and antioxidant treatment both evoke changes in central arterial elasticity, which may induce BRS alterations. It is feasible to determine both carotid and aortic distensibility using WTS, by which method barosensory vessel wall properties can be studied in their complexity.

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### 1/3. PROGRAM

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## BIOLOGICAL EFFECTS OF IONIZING AND NON-IONIZING RADIATION

### *Coordinator*

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Biological effects, induced by different physical and chemical environmental factors (e.g. ionizing and non-ionizing radiations, pollutants) endanger the whole biosphere including humans. The conscious environmental protection and the prevention of several human diseases due to these environmental factors can be effective only knowing and quantifying the sources (natural radiation background, nuclear disaster, solar radiation, ozone depletion, chemical pollution, etc.) In this Program field and laboratory measurements of monitoring of ionizing and ultraviolet radiations are offered with particular interest to the quantification of their biological effects on global, cellular and molecular level.

### *Sub-programs*

Studies of radiation resistance influencing effects of peptides and peptide like compounds on human red blood cell membrane

Mechanism of effect of membrane active drugs acting on red blood cell membrane

Role of photoactivation in genotoxicity of drugs and chemicals

Methods in molecular biology to detect DNA damages

Spectroscopic methods in the study of biological effect of endogenous and exogenous chromophores

Determination of biological dose of natural/artificial UV light

Cytogenetic studies on the effect of environmental factors

Cellular biological studies of the effect of environmental factors

Detection of the effect of terrestrial and extraterrestrial solar radiation, estimation of biological risk

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**TIBOR JÁNOS KOVÁCS (2005)****Activity concentration of  $^{222}\text{Rn}$ ,  $^{226}\text{Ra}$  and  $^{210}\text{Po}$  in Hungarian drinking waters and their contribution to the ingested dose***Supervisor: Dr. Györgyi Rontó*

The quality of drinking water consumed by mankind is more and more getting into the focus of attention. Radionuclides occurring in nature may solve into drinking waters and with this contaminating the water, and causing radiant dose when consumed. Therefore during the examination of the quality of drinking waters the radiological qualification is very important. During my work I worked out a procedure available for carrying out the recommended qualification process. I made the applied nuclear measurement technology procedures finer and more exact on several points. I determined the  $^{222}\text{Rn}$ , total alpha-beta activity,  $^{226}\text{Ra}$  and  $^{210}\text{Po}$  concentration of approximately 100 water samples. Southern Great Plain well-waters. Bottled mineral waters. Tap waters in the vicinity of Veszprém. Lake Balaton highland spring-waters

During the measurements I found extremely high radionuclide ( $^{226}\text{Ra}$ ) concentration only in the case of bottled mineral waters. In one case it reached 3000 mBq/L.  $^{226}\text{Ra}$  concentration, the producer company was notified about this fact, they introduced a decreasing process. Taking the dose conversion factors recommended by WHO and IAEA into consideration I estimated the expected dose from radionuclides during average water consumption. Measurements so far have proved that the radiological qualification of waters used for satisfying water demand originating from different sources is essential. Most of the countries introduced the recommendations of the EU and WHO as these do not apply to spring waters and bottled mineral waters, however, the consumption of these increases by leaps and bounds with the development of a healthier outlook on life, and the actual decrease of effective dose of the population can only be provided for by the expansion of the regulations.

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- Kovács T, Bodrogi E, Dombovari P, Somlai J, Nemeth Cs, Capote A, Tarján S (2004)  $^{238}\text{U}$ ,  $^{226}\text{Ra}$ ,  $^{210}\text{Po}$  concentrations of bottled mineral waters in Hungary and their committed effective dose. *Radiat Prot Dosim* 108:175-181.
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**ZSÓFIA SZABÓ (2004)**

## **Investigation of the mechanism of cell membrane-active compounds**

*Supervisor: Dr. Katalin Blaskó*

The cyclic lipodepsipeptides produced by *Pseudomonas syringae* pv. *syringae* possess fungicide properties. They inhibit much of the cell functions, presumably on the basis of their pore-forming activity. The syringomycin E, studied earlier by our research group, formed pores on human red blood cells (RBC), and caused also partially hemolysis. To reach selective toxicity, knowledge of the relationship between the structure and function of the CLPs is required. To fulfill this requirement, the poreforming properties of two other CLPs, the syringopeptin22A (SP22A) and the syringotoxin (ST), were investigated on RBCs. On the basis of our transport kinetic measurements on RBC, the SP22A and the ST formed pores built up by few monomers. ST pores inactivated at 37 °C, and at 20 °C, however, they remained stable at 8 °C, similarly to the SRE. Inactivation of the SRE and ST pores was faster at 37 °C than at 20 °C. SP22A pores did not inactivate even at 37 °C. On the basis of our results, we concluded, that fluidity has a crucial role in the process and the dimension of the hydrofobic part and the number of the positive charges of the polar headgroup influence the stability of the pores. To study the interactions of CLPs with membranes on molecular level we applied EPR spectroscopy and spectral simulation methods for liposomes of different composition. CLPs decreased the fluidity at all depths of the membrane: the motional and rotational freedom of the lipid molecules decreased, while their ordering increased. These observations in conjunction with the concentration dependence of the CLP's action suggested that CLPs form pores involving the lipids, too. Investigation of the temperature dependence of the CLPs' action showed that to get their complete effect a given temperature range is required. It corroborated that the fluidity has a crucial role in the interactions between CLPs and membrane lipids. From the results, obtained with DPPC-DOPC or DPPC-cholesterol liposomes we concluded that the membrane-inhomogeneity decreases the effectiveness of the CLPs. Dynamic light scattering measurements were used to determine the size-distribution of the liposomes. According to our experiences CLPs provoke fusion and/or aggregation, which can have a role in the cell-killing properties of the CLPs.

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**1/4. PROGRAM****NEPHROLOGY AND HYPERTENSION FROM MOLECULAR LEVELS TO BEDSIDE****Coordinator****László ROSIVALL**

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This program received accreditation in 1993. Basic and clinical nephrology research in Hungary has been traditionally strong and has gained international recognition, upon which this program has been established. The faculty represents various fields of physiology, pathophysiology, internal medicine, pediatrics, transplantation, clinical nephrology; they are immunologist and hypertension experts known for their multidisciplinary approach in research and education. Special emphasis is placed on the regulating mechanisms of fluid and electrolyte balance, blood pressure and kidney function. Modern experimental techniques are used at various levels from molecules to bedside, representing translational research. Our research team has gained international recognition and is a leading expert on the regulation of renal hemodynamics and microcirculation, the mechanisms and progression of various kidney diseases including chronic renal failure, diabetic nephropathy, renal fibrosis, and kidney allograft rejection. We are studying intracellular signal mechanisms, cell-cell communication, TGF-beta, VEGF, relaxin and the renin-angiotensin system, and their interactive roles in the control of renal hemodynamics. By studying the morphology and function of the afferent arteriole and juxtaglomerular apparatus, we have described a novel regulatory mechanism of glomerular filtration.

*Sub-programs**Supervisors***Molecular nephrology**

Intracellular signalling mechanisms of transforming growth factor-beta and angiotensin II

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István MUCSI

Physiology and pathophysiology of renal function in transforming growth factor-beta transgenic mice

Miklós MÓZES

Relaxin transgenic animal model and its renal function

Miklós MÓZES,

László ROSIVALL

**Regulation of fluid and electrolyte balance and glomerular hemodynamics**

The renin-angiotensin system

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János PETI PETERDI

Tubuloglomerular feedback signalling

László ROSIVALL,

János PETI PETERDI

Early changes in tissue gene expression in the course of cardiac hypertrophy: natriuretic peptides and growth factors

Miklós TÓTH

Effects of vascular endothelial growth factor on the development of endothelial fenestration

László ROSIVALL

**Hypertension**

Endogenous natriuretic substances in cardiac hypertrophy clinical and experimental investigations

Rudolf de CHÂTEL,

Miklós TÓTH

Effect of hypertension on microcirculation

Ákos KOLLER

Hypertension in pregnancy and molecular mechanisms of the toxicosis	Miklós MOLNÁR
<b>Renal transplantation</b>	
Molecular mechanism in rejection of transplanted kidney	Péter HAMAR
The role of the complement system in renal and circulation diseases	János SZEBENI
<b>Progression of renal diseases</b>	
Cell-cell and cell-matrix interactions in the progression of chronic renal fibrosis	István MUCSI
Molecular mechanisms in progression of renal diseases	László ROSIVALL
aDevelopment and evaluation of transgenic animal model	Péter HAMAR
Electrolyte transport	
Role of angiotensin in electrolyte transport of tubular epithelial cells	László ROSIVALL, Ákos ZSEMBERY
Disturbances of electrolyte transport in cystic fibrosis	Ákos ZSEMBERY
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Diagnosis and treatment of renal osteodystrophy	István MUCSI
Investigation of the disorder of calcium metabolism in experimental renal failure	András SZABÓ
Dialysis, biocompatibility and quality of life	László ROSIVALL

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PÉTER BARTA (2005)

## Novel experimental data in blood pressure regulation

Supervisor: Dr. György Losonczy

The thesis introduces novel experimental data about the pathogenesis of hypertension, focusing on salt-related renal effects and the pathogenesis of preeclampsia syndrome. Hypertension as a determinant of human morbidity and mortality has been described more than 100 years ago. Sodium excess, renal adaptation and renin-angiotensin-aldosterone system are intimately involved in the pathogenesis. Preeclampsia, the human pregnancy specific form of hypertension is a syndrome in that virtually every organ system can be affected. Organ dysfunction in salt sensitivity hypertension and preeclampsia are not confined merely to blood pressure. Association of salt-load with insulin resistance, accelerated nephrosclerosis, increased cardiovascular damage or asthma suggests that physiologic and pathophysiologic responses elicited by sodium-chloride are rather systemic. Recent evidence indicate that both salt-related and pregnancy-induced hypertension syndrome are characterized by disturbances of the renin-angiotensin system, elevated oxidative stress, insulin resistance, endothelial dysfunction and vascular inflammation. Underlying molecular mechanisms might be complex and remain to be elucidated.

To identify unexpected gene targets in rat kidneys in response to altered salt intake we used oligonucleotide microarray technology. We hypothesized that oligonucleotide microarray (Affymetrix GeneChip) has utility in identifying novel, unexpected gene targets in rat kidneys in response to altered salt intake. We showed the utility and feasibility of the technique on the whole organ level. We detected a specific set of genes differentially regulated by high-salt diet in renal tissue, comprising less than 1% of the screened 8800 transcripts. We found 35 genes that were upregulated by a high-salt intake, while 30 were downregulated. Of particular interest, salt-induced regulation of PI3 kinase regulatory subunits, FABP, PTP2 and annexin VI might play novel, yet undescribed role in renal ion channel regulation. Other genes regulated by salt-diet play essential role in insulin signaling, lipid metabolism. Sodium chloride induced change in the expression level of HMG-CoA reductase and lanosterol demethylase) raises the possibility of a close genomic link between salt-induced adaptation and metabolic changes. The calcium channel beta subunit III and AT<sub>1</sub> receptor were also regulated by differential salt intake. Based on the current available evidence, these genes may be important determinant of salt sensitive blood pressure regulation. Were we to pursue any of these putative candidates in further studies, extensive validation on protein level would be necessary. We propose further experiments, using the classic candidate-gene approach, to elucidate the role of our putative candidate genes in renal physiology and sodium related multi-organ physiologic adaptation.

We made previously observations that serum from preeclamptic women contains an IgG autoantibody (AT<sub>1</sub>-AA) that reacts with the AT<sub>1</sub> receptor in a stimulatory fashion. We postulated that AT<sub>1</sub>-AA-induced AT<sub>1</sub> receptor stimulation, NADPH oxidase activation, ROS production, NF-κB transactivation and increased oxidative stress are involved in the pathogenesis of preeclampsia. We tested whether or not AT<sub>1</sub>-AA activate NADPH oxidase, ROS generation and NF-κB in primary human VSMC and trophoblasts. Finally, we hypothesized that preeclamptic placentas also exhibit upregulation of NADPH oxidase components, ROS production and activation of NF-κB compared to normal placentas. Five new aspects about preeclampsia emanated from our study. First, AT<sub>1</sub>-AA from preeclamptic women induces intracellular ROS in VSMC and trophoblasts, an induction that is mediated by NADPH oxidase. Second, AT<sub>1</sub>-AA activates NF-κB, the major inflammatory transcription factor, as a downstream target of NADPH oxidase-derived ROS. Third, ROS production is increased in preeclamptic placentas. NADPH oxidases are present in the placenta and are massively stimulated in preeclampsia. Finally, NF-κB is markedly upregulated in preeclamptic placentas. These cumulative data strongly suggest, that AT<sub>1</sub>-AA contribute to the pathogenesis of preeclampsia and could play central role in the development of maternal vascular inflammatory response and endothelial dysfunction. However, whether or not AT<sub>1</sub>-AA is the cause or the result of vascular damage in preeclampsia will require additional investigations.

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## KATALIN FÖLDES (2003)

### Metabolic changes in kidney transplanted patients

*Supervisor: Dr. Rudolf de Châtel*

In the background of increased cardiovascular morbidity and mortality following successful renal transplantation metabolic alterations have been revealed which lead to diabetes mellitus, dyslipidemia and hypertension. In addition after transplantation a change in bone metabolism was also detected. Applying serum C-peptide and islet-cell antibody (ICA) methods it was demonstrated that posttransplant diabetes mellitus (PTDM) is characterised by preserved beta-cell function and insulin resistance, similar to NIDDM. It was demonstrated that posttransplant disturbances in glucose metabolism are more frequent than have been previously suggested and to detect it an application of oral glucose tolerance test is further on indispensable. PTDM is frequently associated with dyslipidemia and hypertension. We were among the first authors who gave evidence that an improvement in lipid concentrations can be achieved by the administration of fluvastatin in dyslipidemic posttransplant diabetic patients. We demonstrated that long-term combined CsA and fluvastatin (20 mg/die) therapy can be safely given to posttransplant patients, it does not lead to myotoxic symptoms, on the other hand, the administration of fluvastatin during CsA therapy does not change its serum level. Applying 24 hour ambulatory blood pressure monitoring (ABPM) we demonstrated a change of the circadian rhythm of their blood pressure. Our data showed that reduced (or even inverse) diurnal indices occur with a high frequency in PTDM patients, the prevalence of absolute nocturnal hypertension was higher in these subjects than in patients with essential hypertension, and sustained-release calcium channel blocker, isradipine, significantly improved the daily blood pressure profile. It is well known that in the first year following renal transplantation a rapid bone loss can be detected. Our aim was to elucidate its background. Applying markers reflecting bone metabolism our data refer to an imbalance in bone remodelling favoring net bone loss which is presumably mainly due to the administration of immunosuppressive drugs given in high doses. In the development of rapid bone loss, however, the slowly resolving preexisting secondary hyperparathyroidism and the decreased production of dehydroepiandrosterone sulphate (DHEAS) may also play a role.

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**MÓNIKA GÖÖZ (2004)**

## **Regulation of endogenous ouabain-like factor production in the adrenal gland and in volume expanded physiological and pathophysiological states**

*Supervisor: Dr. Miklós Tóth*

The aim of the present work was to study regulation of endogenous ouabain-like factor production. We raised a specific antibody against ouabain-*bsa* conjugate in rabbit. Using this antibody we showed presence of olf immunopositive cells in rat adrenals, and developed a highly sensitive ouabain radioimmunoassay. Our *in vitro* experiments using dispersed rat adrenocortical cells provided evidence that not only the glomerulosa but also the fasciculata/reticularis cells produce olf. Further, we showed that besides *acth* the extracellular [k+] modulates olf secretion both in the human and rat adrenals. As “the major regulator” of endogenous ouabain we showed the striking stimulatory effect of nicotine on rat adrenocortical cells. Studying the role of endogenous olf in the adrenals we found that ouabain interacts with *anp* and *angiotensin-ii* at different extracellular [k+] on the aldosterone secretion, which could help to explain the interactions of these hormones in both physiological and pathophysiological states. In our *in vivo* experiments we found that in volume overloaded rats during the development of cardiac hypertrophy endogenous olf and *adrenomedullin* are substituted after adrenalectomy from other organs to provide positive inotropic substances to the failing heart. Analyzing olf in volume expanded states we found elevated plasma olf levels in patients with moderate form of congestive heart failure, which may contribute to the compensated state of this disease. We also showed that during pregnancy diabetes further augments plasma and urinary olf concentrations. As the plasma olf level was more elevated in gestational diabetes than in pregnant women with *idm*, our data may explain why gestational diabetes predisposes more often to pre-eclampsia. As another novel result we found correlation between gestational age and plasma immunoreactive olf levels in newborns and showed that mature infants have lower olf level at birth than premature newborns, which can provide important new information for newborn physiology. In conclusion, we provided new evidence on localization, intra-adrenal regulation of endogenous ouabain-like factor, and its interaction with endogenous vasoactive substances. Also, we identified nicotinic regulation as the major modulator of olf secretion, and provided data for its role in diabetes, during development of cardiac hypertrophy, and in newborns.

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**GERGELY KOVÁCS (2003)**

## **Regulation of the apical NA-Cl-K cotransporter in macula densa**

*Supervisor: Dr. László Rosivall*

Macula densa (MD) cells, the sensor element of tubuloglomerular feedback mechanism (TGF), detect changes in distal tubular sodium chloride concentration ( $[NaCl]_l$ ) via the apical Na-2Cl-K cotransporter (NKCC). This apical cotransporter might be a site for regulation of TGF. Nitric oxide (NO) and *angiotensin II* (Ang II) are well known positive and negative modulators, respectively. Expression of the neuronal nitric oxide synthase (nNOS) is abundant in MD cells. MD cells also possess

Ang II receptors. Therefore, in our studies, we investigated the regulation of NKCC activity by nNOS, Ang II and the possible interaction of NO with Ang. II. MD cell  $\text{Na}^+$  concentration ( $[\text{Na}^+]_i$ ) and NO production was measured using SBF1 and DAF-FM DA, respectively, with fluorescent microscopy. NKCC activity was assessed by examining the furosemide-sensitive part of the initial rate of increase in  $\text{Na}^+$ , when luminal  $[\text{NaCl}]$  was increased from 25 to 150 mM. We found that nanomolar Ang II stimulated NKCC activity via apical  $\text{AT}_1$  receptors. This stimulatory phenomenon was also observed when Ang II was added to the basolateral side. However, at micromolar concentrations of  $\text{AT}_2$  receptors were also activated which in turn blocked this stimulatory effect. Specific inhibition of nNOS increased NKCC activity tremendously. Combination of various concentrations of Ang II and nNOS inhibition had no additional effect. Addition of L-arginine (a substrate of nNOS) did not affect NKCC activity and did not increase NO production significantly suggesting a high basal NO production in MD cells. Finally, Ang II failed to stimulate NO production by MD cells. Our results indicate that 1) Ang II may modulate TGF, at least in part, though directly regulating NKCC in MD cells, 2) high resting NO production by the MD tonically inhibits Na-2Cl-K cotransport and TGF, 3) no positive interaction was shown between Ang II and nNOS.

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## ATTILA BALÁZS PATÓCS (2005)

### Gene mutations and polymorphisms in adrenal tumors

Supervisor: Dr. Károly Rácz

The aim of this study was to examine genetic abnormalities thought to be potentially important in the pathomechanism of hormonally inactive adrenocortical adenomas and pheochromocytomas, and to compare the results with clinical and biochemical parameters present in patients with these adrenal tumors.

Using an allele-specific PCR method we showed that 16.1 % of unilateral and 21.1 % of bilateral hormonally inactive adrenocortical adenomas had mutations of the *CYP21B* gene. Based on these data we presumed that the presence of *CYP21B* mutations in these patients may result in a partially decreased activity of the 21-hydroxylase enzyme and that a subsequent overproduction of ACTH may, therefore, play a causal role in the development of these tumors. However, the lack of close relationship between the presence of *CYP21B* mutations and ACTH-stimulated plasma 17-OHP concentrations in several patients indicated that the role of *CYP21B* mutations on tumor formation may involve mechanisms independent of ACTH-induced changes in 17-OHP secretion. Sequencing of all exons of *CYP17* in germline DNA of a patient with bilateral adrenal adenomas revealed a previously undescribed disease-causing mutation of the *CYP17* gene (Arg440Cys) and 5 different *CYP17* polymorphisms, of which two polymorphisms (Asp192Asp és Ser283Ser) were described by our *in silico* approach. This confirms the usefulness of this approach to search for novel *CYP17* variants.

In the course of our studies on mutation screening of the *RET* gene, patients with pheochromocytomas having mutations at codon 609 have been identified and described as a new MEN 2A phenotype associated with this mutation. Our studies on a large kindred with FMTC consisting 80 members indicated that co-segregation of a known disease-causing *RET* mutation (Val804Leu) and a *RET* polymorphism presumably involved in the pathomechanism of sporadic medullary thyroid cancer (Ser836Ser) failed to have an impact on the clinical course of FMTC. Our extended mutational screening studies for MEN 2 syndrome and vonHippel-Lindau syndrome, together with clinical screening for type 1 neurofibromatosis indicated that the prevalence of hereditary cases in non-selected patients with pheochromocytomas is as high as 28 %. We showed that clinical and laboratory parameters do not offer a great help to differentiate between patients with sporadic and hereditary pheochromocytomas.

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## 1/5. PROGRAM

### CLINICAL AND EXPERIMENTAL CARDIOLOGY/ATHEROSCLEROSIS

#### Coordinator

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The aim of the Program is to carry out experimental and clinical studies on the pathological mechanisms responsible for the cardiac and vascular disorders leading the mortality statistics. Metabolic disturbance, as diabetes, is one of the most important risk factor in this process – therefore it is used as a model. The different approaches are given by the sub-programs.

#### Sub-programs

Investigation of interactions between endogen cardiovascular agents in hypoxia and abnormal metabolic states  
intblInvestigation of electrophysiological effects of endogen cardiovascular agents and therapeutics in isolated heart preparations  
Functional morphology and toxicology of environmental and civilization risk factors of cardiovascular system  
Lipidology (The pathobiochemistry, pathophysiology and clinical investigation of lipid- and lipoprotein-metabolic disturbances)  
The effect of food components on atherogenesis and the formation of the so-called atherogen lipoproteins  
Local regulation of blood flow in metabolic diseases and hypertension  
Investigation of electrical properties of smooth muscle and the possibility of electrical treatment (non-pharmacological) of certain diseases  
Thrombotic aspects of coronary disease – prothrombotic states and their modulation in the clinical practice. Role of receptors of arterial endothelium in atherothrombosis

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**CSONGOR CSEKŐ (2005)****Role of hydrogen peroxide in the local regulation of microvascular tone**

*Supervisor: Dr. Ákos Koller*

It has been demonstrated that in various diseases affecting the cardiovascular system, such as hypertonia, diabetes mellitus, hyperhomocysteinemia there is an increased formation of superoxide anion and hydrogen peroxide ( $H_2O_2$ ) by vascular tissues and activated leukocytes. Several vascular effects of  $H_2O_2$  have been explored, but its direct effect on the myogenic tone of arterioles, determining tissue perfusion, is not yet known. Previous studies suggest that in certain conditions  $H_2O_2$  may act as endothelium-derived mediator.

Thus, we hypothesized that  $H_2O_2$  has an important role in the local regulation of skeletal muscle blood flow. To test this hypothesis *first*, effects of extraluminally administered  $H_2O_2$  on the diameter of isolated, pressurized (at 80 mmHg) rat gracilis skeletal muscle arterioles (diameter  $\sim 140 \mu m$ ) were investigated. Lower concentrations of  $H_2O_2$  ( $10^{-6}$ - $3 \times 10^{-5}$  M) elicited constrictions, whereas higher concentrations of  $H_2O_2$  ( $6 \times 10^{-5}$ - $2 \times 10^{-4}$  M), after initial constrictions, caused dilations of arterioles ( $10^{-4}$  M  $H_2O_2$ :  $-19 \pm 1\%$  and  $66 \pm 4\%$ ). Endothelium removal reduced both constrictions (max:  $-10 \pm 1\%$ ) and dilations ( $33 \pm 3\%$ ) to  $H_2O_2$ . Constrictions to  $H_2O_2$  were completely abolished by indomethacin and the prostaglandin  $H_2$ /thromboxane  $A_2$  receptor antagonist SQ29548. Dilations to  $H_2O_2$  were significantly reduced by inhibition of nitric oxide synthase (to  $38 \pm 7\%$ ) but were unaffected by clotrimazole or sulfaphenazole (inhibitors of cytochrom P450 enzymes), indomethacin or SQ29548. In endothelium-denuded arterioles clotrimazole had no effect, whereas  $H_2O_2$ -induced dilations were significantly reduced by charybdotoxin plus apamin, inhibitors of  $K_{Ca}$ -channels (to  $24 \pm 3\%$ ) and by glybenclamide selective blocker of  $K_{ATP}$  channels (to  $14 \pm 2\%$ ), and the non-selective  $K^+$ -channel inhibitor tetrabutyl-ammonium (to  $-1 \pm 1\%$ ). Intraluminally administered  $H_2O_2$  caused biphasic changes in diameter: lower conc. ( $10^{-6}$ - $3 \times 10^{-5}$  M) elicited only constrictions (max:  $22 \pm 3\%$ ), whereas higher conc. ( $10^{-4}$  M) caused, after initial constrictions, dilations (max.:  $43 \pm 5\%$ ). *Second*, several doses and types of catalases (500 U/ml, powder; 1500 U/ml solution; 250 U/ml PEG-catalase) were administered extraluminally. All forms and doses of catalases inhibited both constrictions and dilations to  $H_2O_2$ . *Third*, in control, increases in intraluminal pressure 1) in a stepwise (20 mmHg/step) manner from 20-140 mmHg, or 2) in one step (80 mmHg/step) manner from 10-90 mmHg elicited constrictions. The pressure-diameter curve was shifted by various forms of catalases, but there were

no differences in the slope of pressure-diameter changes and the myogenic index curves of control and catalase-incubated arterioles, regardless of the type of pressure steps. *Finally*, in control, 1) step-wise (10 mmHg/step) from 0-40 mmHg, or 2) one step (40 mmHg/step) from 0-40 mmHg increases in pressure difference of in- and outflow canules elicited flow-induced dilations (max:  $39 \pm 3 \mu\text{m}$  at 40  $\mu\text{L}/\text{min}$ ). In the presence of various cataleses there were no differences in the slope of curves and the wall shear stress-induced diameter changes, regardless of the type of flow induction. In conclusion, our data show that extraluminal and intraluminal administration of  $\text{H}_2\text{O}_2$ , elicits the release of 1) prostaglandin  $\text{H}_2/\text{thromboxane A}_2$  both from endothelium and smooth muscle, 2) nitric oxide from the endothelium and 3) activates potassium channels, such as  $\text{K}_{\text{Ca}}-\text{K}_{\text{ATP}}$  channels in the smooth muscle resulting in biphasic changes of arteriolar diameter. In the range studied, however  $\text{H}_2\text{O}_2$  does not seem to be involved in the mediation of pressure and flow dependent responses. Because  $\text{H}_2\text{O}_2$  already at low micromolar concentrations activates several intrinsic mechanisms we suggest that  $\text{H}_2\text{O}_2$  released from various tissues or cells by eliciting changes in arteriolar diameter contributes to the local regulation of skeletal muscle blood flow in various physiological and pathophysiological conditions.

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## ZSOLT BAGI (2004)

### Effects of metabolic diseases on microvascular function: the role of oxidative stress and nitric oxide interaction

Supervisor: Dr. Ákos Koller

Clinical and epidemiological studies have shown that pathologic alterations in carbohydrate and amino acid metabolism play an important role in the development of vascular diseases. In diabetes mellitus (DM) and hyperhomocysteinemia (HHcy) alterations of microvascular function can contribute to the development of diabetic microangiopathy or HHcy-induced peripheral occlusive vascular diseases. However the nature of underlying pathological mechanisms is not completely understood. Thus, in rat gracilis skeletal muscle arterioles isolated from streptozotocin-induced type 1 DM and methionine-reach diet-induced HHcy rats endothelium-dependent vasodilation in response to increases in intraluminal flow were studied, a mechanism, which play an important role in the local regulation of tissue blood flow.

We have found that in arterioles of DM rats the flow-induced dilation was significantly decreased compared to that of control vessels, whereas in arterioles of HHcy rats increases in flow, instead of dilations observed in control vessels, resulted in constrictions. The reduced flow-induced arteriolar dilation of DM rats was due to the reduced bioavailability of tetrahydrobiopterin, which is responsible for the decreased synthesis of nitric oxide (NO) by NO synthase. In HHcy the NO synthesis seems to be unaltered, but the enhanced level of superoxide produced by vascular xanthinoxidase eliminates NO by forming peroxynitrite, which then promotes the formation of thromboxaneA2 eliciting flow-induced arteriolar constriction.

On the basis of the present study we hypothesize that decreased flow-induced dilation or constriction in resistance size vessels could lead to the impairment of local regulation of tissue blood flow, which may contribute to the development peripheral microvascular diseases in DM and HHcy. Mechanisms revealed by our studies may help to develop specific therapies that by reducing oxidative stress and/or restoring cellular level of tetrahydrobiopterin could prevent and/or treat DM and HHcy-induced microvascular dysfunctions, thereby reducing the vascular morbidity and mortality in these metabolic diseases.

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ANNA CSISZÁR (2003)

## Hyperhomocysteinemia-induced pro-inflammatory and pro-atherosclerotic changes in coronary arteries

Supervisor: Dr. Ákos Koller

Hyperhomocysteinemia (HHcy) is a newly recognized risk factor for myocardial infarction, however, the effect of HHcy on the function and phenotype of coronary arteries and the mechanisms responsible for HHcy-induced vascular alterations is not known. On the basis of previous studies we hypothesized that in coronary arteries HHcy-induced impairment of vascular functions is due to an increased oxidative stress and a pro-inflammatory, pro-atherosclerotic shift in gene expression profile. Impaired flow-induced NO-mediated dilation: 1) Increases in intraluminal flow elicited dilations of C vessels, responses that were absent in HHcy arteries. The nitric oxide synthase inhibitor L-NAME inhibited flow-induced dilation of C coronaries, whereas it had no effect on responses of HHcy arteries. Dilations of C and HHcy arteries to the NO donor SNP were not different. Responses to flow in HHcy coronary arteries were unaffected by administration of L-arginine or the prostaglandin H<sub>2</sub>/thromboxane A<sub>2</sub> receptor antagonist SQ 29,548. 2) However, in the presence of superoxide dismutase or the superoxide scavenger Tiron increases in flow elicited L-NAME-sensitive dilations of HHcy coronaries. Increased oxidative stress (O<sub>2</sub><sup>-</sup> production, peroxynitrite generation): 3) In HHcy coronary arteries O<sub>2</sub><sup>-</sup> generation was significantly increased, which could be inhibited by SOD or the NAD(P)H oxidase inhibitors diphenyleneiodonium (DPI) and apocynin. NADPH-driven O<sub>2</sub><sup>-</sup> generation was also significantly increased in HHcy vessel homogenates. Hydroethidine staining showed that increased O<sub>2</sub><sup>-</sup> levels are present in the media of HHcy vessels. 4) Western blotting showed an increased tyrosine nitrosation (a stable biomarker of tissue peroxynitrite formation) in HHcy coronaries. Also, extensive prevalence of 3-nitrotyrosine immunoreactivity was observed in HHcy coronaries. It was confined primarily to the subendothelial layers of smooth muscle. Pro-oxidant changes in protein and gene expression profile: 5) Using Western blotting, immunohistochemistry and QRT-PCR we demonstrated a pro-oxidant shift in vascular phenotype. In HHcy coronary arteries expression of the NAD(P)H oxidase subunit nox1 was significantly increased and was predominantly confined to the arterial smooth muscle. Protein expression of p67<sup>phox</sup>, p22<sup>phox</sup> and p47<sup>phox</sup> subunits and that of eNOS, Cu,Zn-SOD, Mn-SOD, and xanthine oxidase were unchanged. Expression of iNOS was significantly increased in HHcy. Pro-inflammatory changes in gene expression profile: 6) Using cDNA based microarray screening we found a pro-inflammatory shift in the vascular phenotype. In coronary arteries of HHcy rats expression of several pro-inflammatory cytokines, such as TNF $\alpha$  and TNF $\beta$ , IL-6, IL-11, IL-20 and their receptors like IL-2R $\beta$  and IL-2R $\gamma$ , TNFR1, IL-15R $\alpha$  was significantly increased. Protein expression of TNF $\alpha$  was also significantly increased in HHcy arteries and was localized in the smooth muscle. The expression of various chemokines, such as MCP-3, lungkine, exodus-2 was also significantly increased in HHcy coronaries. Increased expression of TNF $\alpha$  upregulates NAD(P)H oxidase-dependent O<sub>2</sub><sup>-</sup> production. 7) Inhibition of TNF $\alpha$  substantially (by ~40%) decreased O<sub>2</sub><sup>-</sup> generation in HHcy coronary vessels in culture. Also, *in vitro* incubation of control vessel segments (18 h) with recombinant TNF $\alpha$  significantly increased DPI-inhibitable O<sub>2</sub><sup>-</sup> generation. *Conclusion:* All these changes suggest that in coronary arteries HHcy elicit a pro-inflammatory shift in vascular phenotype. The increased expression of TNF $\alpha$  may upregulate the expression/activity of a nox1-based NAD(P)H oxidase and iNOS increasing O<sub>2</sub><sup>-</sup> production and reducing the bioavail-

abimagaslity of NO (by forming ONOO<sup>-</sup>). These functional and phenotypic changes likely contribute to the development of coronary atherosclerosis.

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**TIBOR HIDVÉGI (2003)**

## Clinical characteristics of the metabolic syndrome in Hungary

Supervisor: Dr. Lajos Szollár

The accelerated atherosclerosis as a vascular consequence of metabolic syndrome is of great clinical importance. In order to detect subjects with metabolic syndrome a mass-screening was performed. Subjects aged from 20 to 65 years exhibited at least one of the following clinical characteristics: abnormal body mass index (BMI) elevated waist-hip ratio, or hypertension. In subjects available for complete statistical analysis (n=944; women/men: 545/399; age: 46.1±7.3 years; x±SD) hyperinsulinemia (HI) was detected in 52.9%. Early stage of metabolic syndrome (hyperinsulinaemia with normal glucose tolerance) was more often (33.2%) detected while later stages (hyperinsulinaemia with IGT or diabetes) were in a lower proportion found (13.0% and 6.7%, respectively). Glucose intolerance (IGT or diabetes mellitus) could be found in 23.6% of subjects. Metabolic syndrome was diagnosed according to the modified criteria of WHO in 35.2% of subjects with a male predominance (men: 40.6%; women: 31.2%). According to the Adult Treatment Panel III (ATP-III) guidelines metabolic syndrome was present in 68.5% with no significant difference in gender. Clustering of the clinical features of metabolic syndrome proved to be associated with lower education level in a large cohort of subjects, particularly in women. Carotid intima media thickness (IMT) was determined by high-resolution B-mode ultrasonography in subjects who proved to be hyperinsulinemic (n=91; men/women: 35/56; age: 47.7±4.4 years). The values of IMT were higher in hyperinsulinemic subjects than those of controls. There was no significant difference between hyperinsulinaemic and control groups regarding lumen diameter of the common carotid arteries, however, the lumen diameter of the internal carotid arteries proved to be smaller than that of control subjects. There was no significant difference between hyperinsulinaemic and control subjects regarding plasma homocystein, folic acid and vitamin B<sub>12</sub> levels. Plasma homocystein values independently from the stages of glucose intolerance were not elevated in hyperinsulinaemic subjects. The reliability of the new fasting blood glucose criteria was also analysed and it was found that 2-h post-glucose challenge criteria during an oral glucose tolerance test should be used for the diagnosis of different categories of glucose intolerance when screening for metabolic syndrome. Routine clinical and laboratory investigations (measuring anthropometric data, blood pressure and performing oral glucose tolerance test) are simple but useful for identifying subjects with metabolic syndrome enabling a prevention strategy of cardiovascular morbidity to implement.

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ZSOLT DÁNIEL IVÁNYI (2005)

## Evaluation of the mechanism of the systemic inflammation induced organ dysfunction in animal sepsis model

Supervisor: Dr. Péter Gergely

Our *hypothesis* was that therapeutic options, targeting the cell energetic machinery or the vascular reactivity could improve the energetic and oxygen balance during severe septic shock. To test our hypothesis, we evaluated the hepato-splanchnic-hemodynamic, oxygen-exchange and metabolic effects of various therapeutic options, targeting the cell energetic homeostasis and / or the vascular dysfunction. In order to get clinical relevant data, a delayed post-treatment approach has been used in a model of hyperdynamic porcine endotoxemia, which fulfils the criteria of hyperdynamic human sepsis. The following therapeutic options were tested: Influencing the cell energetic machinery: N-acetyl-cystein (NAC) a free radical scavenger. Inhibition of PARP-1 enzyme in order to prevent increased energy consumption, and the mitochondrial failure due to the lack of substrate (NAD). Influencing the microcirculatory dysfunction: ATP-sensitive potassium channels (KATP) inhibition, depleted serum vasopressin replacement (under publication). *Measurements and Calculations:* We measured cardiac output, intrathoracic blood volume, extravascular lung water, portal venous and hepatic arterial blood flow which were summed to yield total hepatic blood flow. Arterial, mixed, hepatic and portal venous blood samples were analyzed for bloodgases, total haemoglobin, haemoglobin O<sub>2</sub> saturation, lactate, and pyruvate concentrations. From these data, systemic intestinal and hepatic O<sub>2</sub> delivery, uptake, O<sub>2</sub> extraction and lactate fluxes were calculated. Ileal mucosal PCO<sub>2</sub> was measured continuously and ileal mucosal – arterial PCO<sub>2</sub> gap was calculated. The key findings were that: NAC infusion had no beneficial effects on global or regional hemodynamics, gas exchange, or metabolism. PJ34 infusion improved cardiac output as a result of an increased stroke volume, and attenuated the otherwise progressive LPS-induced deterioration of intestinal energy balance assessed by the ileal mucosal– arterial PCO<sub>2</sub> gap. HMR1402 infusion transiently increased mean blood pressure, decreased cardiac output due to a significant reduction in heart rate without altering mean arterial pressure, enhanced lactate production, increased lactate/pyruvate ratios suggesting a disturbed cytosolic redox potential. *Conclusion:* In our model, therapies designated to reveal the vascular dysfunction or the cellular energetic failure resulted in different therapeutic conclusions, suggesting that these mechanisms may have different levels of importance in hyperdynamic endotoxin shock states.

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ÉVA NIESZNER (2003)

## Cardiovascular effects of sulphonyl ureas experimental and clinical experiences

Supervisor: Dr. Gábor Pogátsa

The participation of K<sup>+</sup> ATP channels in the cardioprotective procedure that is in preconditioning is proved. Either being a signal or the main participant of this procedure sulphonylurea treatment can modify the activity of K<sup>+</sup> ATP channels. In this way the efficacy of preconditioning can be modified. Modification of channel activity and so the influence of preconditioning on myocardial infarction are modified by effects and sulphonylurea treatment as well. Both effects can be assessed by clinical diagnostic equipments, so it is possible today to choose the proper, personal therapy.

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ANDREA SZÉKELY (2003)

## The role of anesthetics in the prevention of ischaemia and reperfusion injury

Supervisor: Dr. Lajos Szollár

Adhesion of polymorphonuclear neutrophils (PMN) to the coronary endothelium is a crucial step in the development of ischemic myocardial injury. We tested the possible effects of six widely used anesthetics on non- and postischemic coronary adhesion of PMN in isolated perfused guinea pig hearts. In addition, the ability of drugs to influence oxidative burst reaction was assessed by measuring luminol enhanced chemiluminescence. The postischemic stimulation of adhesion was fully prevented by ketamine, thiopental and midazolam. Propofol gave inconsistent results. However, etomidate, fentanyl had no effects. Ketamine, midazolam and propofol did not significantly influence oxidant production by PMN, etomidate and lipid solvent enhanced burst reaction. This activating effect could explain the biphasic behavior of propofol emulsion. Ketamine is a racemic mixture of two optical enantiomers, we looked for possible differences in action between stereoisomers. Applying the same model we demonstrated stereoselective differences in the biological action, S(+)-ketamine inhibited PMN adherence, whereas R(-)-ketamine worsened coronary vascular leak. In a retrospective clinical study we evaluated the possible differences of anesthetics in pediatric patients underwent open heart surgery. We found, that isoflurane and sevoflurane were both effective and safe in their action during anaesthesia. In the postoperative period we compared the influence of propofol with midazolam by analyzing the hemodynamic, metabolic and antiinflammatory response. We demonstrated, that there were no difference in the hemodynamic and metabolic conditions between the groups, however, propofol reduced the immune response more than midazolam, assessed by cortisol values and fewer occurrence of fever and pleural effusions. At the same time, propofol increased significantly the creatin-kinase, but not creatin-kinase MB, activity.

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## 2. PH.D. SCHOOL OF CLINICAL MEDICAL SCIENCES

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### **2/1. PROGRAM**

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## **IMMUNOLOGICAL PROCESS AND FREE RADICAL REACTION IN LIVER DISEASES**

### *Coordinator*

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Evidence accumulates that natural (vitamins, flavonoid type molecules) and synthetic (butylated hydroxytoluene, dihydro-quinolin-type molecules) antioxidants exert a preventive effect on local oxidative damage in several models in vitro and in vivo. Therefore, the aim of our Program is to investigate the role of oxidative stress and the shift in pro/antioxidant balance in the pathogenesis of several gastrointestinal and immunological diseases, metabolic disorders and drug side effects by direct and indirect methods. The ongoing experiments focus on steatosis, hepatitis, cirrhosis, hepatocellular carcinoma, gallstone formation, cholestasis, inflammatory bowel diseases, colon neoplasm and metabolic disorders (carbohydrate, lipid) as well as amiodarone toxicity.

### *Sub-programs*

Alcoholic liver disease

Role of free radical reactions and antioxidants in the regeneration of liver and bowel mucosa

Ursodeoxycholic acid treatment in hepatobiliary diseases

Interferon treatment and oxidative stress in viral hepatitis

Immunohistochemical and electronmicroscopical investigation of NO radicals in the gastrointestinal tract

Investigations of gene expressions in liver diseases

Investigation of ethiopathogenesis of hepatocellular carcinoma

Carbohydrate and lipid metabolism in connection with free radical reaction, atherosclerosis

Role of free radical reactions in the pathogenesis of amiodarone toxicity

Role of free radicals in acute and chronic pancreatic diseases

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a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

**KÁROLY ALTDORFER (2003)****Nitric oxide containing nerve elements***Supervisor: Dr. Erzsébet Fehér*

The following new observations were made in our immunohistochemical and immunocytochemical investigations of the neural elements of the gastrointestinal tract: The distribution and density of the nitrergic nerve elements are different in the gastrointestinal tract but greater numbers of NO containing nerve elements are found in the myenteric plexuses and in the inner, circular smooth muscle layer of the sphincter-regions. This distribution pattern suggests that nitrergic nerves cause smooth muscle relaxation in the sphincter regions. In the pylorus we have demonstrated NOS immunoreactive nerve terminals in both intramural nerve plexuses forming intraneuronal synapses. These results suggest the postsynaptic effect of NO on these plexus neurones and thus participation in the local neural reflexes. The similar distribution and localization of VIP and NOS immunoreactive nerve elements suggest the possible colocalization of these transmitters in the inhibitory neurons of the pylorus. We have observed nitrergic nerve terminals in close apposition to the smooth muscle cells of the blood vessels, suggesting the regulating effect of NO on the blood flow and thus on the gastroprotection. NO containing nerve terminals were also found around the pyloric glands and be-

neath the surface epithelium suggesting the nitrergic modulation of gastric secretion. In our experimental colitis model we have demonstrated the marked decrease of the number of nitrergic nerve terminals and their synapses during moderate inflammation. We found that some of the interstitial cells of Cajal in the inner, circular muscle layer of the inflamed colon became NOS immunoreactive. This change can modulate the motility during inflammation and can be a sign of innervation plasticity of the enteral system.

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**GÁBOR BÉKÉSI (2004)**

## **Steroids as antioxidants and the clinical importance of this in hormone replacement therapy**

*Supervisor: Dr. János Fehér*

*Background:* There are numerous experimental and clinical data to support the view that changes in physiological and pathological steroids' status can play an important role in forming susceptibility to certain diseases or in increasing the frequency of such illnesses. Steroids are capable of exerting an influence on many essential biochemical mechanisms and thereby of playing a substantial role in essential physiological processes. Apart from the specific endocrinological effects, the most significant example of this in the case of estrogens is their role in lipid and bone metabolisms. In recent years, we have come across more and more data in the literature referring to the antioxidant role of steroid hormones. Alongside with other factors, free radicals have achieved a substantial role in the pathogenesis of diseases at the forefront of worldwide morbidity and mortality statistics. Besides, neutrophil granulocytes represent one of the most important locations for production of radicals. Therefore, it seemed logical to make a summary of our interest in how steroid hormones influence the radical producing activities of these cells and what the consequences might be in respect to one of the leading killers, cardiovascular diseases. *Objectives:* To examine (1) whether the menopause implies changes in the activities of the myeloperoxidase enzyme, which figures in free radical reactions and can be found in neutrophil granulocytes, (2) whether hormone replacement treatment has an effect in terms of the quantity and activity of this enzyme and (3) whether this has consequences in respect of the production of free radicals. To assess (4) how plasma concentration of the myeloperoxidase enzyme changes with age and by gender and (5) whether a connection exists between the antioxidant role of steroid hormones, as mentioned above, and its effect on the myeloperoxidase enzyme. *Methods:* We determined the levels of intracellular myeloperoxidase activity in randomly selected patients at the II. Medical Clinic of Semmelweis University during blood count examinations carried out for other purposes, by compiling mean peroxidase index values measured automatically by a Technicon H-3 haematology device. We also used this machine to assess the effects of oestrogen and prednisolone on myeloperoxidase activity. We applied the ELISA method to measure quantities of the myeloperoxidase enzyme in plasmas or in supernatants of neutrophil cells obtained from healthy volunteers. Superoxide anion was determined by photometry during examination of the role of MPO, MPO-inhibitors and certain steroids in the superoxide generation. *Results and new claims:* Intracellular myeloperoxidase activity and the plasma level of the enzyme show a significant reduction for people of both sexes in the sixth decade, compared with previous years. But for menopausal women, following oestrogen replacement, there is a significant rise in enzyme activity and quantities released from neutrophils. We observed a similar increase in intracellular enzyme activity after prednisolone administration and in release of the enzyme from neutrophils by adding testosterone. A significant reduction could be achieved in quantities of the free radical known as superoxide anion on the effect of myeloperoxidase mixed in rising quantities and in vitro with neutrophil granulocytes. We found a significant increase in free radical production through in vitro incubation of myeloperoxidase inhibitors with neutrophil cells, furthermore, these inhibitors stopped the reducing effect of several steroid hormones on superoxide anion production. However,

hypochlorite anion, the end product of the reaction catalyzed by the myeloperoxidase enzyme, also caused a reduction in superoxide radical production in our methodology. *Conclusions:* Our results support the view that certain steroid structures have an antioxidant effect, and this effect is probably realized at least in part through an increase in activity and quantity of the myeloperoxidase enzyme. Thus any attempt to target obstruction of a physiological reduction in the level of antioxidant steroids, replacement of quantities, or even an increase in the activity or quantity of the myeloperoxidase enzyme appears promising in the fight against the many diseases transmitted by free radicals. In the non-too-distant future and after further research the myeloperoxidase enzyme might also be thought of as a kind of new indicator of antioxidant capability and even a predictor of diseases originating from free radicals.

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## KLÁRA FARKAS (2005)

### Nitric-oxide and oxidative stress in diabetes mellitus

*Supervisor: Dr. Anikó Somogyi*

Accelerated atherosclerosis is central to the increased morbidity and mortality associated with diabetes mellitus. Endothelial dysfunction is an early sign of vascular damage that precedes the morphological changes of the vessel wall. It is characterised by the alteration of the bioavailability of endothelial vasoactive factors resulting in an impaired vasoreactivity. One of the most important vasoactive factors is endothelial nitric oxide (NO). Hyperglycaemia and hyperlipidaemia induced increased oxidative stress – leading to the imbalance of the prooxidant-antioxidant factors –may contribute to the impairment of the bioavailability of endothelial NO. The aim of our research was to detect quantitative and qualitative changes in NO bioavailability and their connection with parameters of oxidative stress in different disturbances of a carbohydrate metabolism (type 1 and 2 diabetes mellitus, impaired glucose regulation). According to our results in well controlled type 1 diabetes mellitus there was no sign of prooxidant-antioxidant imbalance or disturbance in the nitric oxide metabolism either in patients with or without late diabetes specific complications. Poor metabolic control was found to be associated with increased oxidative stress and the impairment of the NO/cGMP pathway was shown to be present both in the fasting and the postprandial state regardless of the presence of late complications. These alterations - detectable before the onset of microalbuminuria - may represent an early sign of hyperglycaemia induced vascular damage. In type 2 diabetes mellitus poor glycaemic control was associated with a prooxidant-antioxidant imbalance and changes in the NO metabolism correlated with the actual metabolic parameters and the presence of hypertension, therefore may serve as indicators of increased vascular risk. In impaired glucose regulation - which is considered pathogenetically to be between normal glucose regulation and diabetes mellitus –impaired endothelium dependent vasodilation and increased nitrotyrosine concentration indicating oxidative damage of NO were found in the postload state demonstrating a hyperglycaemia initiated increased vascular risk.

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LÁSZLÓ KÓBORI (2004)

## Role of the oxidative stress and arterial blood supply in the function of the transplanted liver graft

Supervisor: Dr. János Fehér

Reperfusion injury and hepatic artery thrombosis are major causes of graft failure after liver transplantation. The magnitude of oxidative stress increases after reperfusion and the appearance of an arterial thrombosis presents a higher risk for the graft and patient survival. The incidence of hepatic artery thrombosis is over 10% and is usually higher in pediatric recipients. For the monitoring of the oxidative stress MPO was measured in our study group. This study provides evidence of increased oxidative stress before liver transplantation. The magnitude of these changes increased after the operation, mostly in cases with acute liver failure and hepatic artery thrombosis. No significant elevation of MPO was measured under rejection periods. The incidence of splenic artery aneurysms in our liver transplant patients was 8-13%. The aneurysms were generally multiple and located in the distal third of the splenic artery. The incidence was higher in woman and in patients with parenchymal liver disease and portal hypertension. The incidence of rupture was 4%. The diameter of the splenic artery was bigger than the diameter of the hepatic artery in all cases, representing a potential technical problem in the time of transplantation. Use of donor interponates for arterial reconstruction are common methods in liver transplantation, but results are controversial. Autologous, tubular graft lined with mesothelial cells, prepared from the posterior rectus fascia sheath was used for experimental artery replacement. The grafts presented good long-term patency rate and low thrombogenicity without risk of rejection. In conclusion, reducing the level of oxidative stress and performing an „ideal” arterial supply for the liver graft present better survival after transplantation.

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IBOLYA KOCSIS (2005)

## Monitoring redox-homeostasis. Importance in human studies and in animal experiments

Supervisor: Dr. Anna Blázovics

Introduction of the term “oxidative stress” denotes an imbalance between the production of reactive oxidative species and the antioxidant defense systems of the organism. The assay of oxidative stress parameters has brought substantial insight into the pathogenesis of most of the diseases in humans, by demonstrating the involvement of free radicals and /or the decrease of antioxidant mechanisms. Aims: With the help of monitoring redox homeostasis in animal experiments, the possible beneficial effects of natural antioxidants of plant origin were investigated. *Cichorium intybus* (L.) extract, juice of black radish root (*Raphanus sativus* L. var niger) and commercially available Beiqishen tea product were examined in experimental hyperlipidaemia, and the main differences in their antioxidant effect were taken into consideration. The information values of some oxidative stress parameters were evaluated in human studies, monitoring of changes and the differences of redox homeostasis in inflammatory bowel diseases (IBD), in diabetes and in the condition of alcoholic (ASH) or non-alcoholic steatohepatitis (NASH). Methods and materials: Male wistar rats were treated with lipid rich diet, the changes of lipid metabolism were detected by the values of main metabolites [cholesterol (CHOL), triacylglycerols (TG)]. The liver function was monitored by the values of the transaminases

and alkaline phosphatase. The antioxidant status of serum and liver tissue were examined by methods of spectrophotometry and luminometry. The metal ion content was determined in plant extracts and also in the liver tissue of the animals by inductively coupled plasma emission spectrometry (ICP-OES). The degree of the liver tissue steatosis was examined by histochemical reactions, observed by microscopy. In human studies the redox homeostasis was monitored by the most important metabolic parameters [albumin (ALB), uric acid (UA)], and were correlated with the values of special antioxidant parameters [total antioxidant status (TAS), total scavenging capacity (TSC), free-SH-groups] in sera and in erythrocytes. Results and discussion: It was verified in our experiments, that black radish juice and chicory extract significantly influenced lipid-metabolism in rats. In the cases of CHOL and TG, beneficial changes were detected. In chicory extract treated animals the TSC of pancreas tissue was significantly higher than in the controls. Significantly higher concentrations of Fe, Cu, Na and K could be detected in the liver tissue of chicory treated animals. Metal ions detected in Beiqishen tea (Ni, As, Mo), injured the total scavenger capacity of liver tissue significantly. Evaluating the different antioxidant parameters in case of IBD, albumin, TAS and total scavenger capacity could well confirmed each other in their diagnostic information values. Strong correlation was found between TAS and ALB, or TAS and UA parameters in colitis ulcerosa. Significant decrease was evaluated of TAS in diabetes in relation with the time of clinical duration.

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ÉVA RUZICKSA (2004)

## Diabetes and natriuretic substances

Supervisor: Dr. Anikó Somogyi

*Aims, hypothesis.* Diabetes mellitus is associated with an excessive cardiovascular morbidity and mortality. Many factors including hypertension contribute to the high prevalence of cardiovascular diseases. In order to gain insight into the cardiac adaptive mechanisms in diabetes we studied if angiotensin II (AII) alters atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP) and adrenomedullin (AM) gene expressions in the left ventricle of the diabetic rat heart. The cardiac gene expressions of  $\alpha$ -myosin heavy chain ( $\alpha$ -MHC),  $\beta$ -MHC, skeletal actin (SA) and cardiac actin (CA) were also assayed. *Methods.* Diabetes was induced by streptozotocin (STZ; 60 mg/kg b.w., i.v.), AII (33  $\mu$ g/kg/hour) was administered for the last 24 hours via osmotic minipumps 2,5 or 7 weeks after treatment of male Wistar rats with STZ or vehicle. Mean arterial pressure (BP) was measured by radiotelemetry. Ultralente insulin (I) was used subcutaneously. Left ventricular expressions of genes were measured by total RNA isolation by guanidine isothiocyanate-CsCl method and by Northern-blotting. Serum glucose concentration was measured using a reagent kit from Boehringer Mannheim and serum fructosamine concentration was measured using a reagent kit from Roche Diagnostics GmbH on a Hitachi 907 analyser. *Results.* All groups of diabetic animals had significantly higher blood glucose and fructosamine values compared to controls and AII treated groups ( $p < 0,001$ ). Diabetes elevated left ventricular weight-to-body weight (LV/BW) ratio, an index of left ventricular hypertrophy, at week 7 but not at week 2,5, and increased ANP mRNA content at 2,5 weeks whereas did not alter AM and BNP gene expressions. BP and LV/BW ratio were increased by AII in all groups except in the 7-week diabetic group. ANP mRNA levels were 4-fold ( $p < 0,001$ ) and 3-fold ( $p < 0,05$ ) increased by AII at 2,5 and 7 weeks in control animals, respectively, and 11-fold ( $p < 0,001$ ) and 7-fold ( $p < 0,001$ ) at 2,5 and 7 weeks in diabetic animals, respectively. AII increased BNP ventricular mRNA concentration in control and diabetic animals at 2,5 (1,3-fold,  $p < 0,001$ ; and 1,6 fold,  $p < 0,001$ ) and at 7 weeks (1,3-fold,  $p < 0,05$ ; and 1,8-fold,  $p < 0,001$ ) respectively. Left ventricular AM mRNA levels were increased by treatment with AII for 24 hours in 2,5-week diabetic ani-

mals. In the second series of the experiments, insulin significantly lowered blood glucose and fructosamine levels ( $p < 0,001$ ). Insulin elevated LV/BW ratio more than AII or diabetes did it alone, both at weeks 7 and 2,5 ( $p < 0,001$ ), however at week 7, diabetes and AII together elevated this ratio ( $p < 0,05$ ). Left ventricular ANP expression was elevated by AII and diabetes together at 2,5 week ( $p < 0,001$ ). At week 7 diabetes elevated the ANP mRNA levels ( $p < 0,0049$ ), diabetes and AII together further elevated this value ( $p < 0,01$ ). Interestingly, insulin did not elevated further this level in diabetic AII treated animals ( $p < 0,001$ ). Changes in left ventricular BNP expression were similar but smaller, it was elevated by AII and diabetes together at week 2,5 ( $p < 0,05$ ) and insulin did not elevated further this level in diabetic AII treated animals ( $p < 0,001$ ). At week 7 same changes occurred, however AII treatment did not elevated this value compared to insulin treated diabetic animals. *Conclusion.* Angiotensin II markedly increased natriuretic peptides mRNA levels in the left ventricle of normal and diabetic rat hearts, whereas it elevated adrenomedullin mRNA levels just in week 2,5 diabetic rats and failed to cause hypertension in 7-week diabetic rats. Left ventricular ANP and BNP mRNA levels were elevated by AII in diabetic animals more than the additive effects of diabetes and AII alone, showing that AII induced an amplified response in cardiac ANP and BNP levels in diabetes. Long-term insulin treatment administered once daily lead to cardiac hypertrophy due to its growth hormone effect. However in insulin treated animals the elevation of the natriuretic peptides was smaller or missed than compared to diabetic or AII treated diabetic animals. Partial reversion of hypertension-induced changes in cardiac protein expression by insulin treatment may reflect beneficial effects contributing to enhancing readaptation of the heart to overload.

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**KRISZTIÁN STADLER (2005)**

## **Role of free radicals in the development of late complications of experimental diabetes mellitus in rats**

*Supervisor: Dr. Judit Jakus*

*Diabetes mellitus* is a chronic disease and one of the most important health problems in the world. Nowadays, life quality of diabetic patients is mainly determined by the development of late complications: not by hypo- or hyperglycemia. Several studies have discussed that oxidative stress is developed during malfunctions of the carbohydrate metabolism. Free radical mechanisms and the possible sources of oxidative stress in the pathogenesis of diabetes have been extensively studied, but the relationship between free radical formation and the complications of diabetes is still controversial. This work tries to contribute new data to answering the question whether free radical production is related to the appearance of diabetic complications. Our most important theses are as follows: 1. Accelerated oxidative processes and altered free radical status were found at an early stage of streptozotocin-induced diabetes, especially regarding nitric oxide overproduction was. 2. It has been concluded that high nitric oxide levels and oxidative stress lead to the formation of high amounts of peroxynitrite in the diabetic aorta. 3. We determined that nitric oxide and peroxynitrite generation in healthy aorta of older rats showed a similar increase as in diabetic aorta, but with a timeshift, suggesting a kind of premature aging of the vasculature in case of carbohydrate metabolism disorder. 4. Increased free radical production and other oxidative stress-related biochemical processes developed before the evolution of any histopathological signs of late complications detected by light microscopy. It is possible therefore, that free radical processes could play a decisive role in the development of late complications. 5. We have determined that both aminoguanidine, a NOS inhibitor, and ultratard insulin treatment suppressed reactive nitrogen species overproduction, but probably their

mechanism of action is different. 6. It has been concluded that only aminoguanidine was able to prevent cardiac hypertrophy in diabetes, while insulin treatment was not. 7. Insulin treatment was able to improve most of the carbohydrate metabolism parameters in diabetic rats, while aminoguanidine moderated oxidative stress through inhibition of glycation. Its combination with insulin treatment, therefore, may offer new therapeutic options in the prevention of certain complications in diabetes.

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## GÉZA TELEK (2004)

### Oxidative stress and cellular adhesion molecules in acute pancreatitis

Supervisor: Dr. János Fehér

In acute pancreatitis (AP), parallel with the intracellular activation of pancreatic digestive enzymes, an uncontrolled inflammatory explosion is set forth at local and systemic levels. In our work we investigated yet unexplored elements of this process in the taurocholate experimental AP model and in the human disease. The exact source of oxygen free radicals (OFRs) formed in acute pancreatitis has long been unclear. We have developed an effective technique for the *in vivo*, or *in vitro* demonstration of OFR production on a histological level which is based on cerium capture free radical cyto/histochemistry combined with reflectance confocal microscopy detection. We have verified and standardized the specificity and reproducibility of this method. We have shown an apparent OFR source switch in the pancreas from acinar cells dominating the early period to leukocytes as the disease progressed. Later the OFRs enter the blood stream as suggested by the intracapillary cerium deposits. We observed a generalized, time dependent OFR production by blood and ascites derived polymorphonuclear (PMN) leukocytes, and using an image analysis based quantification method we showed that the activation of these cells reached a maximal plateau phase as early as 2 hours post AP induction. During our investigation of cellular adhesion molecules (CAMs) for leukocytes we have demonstrated that they are differentially upregulated at the sites of oxidative stress in the pancreatic tissue. The taurocholate AP model is characterized by a strong and rapid P selectin, as well as ICAM upregulation on the levels of protein expression, and gene activation, whereas only a modest, and delayed augmentation of E selectin and VCAM expression occurred. We have provided for the first time histological confirmation of early intrapancreatic NF- $\kappa$ B activation, furthermore, its co-localization with the oxidative stress suggested the direct activating role of OFRs as signal transmitters. The characteristics of the OFR production, CAM upregulation and NF- $\kappa$ B activation in the human acute pancreatitis sample were very similar to those of the taurocholate experimental model, furthermore all features were concurrently present in the human tissue. We have detected considerable numbers of PMNs with damaged nuclear morphology in the late course of experimental AP. Based on the cerium capture/CLSM and Annexin V methods we conclude that these cells represent the development of leukocyte "activation induced cell death" in the disease. The extensive nature of the phenomenon evokes the possibility that (besides the gut barrier failure) this special form of leukocyte death plays a role in the AP associated relative immune-deficiency, and consequently in the development of infectious complications. These early elements of AP pathophysiology represent attractive fields for continued investigation to explore novel therapeutic modalities.

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## ZSOLT EMBER (2003)

# Detection and role of the free radical nitric oxide in the exocrine pancreas

*Supervisor: Dr. Erzsébet Fehér*

Nitric oxide (NO) is a small free radical molecule that has several functions in various tissues. NO is well distributed in the gastrointestinal system including the pancreas and thought to be involved in the regulation of blood flow, motility and secretion. Although morphological reports provide evidence for the intrapancreatic production of NO, much less is known about the direct actions of NO exerted on the exocrine pancreatic tissues. In order to localize the neuronal isoform of the enzyme of NO production, the neuronal nitric oxide synthase (nNOS), we applied NADPH diaphorase (NADPHD) histochemistry and NOS immunohistochemistry in the rat and porcine pancreas. NADPHD reactivity (R) and/or NOS immunoreactivity (IR) were identified in the pancreatic ganglion cells and in nerve fibers next to acini and blood vessels. NADPHD R was also displayed by the porcine vascular endothelium, whereas the islet cells and the rat ductal epithelium occasionally showed nNOS IR. The distribution of nNOS in the neuronal and especially in the non-neuronal tissues showed age-related changes with increased contents of nNOS in the adult and ageing rat pancreas. In order to demonstrate the direct actions of NO on in vitro rat preparations, we studied the effects of the NO donor sodium nitroprusside (SNP), the NOS substrate L-arginine (L-Arg), the NOS inhibitor NG-nitro-L-arginine (L-NNA) and the cyclic GMP (cGMP) analogue 8-bromo cGMP (8-Br cGMP) on basal and stimulated amylase secretion in isolated rat pancreatic segments. Stimulation was achieved by either acetylcholine (ACh) or electrical field stimulation (EFS). The changes in intracellular free calcium concentration ( $[Ca^{++}]_i$ ) were also investigated in fura-2 loaded isolated rat acinar cells. Both amylase output and  $[Ca^{++}]_i$  were measured by fluorimetric methods. Our results show that both EFS and ACh ( $10^{-5}$  M) resulted in marked increases in amylase output from pancreatic segments. SNP ( $10^{-3}$  M) significantly reduced basal amylase output. This inhibitory effect could be mimicked by 8-Br-cGMP ( $10^{-5}$  M). Combining SNP or 8-Br cGMP with EFS caused a significant decrease in amylase secretion compared with the response with EFS alone. This inhibitory effect was not evident when extracellular  $Ca^{++}$  concentration was elevated from 2.56 to 5 mM. SNP or 8-Br cGMP seemed to have no significant inhibitory effect on ACh-evoked amylase output compared with ACh alone. L-Arg ( $10^{-3}$  M) or L-NNA ( $10^{-3}$  M) had no significant effects on either basal or EFS- or ACh-evoked amylase output from pancreatic segments. In fura-2 loaded isolated acinar cells, ACh induced the typical large increase in  $[Ca^{++}]_i$  whereas SNP and 8-Br cGMP significantly decreased basal  $[Ca^{++}]_i$ . Neither SNP nor 8-Br cGMP elicited any modification in ACh-evoked rise in  $[Ca^{++}]_i$ . The results above indicate the presence of nitrergic pancreatic nerves and the existence of a direct action of exogenous NO on rat acinar cells and also suggest its neuromodulatory role in the regulation of exocrine pancreatic secretion, possibly through the inhibition of ACh release.

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**2/2. PROGRAM****FETAL AND NEONATAL MEDICINE****Coordinator****Zoltán PAPP**

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The Ph.D. Program is designed for medical doctors who wish to specialize in prenatal genetics and fetal medicine. Our aims are: to provide medical and science based students with comprehensive knowledge in the field of genetic and fetal medicine, to provide suitable environment for clinical or laboratory based research project, to enable students for the use of laboratory techniques such as PCR, F-PCR, RFLP, blotting techniques etc., to train students in modern prenatal diagnostic methods, like color-Doppler ultrasound, intrauterine echocardiography etc.

**Sub-programs**

Prenatal genetics  
 Prenatal ultrasonography  
 Embryo-fetopathology  
 Diagnosis of fetal state  
 Cardio-pulmonal adaptation and the attached diseases of the neonate  
 Hypertension in pregnancy  
 Life-threatening conditions of fetal life: diagnosis and therapy

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 Attila Demeter na  
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a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

ZOLTÁN BÁN (2005)

## Detection of fetal aneuploidies from amniotic fluid and chorionic villus samples by quantitative fluorescent polymerase chain reaction

Supervisor: Dr. Zoltán Papp

Traditional cytogenetic analysis is the 'gold standard' in the detection of fetal chromosome abnormalities. Because of the long term culture of the fetal cells the average reporting time is minimum two weeks. The multiplex quantitative fluorescent polymerase chain reaction (QF-PCR) has been shown to be a useful tool in the detection of fetal aneuploidies. It has several advantages over cytogenetic analysis. It is fast and does not require viable cells. QF-PCR has its limitations as it can not detect some fetal chromosome disorders of clinical importance. The aims of the study were to test the reliability of QF-PCR for the prenatal diagnosis of the common aneuploidies, to obtain data on the allele distribution of 7 different short tandem repeats (STR) in Hungarian population, to determine the indications in which QF-PCR can be applied safely in prenatal diagnosis and to analyze the possibilities for decreasing the rate of allele drop-out. In a prospective study of 4875 amniocenteses and 146 chorionic villus samplings, both QF-PCR and karyotyping of the prenatal samples were performed. The results of QF-PCR were compared with those of the conventional cytogenetic study. Allele distribution of the applied STR markers was analyzed. The occurrences of chromosome disorders which can not be detected by QF-PCR were compared in cases with different indications of amniocentesis. 98.2% of the QF-PCR results were informative without false-negative and false-positive results. The rate of homozygosity was over 80% in all investigated STR markers in the Hungarian population. With the use of silica adsorption column based method a significant decrease in the frequency of ADO was observed. In conclusion: We applied a reliable, simple and cost-effective protocol using 7 STRs for the prenatal diagnosis of the common aneuploidies. In our study on almost 5021 prenatal samples, all chromosome disorders of clinical significance were detected in case of advanced maternal age and positive serum screening results. The highest number of chromosome disorders that are not detectable by QF-PCR were in case of structural abnormalities detected by ultrasound. Prenatal QF-PCR diagnosis of the trisomies 21, 18, 13 and sex chromosome anomalies is a reliable alternative to cytogenetic analysis of fetal samples, but the indication of the prenatal diagnosis should be considered prior to its application.

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ARTÚR BEKE (2005)

## Cytogenetic background of fetal minor and major ultrasound anomalies in pregnancy

Supervisor: Dr. Zoltán Papp

In this study the author investigated chromosome abnormalities detected in cases with prior abnormal ultrasound findings. During a ten-year period there were 1907 invasive interventions carried out with the purpose of chromosome analysis. The invasive intervention was genetic amniocentesis in 1619 cases and chorion villus sampling in 288 cases. Karyotyping revealed 103 cases (5.4%) with chromosome abnormalities. Abnormalities with subcutaneous oedema were examined: abnormal

karyotype was found in 20% of cases with non-immune hydrops, 48.1% of cases with cystic hygroma, and 53.8% of cases with non-immune hydrops and cystic hygroma altogether, 8.3% of cases with nuchal oedema in the 1st trimester, and 5.5% in the 2nd trimester. The incidence rate of chromosome abnormalities in cases with cerebral anomalies was 6.3% of cases with ventriculomegaly, 3.6% of cases with choroid plexus cysts, and 15.9% of cases with other cranial anomalies. Regarding abnormalities of the heart; isolated echogenic intracardiac focus and ventricular septal defects were not associated with chromosome abnormality, but, in conjunction with other positive ultrasound findings the incidence rate of chromosome abnormalities were 7.9% and 26.7%, respectively. Other anomalies of the heart and large blood vessels showed an abnormal karyotype incidence rate of 18.2%. In cases of unilateral pyelectasis unassociated with other anomalies, the incidence rate of the chromosome abnormalities was 1%. In cases of bilateral pyelectasis, or pyelectasis associated with other anomalies, the incidence rate was 3%. In terms of anomalies of the abdominal wall and the abdomen; the incidence rate of association with chromosome abnormalities was 9.5% in cases with omphalocele, 11.8% in cases with duodenal atresia, and 5.7% in cases with echogenic bowel. In cases with short femur and humerus the rate of abnormal karyotype was 16%. Conclusions: Ultrasound plays important role in prenatal diagnosis. In cases with positive ultrasound findings, the performance of karyotyping is reasonable.

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## ZORÁN BELICS (2005)

### Correlation between the sonographic measurement of the fetal iliac angle and usual fetal aneuploidies

Supervisor: Dr. Zoltán Papp

The aim of present dissertation was to determine whether iliac wing angle measurement in first and second trimester fetuses is a useful sonographic marker for the detection of trisomy 21, 18 and 13. Fetal iliac angle measurements were performed in 1928 first and second trimester fetuses at the Semmelweis University, I. Department of Obstetrics and Gynecology, between 1999 and 2004. The measurement was taken from a transverse section of the fetal pelvis, which was photo documented during each examination. The iliac angle measurements in fetuses with trisomy 21 (n=50), trisomy 18 (n=16) and trisomy 13 (n=11) were compared with iliac angle measurement in fetuses with normal karyotypes (n=1754). The sensitivity, specificity, positive predictive value, negative predictive value and false positive ration in trisomy 21 fetuses compared for multiple cutoff value. Statistical significance for measurements was estimated for trisomy 21,18 and 13, gestational age, fetal sex, and interobserver reproducibility. In the normal fetuses the mean ( $\pm$  standard deviation [SD]) iliac wing angle was  $63,99^\circ$  ( $\pm$ SD 14,25; with a range from  $24,10^\circ$  to  $108,00^\circ$ ). The mean iliac wing angle in the fetuses with trisomy 21 was  $90,71^\circ$  ( $\pm$ SD 14,50; with a range from  $50,50^\circ$  to  $118,00^\circ$ );  $73,46^\circ$  ( $\pm$ SD 25,11 with a range from  $46,70^\circ$  to  $158,00^\circ$ ) and  $75,64^\circ$  ( $\pm$ SD 16,56 with a range from  $51,00^\circ$  to  $105,50^\circ$ ) in fetuses with trisomy 18 and 13. These values detected in fetuses with trisomy 21 were significantly higher than those seen in normal fetuses. There was no significant difference between the iliac angle measured in fetuses with trisomy 18 and 13 and healthy fetuses. The proven larger iliac wing angle in neonates with Down-syndrome can be demonstrated sonographically during the pregnancy, especially during the second trimester, and may be useful in prenatal screening of trisomy 21. The first time when the measurement is possible is 11th week of gestation. Fetal sex or gestational age did not have a statistically significant influence on iliac angle value. The sonographic measurement of the fetal iliac angle can not be used as a marker for trisomy 18 and 13, fetuses in question, on average, have iliac angles only a few degrees larger than healthy fetuses.

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## ATTILA DEMETER (2005)

### The importance of prognostic factors of common epithelial ovarian tumours based on the therapeutic results

Supervisor: Dr. Zoltán Papp

*Objective.* The outcome and prognosis of apparently similar cases of epithelial ovarian cancers with the same histology and stage is highly variable. The objective was to compare survival and prognostic factors of patients treated at the I. Department of Obstetrics and Gynecology Semmelweis University Faculty of Medicine between 1993-2003 with the similar data of the twenty-fifth Annual Report on the Results of Treatment in Gynecological Cancer of FIGO. In addition the aim was to assess the prognostic value of MMP activities and fibronectin concentration in ovarian tumour patients. *Methods.* The twenty-fifth Annual Report of FIGO included 5694 patients with ovarian tumours from 32 countries diagnosed in 1995-98 and treated in 1996-98. Hungary did not participate in the twenty-fifth Annual Report. Between 1993 and 2003 202 patients with common epithelial ovarian tumours had been treated in our Department. Treatment and survival data were supplemented from medical record review and from the Population Register Office. In order to compare different prognostic factors a multivariate Cox proportional regression analysis was performed. The authors measured MMP activities in 17 surgically removed ovarian tumours, serum and ascites by applying zymographic technique and MMP activities were expressed as integrated arbitrary units (IU) per microgram of proteins. Fibronectin content was identified by immunotechnique blot analysis and quantity estimated in densitometric analysis. *Results.* The 5-year survival was 90% of ovarian tumours with low malignant potential and 30,9% of epithelial ovarian cancers respectively. Multivariate analysis identified adverse prognostic factors including advanced age (>60 years) and stage, high grade and suboptimal operation with residual macroscopic disease and the presence of ascites. However the histological type was not identified to be an adverse prognostic factor in this study. No correlation could be seen between the histology of the ovarian tumours and the elevation of MMP-2/9 activity. More interestingly however ovarian cancer patients who developed recurrent disease, expression of MMP-9 and fibronectin concentration were significantly elevated and the activated forms of both MMP-9 and MMP-2 were more frequent in this group of patients compared with the non-recurrent ones. *Conclusion.* A great deal of effort should be devoted for identification of further prognostic factors to improve treatment of ovarian cancer. These prognostic factors might help to identify those ovarian cancer patients at the time of diagnosis whose disease will have unfavorable outcome. Our data support the notion that high expression of MMP-9 and fibronectin indicates poor prognosis of ovarian cancer patients with similar clinicopathological stage and tumor histology.

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**ERIK HAUZMAN (2005)**

## **Prognostic value of certain endocrine markers in predicting the outcome of in vitro fertilization pregnancies**

*Supervisor: Dr. János Urbancsek*

Pregnancies obtained after in vitro fertilization (IVF) and embryo transfer (ET) are at increased risk for an adverse outcome compared with women who conceive naturally. Multiple gestations also occur more frequently after IVF. Therefore, there is a need for markers that accurately detect the establishment of pregnancy and predict its outcome as early as possible, allowing for modification of monitoring and treatment if required. Ultrasound (US) examination is part of the routine follow-up after IVF, but a gestational sac is not reliably visible until 33–37 days after ovulation induction. As a result of the inability of US to identify very early pregnancy abnormalities, there is an ongoing effort to find endocrine markers that can earlier detect the establishment of pregnancy and forecast its outcome. We aimed to assess the predictive value of the following potential serum markers, measured in the second week after ET: total  $\beta$ -hCG (theoretical post-ET day 11 values, calculated from levels in two samples collected with a difference of two days, based on the mathematical model describing its exponential increase in early pregnancy), inhibin A, CA-125, PAPP-A, and free  $\beta$ -hCG. Based on our results, we conclude that (1) day 11 total  $\beta$ -hCG can be used to compare hCG levels in samples from different sampling days and to predict early pregnancy losses and multiple ongoing pregnancies with high sensitivity and specificity. (2) Inhibin A concentrations are more accurate than day 11 hCG levels for predicting preclinical abortion after IVF but they have no advantage in forecasting ongoing or multiple ongoing pregnancies. (3) Prognostic accuracy of CA-125 measurements for the prediction of pregnancy as well as its outcome is inferior to that achieved with inhibin A. (4) Serum PAPP-A measurements on the second week after ET are not useful in predicting the outcome of IVF treatment. (5) Serum free  $\beta$ -hCG levels on post-ET week 2 cannot be used for predicting IVF outcome because free  $\beta$ -subunit concentrations are below detection limit in the vast majority of samples at this time point. Nevertheless, differences in free  $\beta$ -hCG concentrations among pregnancies with different outcomes seem to correspond to those observed in total  $\beta$ -hCG levels.

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**TAMÁS MARTON (2003)**

## **Post mortem examination of the human fetus**

*Supervisor: Dr. Zoltán Papp*

Firstly the method of fetopathological examination is described and a biometry reference database is published with body measurements and organ weights (280 cases of non-syndromic spontaneous abortion and interruption). The second topic deals with first trimester fetuses. 60 fetal post mortem results were analysed (weight < 70g). Anencephaly (4), craniorachischis (7) spina bifida (5), iniencephaly at 10/40 weeks, body stalk defect (2) and omphalocele (5) were found. 7 chromosome aberrations included 5 trisomy 18, 1 trisomy 13 and 1 Down syndrome caused by imbalanced chromosome translocation. 6 out of 14 spontaneous abortions had a developmental abnormality. Pathological findings in chronic twin to twin transfusion (TTTS) syndrome are described. Six monozygotic twin pairs with severe symptoms of TTTS were examined. Intrauterine hypertension was estimated using the Bernoulli equation, during ultrasound scan, which was confirmed with post mortem examination, myocardial hypertrophy and intrauterine myocardial infarct were described as

a complication of the TTTS and subsequent fetal hypertension. The significance of the MTHFR gene 677C→T mutation in the aetiology of NTD: DNA of 210 healthy controls, 61 pregnant patients with NTD fetuses, and DNA of 41 NTD fetuses were analysed. We did not find any significant difference between the controls and cases in the frequency of the mutation however, the allele frequency was comparable with the figures reported worldwide. In the last part of the paper the use of comparative genomic hybridisation (CGH) in fetopathology is being described first time in Hungary.

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### IMRE PETE (2003)

## Factors predicting the outcome of the cervical cancer in patients underwent radical hysterectomy with lymph node dissection

Supervisor: Dr. Zoltán Papp

The main principals of the treatment of cervical cancer changed during the last twenty years. Instead of the earlier full dose irradiation technique, the surgical approach has been adopted. Considering the metastatic pattern of the cervical cancer, the Wertheim's radical hysterectomy with bilateral lymphnode dissection has become the appropriate approach. Histological data gathered during this management and their connection to the outcome of the patients revealed the need of comprehensive evaluation of the so called histological prognostic factors. The author examined the role of these histological prognostic factors, collected from 308 patients who underwent radical Wertheim's hysterectomy at the National Institute of Cancer, Hungary, Budapest between 1989 and 1997.

Special attention was paid to the following questions: a. How do these factors work? b. Which of them is the most appropriate predictor of the patient's outcome? c. Is it possible to improve the prediction power by adding such parameters as ploidy or heterogeneity of cervical cancer? d. How often can the HPV-infection be detected in cervical cancer, and can the PCR technique be replaced by any other cost-saving method, like gathering direct and indirect histological signs of infection in the paraffin embedded slides? e. How can the prognostic factors be used to evaluate the patients fit for minimal radical surgery?

To answer these questions comprehensive statistical analysis was used, and a new examination technique (DNACE) introduced. The presence of HPV infection was examined in patients who underwent cone biopsy because of in situ cervical cancer. For evaluating the presence of high-risk HPV infection PCR technique was applied. Putting together the data the following conclusions were drawn: a. Introduction of prognostic factors serves the patients benefit. b. The most important predictors of the postoperative treatment depends on are parametrial infiltration, the tumour presence in the surgical margin, vascular/lymphatic space invasion, the grade, the presence of lymphnode metastasis. c. Adding new factors like data coming from DNACE examination does not improve the prognostic prediction. d. PCR evaluation of HPV can't be replaced on cost-saving base by any other technique. e. Prognostic factors can evaluate which patient should undergo minimal radical surgery, but their prediction power needs further improvement.

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**ISTVAN SZABÓ (2003)**

## **Analysis of uterine circulation with color and pulse Doppler ultrasonography in normal and pathologic pregnancies**

*Supervisor: Dr. Zoltán Papp*

The recent advent of transvaginal (TVCD) and transabdominal (TACD) probes with color and pulsed Doppler capabilities has permitted accurate studies of the circulatory changes in the female reproductive organs. It has provided more information about physiologic and pathologic processes during pregnancy than all the non-invasive methods previously developed. We investigated the uterine blood flow of 651 patients in 857 normal or complicated pregnancies between 5 and 40 weeks of gestation. Color Doppler imaging was used to identify the main uterine arteries and their branches for subsequent pulsed Doppler studies. Blood flow velocity waveforms were obtained and impedance indices [resistance index (RI), pulsatility index (PI)], peak systolic velocity (PSV) and time-average mean velocity (TAV) were measured. As a result of trophoblast induction, the branches of the uterine arteries can be detected by TVCD parallel to the implantation and characteristic flow velocity waveforms can be identified on each part of this network. The impedance to flow in the main uterine artery was significantly lower and TAV was significantly higher in patients with normal early pregnancy than in non-gravid patients. The impedance indices in the uterine branches decreased with gestation and there was a progressive fall in these indices from the uterine artery to the spiral arteries. TAV in the uterine artery increased with gestation. Abnormal implantation and tubal trophoblast invasion in ectopic pregnancy can cause more marked blood flow changes in the adjacent supplying vessels than in the main uterine arteries. There was a significant increase in blood flow on the side with the tubal gestation. The blood flow parameters of the uterine and tubal arteries did not change with gestational age in ectopic pregnancies. Differences between sides were higher in the tubal arteries than in the main uterine arteries and showed no dependence upon gestational age. In normal singleton pregnancies, the impedance indices of the uterine arteries decreased whereas blood velocity significantly increased with gestational age between 19 and 40 weeks. Furthermore, impedance to flow was lower and velocity was higher in the placental uterine artery (closest to the main bulk of the placenta), than in the non-placental artery. The changes in the uterine blood flow during normal pregnancy give indirect proof that increased maternal blood supply is needed for normal intrauterine development. Definition of the reference ranges of the parameters used to characterize blood flow velocity waveforms is important for the investigation of complicated pregnancies. Significant change in uterine artery PI was observed in pregnancies complicated by hypertension. PI values were significantly higher in patients with pre-eclampsia, superimposed pre-eclampsia and normotensive pregnancies complicated with intrauterine growth retardation (IUGR) than they were in patients with normal pregnancies. Marked changes of the uterine blood flow velocity waveform were identified in pre-eclampsia. The presence of an early diastolic notch in the uterine artery velocity waveform, especially bilaterally, is a better independent indicator of pre-eclampsia than any other Doppler parameter. Elevated impedance indices and decreased TAV of the uterine arteries in pre-eclampsia and/or IUGR are signs of increased uterine vascular resistance and impaired uterine circulation. A strong correlation can be found between birth-weight and uterine artery impedance indices in gestations with pre-eclampsia. Because of this correlation, the measurement of these indices makes the prediction of IUGR possible.

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ANDRÁS SZÁNTÓ (2003)

## National incidence of malignant gynaecological tumours treatable with cytostatic drugs and results in the diagnosis and treatment of such pathological processes at our department

Supervisor: Dr. Zoltán Papp

To exactly survey malignant gynaecological tumours occurring in Hungary, 3327 case sheets have been evaluated. In addition to re-staging patients with cancers of the cervix and body of the uterus, as well as the ones with ovarian cancer detected in a one-year period the following aspects have been examined in the survey: where and how the disease was detected; relationship between the patients' age and the stage of the disease; methods of treatment; types of surgery; size of residual tumour; and types of cytostatic therapy. It can be concluded that there has been a decrease in the incidence of cervical cancer, but an increase has been observed in the incidence of the malignant tumours of the uterine corpus. In Hungary today, the two most frequent genital tumours, cervical cancer and cancer of the uterine corpus, occur at approximately the same rate, however, in patients with cervical cancer the mean age per stage has shifted to an earlier age, especially in the precancerous stage. The diagnostic values of colour coded ultrasonography (Color Doppler) have been established in recognising the malignant changes of the uterus (cancer of the body of the uterus, uterine sarcoma), preoperative staging of the disease, patients' follow-up and early recognition of relapses. The dissertation has also covered the effectiveness of cytostatic treatments of different combination applied in malignant gynaecological tumours. The retrospective comparison of 146 patients in the first group, suffering from ovarian cancer, has been carried out in view of the cisplatin/cyclophosphamide and carboplatin/taxol protocols in first-line and second-line chemotherapy. The effect of the size of the residual tumour on the effectiveness of postoperative cytostatic chemotherapy has also been studied. Taxan containing combinations and optimally performed operations have yielded significantly better results. The applicability of tumour marker CA 125 in the follow-up of a large sample of patients with ovarian cancer has been surveyed. In the second group, the effectiveness of adjuvant and curative cytostatic therapy (CYVADIC protocol) of 29 patients operated for uterine sarcoma was evaluated by the author, also establishing the effectiveness of this drug combination. Evaluation of the effectiveness of curative cytostatic treatment, applied for the first time (MIC protocol), has been done in a retrospective study of patients with relapsed cervical carcinoma who received combined radiotherapy after radical hysterectomy and regional lymphadenectomy. The study has confirmed remission in 37%, as a result of treatment.

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ERNŐ TÓTH-PÁL (2003)

## Genetic amniocentesis in single and multiple pregnancies

Supervisor: Dr. Zoltán Papp

Classical second trimester genetic amniocentesis is the most frequently used invasive prenatal diagnostic technique today. It is important to know when is the lowest complication rate related to the procedure as well as for how long has to be taken that into consideration. It is widely accepted that these interventions in single pregnancies contribute to fetal loss by 0.5-1%. The relative proportion and absolute number of multiple pregnancies have gradually increased over the past 15 years. As a

direct consequence of that the number of couples and women with single and multiple pregnancies seeking genetic counseling and requesting prenatal diagnosis has been on the rise. It has been a major challenge to our profession to determine the genetic risk, explore the hazards, and define the appropriate type and time of invasive diagnostic procedures in multiple pregnancies. Over the past 21 years the author has collected large amount of data (9071 cases), which is one of the largest experiences in the literature, about genetic amniocentesis performed in single pregnancies. The author has been examining his experiences on genetic amniocentesis with special regards to outcome of pregnancies, the changes of indications the cumulative fetal loss respectively, in relation to maternal age as well as the gestational age. He has also been examining his experiences on 184 genetic amniocenteses performed in multiple pregnancies over the past decade with special regards to hazards of the intervention. He has determined the spontaneous fetal loss in multiple pregnancies and the maternal age related incidence of fetal trisomies.

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## 2/3. PROGRAM

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### PREVENTION OF CHRONIC DISEASES IN CHILDHOOD

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The present research and doctoral program consists various topics of paediatrics, prevention creates the common basis of the program. No reliable method is available to determine the beginning of a chronic disease. In Hungary the causes of the majority of the leading fatal diseases are to be found already in childhood, although without clinical signs. Their progression gradually leads to a permanent manifest disease with expressed clinical symptoms. A fundamental precondition of preventing the development of chronic diseases is to detect the possibly existing risk factors. Getting to know the cellular and subcellular mechanisms promoting the development of a disease may be of help not only in the prevention, but also in the successful therapy and in eliminating the complications, as well.

The doctoral Program is dealing with research fields having outstanding significance in adult cardiovascular morbidity and mortality and where the identification and elimination of risk factors could prevent long-lasting impairments. In the pathogenesis of cardiovascular diseases sodium homeostasis and its cellular regulation are of utmost importance. Within the doctoral Program this question is dealt with in 3 sub-programs. The research work is aimed to study the altered activity, structural changes and genetic regulation of Na/K/ATP-ase enzyme in diseases accompanied by irregular sodium homeostasis. In insulin dependent diabetes mellitus the prevention of late complications: vascular alterations and hypertonia are of cardinal question. Two sub-programs are devoted to study genetic, metabolic and haemodynamic risk factors in animal experiments and clinical physiological examinations.

The sub-program dealing with the correlation between chronic renal diseases, cardiovascular alterations as well as uraemia and bone metabolism focuses on the regulatory role of the kidney as regards vascular alterations and bone structure deformities. The pre-term birth and treatments applied involve several late complications and hazardous situations. The harmful side effects of oxygenization can be due to the multiplication of oxidative radicals. This hypothesis has been considered as a possible pathogenetic factor in several other diseases, too. The study of this theory in pre-term babies may provide explanations similarly valid in other systems, in a wider sense, as well. The investiga-

tion of postnatal body composition, the hydrodynamic changes accompanied by electrolyte movement may reveal several fundamental regularities. This sub-program offers a completely new approach by using multifrequency bioelectric impedance analysis.

The additional four sub-programs seem to be heterogeneous, however, they have one common aspect, namely, all of them are aimed to detect risk factors thereby improving life's quality. The investigation of the connatal urinary tract malformations in neonates and pre-term babies, in addition to the discovery of basic facts, has directly practical significance: to determine the optimal time and technique of surgical intervention. The number of infantile atopic airway diseases shows an increase proportional with the environmental pollution pointing out the importance of getting to know the natural course and pathomechanism of the disease. The questions of paediatric gastroenterology deal with the immunologic correlation existing between food allergens and intestinal diseases. The deeper knowledge of the pathophysiology of childhood epilepsy syndromes and primary headache disorders may result in a better life quality in adulthood.

*Sub-programs*

**Genetics and inflammation in infancy, childhood and adolescence**

*Topics*

- Functional genetics and short- and longterm complications of preterm babies
- Impact of surgical and anaesthetic interventions on immune functions
- Investigation of risk factors influencing morbidity and mortality in neonatal care
- Genetic, haemodynamic and metabolic risk factors in the development of diabetic nephropathy
- Analysis of single nucleotide polymorphisms for the determination of genetic susceptibility to chronic diabetic complications of children with Type 1 diabetes mellitus."
- "Nonalcoholic fatty liver disease in children suffering from obesity, metabolic syndrome or diabetes mellitus
- Investigation of classification, epidemiology and pathophysiology, as well as that of risk factors and pathomechanism of late complications in children with type 2 diabetes mellitus
- Role of circadian, weekly and seasonal rhythm in the development and progression of acute and chronic complications of diabetes mellitus in the light of clinical, epidemiological, biochemical and molecular biological studies
- Role of inflammation in diabetic complications and in obesity related insulin resistance
- Control of body weight: physiologic and molecular perspectives
- Prevention of renal hyperparathyroidism and osteodystrophy in the early stage renal failure
- Investigation of the effect of 1,25(OH)2D3 upon the parathormone
- Investigation of the apoptosis of parathyroid hyperplasia after calcitriol treatment and renal transplantation
- Investigation of histomorphometric alterations in renal osteodystrophy following the normalisation of calcium regulating hormones
- Investigation of the therapeutical effect of the clinically administered calcitriol on the parathormone levels of patients
- Pediatric nephrology

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Cardiovascular effects of uraemia and kidney transplantation in childhood	György REUSZ
Investigation of the pathomechanism, genetic background and therapy of chronic renal allograft nephropathy	Attila SZABÓ
Prevention of neonatal impairments caused by oxygen generated free radicals	Tamás MACHAY
Effects of early human recombinant erythropoietin administration on the iron status and incidence of retinopathy of prematures	Tamás MACHAY, GYÖRGY SALACZ
The incidence of bronchopulmonary dysplasia of prematures with human recombinant erythropoietin treatment	Tamás MACHAY,
Effects of high frequency oscillatory ventilation, compared with positive pressure ventilation on the incidence of bronchopulmonary dysplasia of prematures	Tamás MACHAY,
Impact of long-term hypothermia on drug metabolism in post-asphyxial newborns	Mikós SZABÓ
Investigation of body composition by multifrequency bioelectronic impedance analysis, with special regard to infantile atrophy as well as to acute and chronic disturbances of water and electrolyte homeostasis	Endre SÜLYÖK
Investigation of exchangeable potassium in the acute phase of water and electrolyte disturbances and during reparation by bioelectronic impedance analysis	Endre SÜLYÖK, Sándor KERPEL-FRONIUS GÉZA HARTMANN
Application of bioelectronic impedance analysis in the definition of the role of the different natriuretic and sodium retaining hormones (ANF, digitalis like substance, dopamine, prostaglandins, endothelins) with the parallel determination of body composition, the corresponding hormone levels and that of the renal function	Endre SÜLYÖK, Sándor KERPEL-FRONIUS Tivadar TULASSAY
Investigation of body composition and electrolyte handling in infantile atrophy by means of bioelectronic impedance analysis	Endre SÜLYÖK, Sándor KERPEL-FRONIUS Géza HARTMANN
Complex investigation of endocrine alterations -, TBW, ECW, FFM -, exchangeable potassium and the Na/K ratio by bioelectronic impedance analysis in the different pathophysiological states characterised by fluid and electrolyte disturbances	Endre SÜLYÖK, Sándor KERPEL-FRONIUS György REUSZ
Congenital organic and functional disturbances of urinary flow - clinical and experimental studies	András PINTÉR
Early postnatal investigation of urinary tract dilatation detected prenatally	Tibor VEREBÉLY
Strategy of surgical and non-surgical therapy for organic and functional obstructions of the urinary tract leading to disorders of urinary flow. New surgical techniques for the treatment of urinary incontinence	András PINTÉR
Diagnosis of neurogenic bladder, conservative and surgical therapy	András PINTÉR
Natural history of atopic respiratory diseases in infancy and childhood; certain questions of pathomechanism and diagnostics	Endre CSERHÁTI
Natural history of bronchial asthma on the basis of follow-ups in adulthood and investigations of asthmatic patients' children	Endre CSERHÁTI, Pál MAGYAR
Eosinophil cationic protein (ECP) as a marker of the activity of obstructive respiratory diseases	Endre CSERHÁTI, Erzsébet KERÉKI
Investigation of cytokines and adhesive molecules in bronchial asthma and food allergy	József KELEMEN, Endre CSERHÁTI

Long time investigation of children with allergic rhinitis	Györgyi MEZEI Ernő MIRISZLAI
Clinical and pollenbiological investigations of aeroallergens which are less allergenic or rarely cause diseases	Györgyi MEZEI, Magda JÁRAI-KOMLÓDI
Pediatric gastroenterology	András ARATÓ
The role of innate immunity in the pathogenesis of coeliac disease and IBD	András ARATÓ
Studying of MHC II expression and innate immunity in children with type 1 diabetes mellitus and coeliac disease	András ARATÓ
Role of innate immunity in the manifestaion of Helicobacter pylori infection	András ARATÓ
Optimal planning of examination of children with chronic liver disease before transplantation and the evaluation of their metabolic state	László SZŐNYI
Electrophysiological follow-up of childhood epilepsy and primary headache disorders. Pathophysiological and epidemiological aspects	Imre SZIRMAI, Ottó KOHLHÉB
Follow-up of EEG background activity in rest in idiopathic and cryptogenic childhood epilepsy	Ottó KOHLHÉB, György KARMOS
Follow-up of EEG background activity in rest in childhood primary headache disorders	Ottó KOHLHÉB, György KARMOS
Epidemiology of childhood primary headache disorders	Ottó KOHLHÉB, Krisztina BODA
Transcranial doppler examinations in childhood primary headache disorders	Imre SZIRMAI, Ottó KOHLHÉB
Investigation of cholinesterase polymorphism in childhood idiopathic epilepsy and primary headache disorders	Györgyi BUJDOSÓ, Viktor FARKAS

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## ANDREA FEKETE (2004)

# The role of gender differences and genetic factors in renal ischemia/reperfusion injury

Supervisor: Dr. György Reusz

Renal ischemia/reperfusion (IR) injury is a well-known feature of several clinically relevant diseases. It remains a leading cause of acute renal failure (ARF) and despite recent progress in treatment the associated mortality still remains over 50-70%. The aim of our study was to reveal unknown pathomechanisms and genetic background that could be helpful in determining the prognosis of ARF and in the invention of new therapeutic strategies, through animal experiments and human observations. While gender disparity in the susceptibility to ischemia exists in several organs (heart, brain, intestine), there is less known about sexual differences in postischemic kidneys. In our experiments we showed that renal I/R injury is associated with significantly higher mortality rates and worse renal hemodynamic parameters in male rats than in females.

Due to I/R injury proximal tubular cells lose their polarity, ATP depletion occurs and these phenomena lead to the inhibition of renal Na<sup>+</sup>/K<sup>+</sup> ATPase (NKA). This results in impaired ion homeostasis and intracellular edema, which further aggravate renal tissue oxygenation. We showed that NKA $\alpha$ 1 subunit mRNA and protein expression and enzyme activity do not change in female rats, while in males all these parameters significantly decrease, which could be additional factors of the worse renal recovery seen after postischemic ARF. In males renal ischemia is associated with a time-dependent redistribution of NKA from the basolateral into the apical membrane of the proximal tubular cells, which causes a temporary inactivation of NKA. Heat shock protein 72 (HSP72) through limiting membrane injury helps to preserve physiological function of NKA. We found that females have higher mRNA and protein levels of HSP72, which might help membrane-reintegration and thereby might ameliorate NKA stabilization, while in males due to lower HSP72 levels this phenomenon is not so relevant. Summarizing our animal experiments, we can conclude that following I/R injury female rats have better survival rates, more stable renal function and renal structure representing lower renal susceptibility, than males. This raises the question whether ischemic renal diseases in men and women futurely need to be differently treated. Besides hormonal factors, genetic polymorphisms were also suggested to influence the production of HSP70s. Our results indicate that the risk of ARF is increased in pre-term neonates carrying HSP72 (1267) GG genetic variation independently of the presence of other risk factors. However, HSP72 (1267) GG genotype, which is associated with low HSP72 inducibility, is also more prevalent in pre-term population than in healthy controls, which could raise further questions about the clinical relevance of HSP polymorphisms in hypoxic diseases.

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- Fekete A, Vannay Á, Vér Á, Vásárhelyi B, Müller V, Reusz Gy, Tulassay T, Szabó AJ (2004) Sex differences in alterations of Na<sup>+</sup>/K<sup>+</sup>-ATPase following ischemia/reperfusion injury in the rat kidney. *J Physiol* 555:471-480.
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BALÁZS GYÓRFFY (2005)

## The significance of vitamin-D receptor gene polymorphisms in chronic diseases

Supervisor: Dr. András Szabó

Recent publications suggest, that high dose Vitamin-D treatment can reduce the risk of the development of type I diabetes (T1DM). However, the role of the Vitamin D receptor (VDR) polymorphisms in the pathogenesis of T1DM is not determined. My aim was to determine the prevalence of the five known VDR polymorphisms, and their correlation with clinical parameters (HbA1c, age at T1DM diagnosis, BMI, microalbuminuria), and their prognostical value in T1DM children. Our results suggest, that the common presence of the BsmI, ApaI and Tru9I polymorphisms highly increases the risk of T1DM in girls, but not in boys. The VDR as a mediator of the effects of calcitriol has a central role in the regulation of the PTH synthesis. In dialysed patients controversial data is available about the influence of VDR polymorphisms on disease complications. Our aim was to determine the prevalence, effects and correlation with parathyroid function of the VDR polymorphisms in hemodialysed patients. No prevalence of a single polymorphism was different compared to the reference group. The ApaI polymorphism misses the HW law in patients, which suggests a correlation between carrier state of the "a" allele and risk on renal failure. The serum iPTH was correlated to a combination of the BsmI, ApaI and Tru9I polymorphisms in male patients. My third aim was to calculate linkage disequilibrium between the VDR polymorphism alleles to assess the correlation between the combinations of VDR polymorphism haplotypes and T1DM and complications in renal failure. Our results indicate a linkage disequilibrium between the BsmI, ApaI and Tru9I polymorphisms. In the investigated conditions the combinations of these polymorphisms were related to the increased risk, whereas no single polymorphism alone had a significant effect. This finding demonstrates the biological importance of the linkage disequilibrium. We have extended my experimental investigations with the *in silico* study of the Hardy-Weinberg equilibrium. Population genetic studies investigating polymorphisms with Mendelian inheritance should always test the Hardy-Weinberg (HW) law. According to my experience several publications fail to present the results of the HW calculation. Our aim was to determine, how the missing HW calculation in the control group of scientific publications questions the published results. I have recalculated HW equilibrium for all sub-genotypes in 540 publications from 10 different journals. We have found all together 211 genotypes in 102 publications, where the deviation from the HW-law was not mentioned by the authors. 69 of these errors were in the control group, where the published results do not meet the null-hypothesis. Therefore the conclusion of these papers needs to be validated by investigating new reference groups. The missing HW equilibrium among the investigated patients indicate a relation between genotype and disease. My aim was to determine, how the missing HW calculation in bi-allelic polymorphisms reduces the detected correlations between genotype and disease. Among the above publications We have found 142 genotypes, where the distribution in an association study, or in the patient group do not fulfill the HW criteria. In these polymorphisms the calculation of the HW law can approve or detect a new correlation with the disease.

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**EDIT JUDIT HIDVÉGI (2003)**

## **The prognostic importance of certain laboratory parameters in children with cow's milk allergy**

*Supervisor: Dr. András Arató*

Of 814 children suspected to have food allergy, 254 were found to have true food protein hypersensitivity. From this population, 54 cow's milk allergic (CMA) patients, whose diagnosis was based on case history and the clinical symptoms, were enrolled to this study. Skin problems were found in 78% of the patients, while 46% had gastrointestinal disturbances and 41% exhibited respiratory symptoms. The prevalence of atopic dermatitis and allergic rhinitis among the parents of CMA children was higher than in the average Hungarian adult population. The serum levels of total IgE (11.46 vs 2.68 kU/l), milk specific IgE and ECP (12.2 vs 7.0  $\mu\text{g/l}$ ) were elevated in the patients compared to the controls, mostly in those who presented positive clinical signs after the cow's milk challenge test (total IgE 22.85 vs 2.68 kU/l, milk specific IgE 0.87 vs 0.32 kU/l) or in those who later become asthmatic (ECP 18.1 vs 9.6  $\mu\text{g/l}$ ). The serum ECP concentration was decreased from 12.2  $\mu\text{g/l}$  to 9.2  $\mu\text{g/l}$  after 2 hours of the challenge. On the basis of this result (and according to other publications), it is probable that eosinophil cells migrate to the bowel, where the allergen attacks and the eosinophils secrete their toxic proteins to the lumen of the bowel. The serum level of IgA and IgG antibodies to different cow's milk protein fractions were determined from the blood drawn from CMA patients at the time of diagnosis. The concentration of IgG to BSA was lower (0.36 vs 2.94 rel. %) compared to the control children's data which may explain by an impaired oral tolerance. The higher level of IgG antibody to alphacasein fraction (2.10 vs 0.89 rel.%) could be a prognostic sign for the prolonged course of CMA. The IgA concentration to BSA was elevated in patients with gastrointestinal manifestations (1.76 vs 0.17 rel. %). There was no correlation between the level of milk specific IgE and the IgA and IgG type antibodies to different cow's milk proteins. In a later study the parameters of bone mineralization were determined in 27 CMA patients older than 3 years. The serum level of Ca, AP, PTH, osteocalcin and beta-crosslaps were within the normal range. The bone mineral density of 10/27 children was under  $-1$  Z score value. The serum AP (610.2 vs 499.7 U/l) and PTH (1.56 vs 0.82 pmol/l) levels were higher, the beta-crosslaps concentration (0.92 vs 1.47 ng/ml) was lower in the CMA patients, as compared to the controls; even though 20/27 of the children were not continuing the milk free diet after the milk challenge test. At this time, 9/27 CMA patients (33%) have become asthmatic. On the basis of these results, new prognostic factors of CMA were determined and the slight decrease of bone mineralization was shown.

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**DÓRA KRIKOVSKY (2005)**

## **Investigation of association between single nucleotide polymorphisms and type 1 diabetes**

*Supervisor: Dr. László Madácsy*

Genetic factors play an important role in the pathogenesis of both type 1 diabetes mellitus (T1DM) and its complications. The genes of the inflammatory proteins which contribute to the development of T1DM may contain certain polymorphisms. These polymorphisms might play an essential role in the pathogenesis and progression of T1DM by influencing the quality or the quantity of the protein coded by the

gene. In our study we investigated the association between TNF $\alpha$  G-308A - and G-238A -, IL-1 $\beta$  C3954T -, TNF $\beta$  A252G -, IL-6 C-174G -, IL-18 G-137C - and C-607A -, HSP72 G1267C -, TLR A896G -, vitamin D receptor - and Gbeta3 C825T single nucleotide polymorphisms (SNP) and T1DM and its complications in 326 T1DM children. The TNF $\alpha$  G-308A, IL-1 $\beta$ , TNF $\beta$ , IL-18 C-607A and HSP72 polymorphisms were associated with T1DM. The alleles which had a higher prevalence among the patients were those associated with a higher secretion of proinflammatory cytokines (TNF $\alpha$ , IL-1 $\beta$ , TNF $\beta$  és IL-18) or a lower production of anti-inflammatory protein (HSP72). Thus these alleles can play a role in both the development and the maintenance of an autoimmune process leading to the destruction of  $\beta$ -cells. The IL-6 G-174C polymorphism correlated with the age at the onset of T1DM. T1DM was diagnosed at an older age in those patients who carried the -174G allele associated with a higher IL-6 secretion. TNF $\alpha$  G-308A polymorphism has shown significant correlation with the standard deviation score (SDS) values of the ambulatory blood pressure monitoring (ABPM) results of T1DM children. The presence of the -308A allele, associated with a higher secretion of TNF $\alpha$  corresponded to lower blood pressure values. The associations found in our work can contribute to the detection of the genetic risk for T1DM and also for the genetic risk of hypertension among T1DM patients.

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- Krikovszky D, Vásárhelyi B, Tóth-Heyn P, Körnerl A, Tulassay T, Madácsy L (2002) Association between G-308A polymorphism of the tumor necrosis factor- $\alpha$  gene and 24-hour ambulatory blood pressure values in type 1 diabetic adolescents. *Clin Genet* 62:474-477.
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## ISTVÁN MÁTTYUS (2005)

### The role of endothelin in the development of diabetic nephropathy

Supervisor: Dr. Anna Körner

In healthy children renal endothelin excretion showed a significant correlation with age and body size. If endothelin excretion was corrected to body surface, it was constant. In term newborns the endothelin concentration of urine was high after birth and decreased to the normal level on the 6th day of life. The renal endothelin excretion of preterm infants is elevated, shows a negative correlation with gestational age, and a positive correlation with diuresis. In diabetic children renal endothelin excretion was significantly higher than in healthy children, with no further elevation in diabetic ketoacidosis (= DKA). In type 1 diabetes mellitus due to the stimulatory effect of the elevated blood glucose, the advanced glycosylation end products, angiotensin II, cytokines and prostaglandins enhance the synthesis of endothelin-1 in almost every cell of the nephron already at the beginning of the disease. The elevation of endothelin synthesis can be detected as elevated urinary excretion, and it seems to be constant. The higher amount of endothelin plays an important role in extracellular matrix production, cellular hyperplasia and hyalinosis. Endothelin, antagonising vasopressin by activating cAMP has a diuretic effect and maintains elevated diuresis until the decrease of GFR lowers urinary output. By antagonising of angiotensin II synthesis the progress of these processes can be inhibited. Partly the effect of ACE inhibition works via inhibition of endothelin synthesis. Endothelin receptor antagonists seem to be also effective in inhibition of development of diabetic nephropathy. Our investigations suggest that elevated renal synthesis of endothelin-1 has a role in the development of diabetic nephropathy. The pathogenesis of diabetic nephropathy is not completely cleared yet. Endothelin-1 is a pathogenic factor, getting in the focus during the last years. Our investigations showed that the renal synthesis of endothelin-1 is enhanced in diabetes mellitus type 1, and it plays a role in the pathogenesis of diabetic nephropathy.

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ÉVA NÉMETH (2004)

## Comparative study of neural blockades in paediatric surgery

*Supervisor: Dr. Tamás Machay*

The I. Department of Paediatrics of Semmelweis University provides perioperative care for about 2400 paediatric patients every year. In order to decrease the intraoperative demand for opioid drugs and to ensure appropriate postoperative analgesia following one-day surgical interventions, the general anaesthesia is usually combined with regional anaesthesia or nerve blockades. We also need to control the haemodynamic and metabolic effects of carbon dioxide diffused from the abdominal cavity to the systemic circulation during laparoscopic surgery. In order to minimise risks and provide safe care, the quality of intra- and postoperative analgesia-anaesthesia must be controlled and monitored properly. In our study four types of anaesthesia/analgesic methods were compared in equal number of patients undergoing one-day surgery. The haemodynamic effects of laparoscopic surgery and the carbon dioxide load were measured in 23 paediatric patients. In addition to the routine haemodynamic parameters, the time course of the heart rate variability was followed during the different interventions in order to reveal how these parameters evolve over time and how they are related with the level of anaesthesia achieved. We found that the length of ilioinguinal blockade produced by using 2 mg/kg ropivacain was significantly shorter than the caudal epidural blockade achieved by administering the same dosage of this drug. The duration of the blockade can be extended if treatment is complemented with 2 µg/kg clonidine used either epidural or intravenously. The duration of analgesia caused by clonidine is typically 1-3 hours longer than that of the sedation achieved. The combination of clonidine and ropivacaine results in patients taking the first painkiller much later than they do if no clonidine is given. Another beneficial effect of clonidine is that it decreases the bleeding during surgery due to its mild hypotensive effect. Clonidine causes a 12.8 per cent fall in blood pressure constituting no anaesthesiological risk whatsoever. We didn't find any differences in the number and proportion of postoperative complications following the different anaesthetic methods. The results were not affected whether clonidine was given epidural or intravenously. The parents reported similar data about the frequency of sleeping and eating problems in the four groups on the day following surgery. We concluded that the haemodynamic effects provoked by the use of carbon dioxide don't constitute a significant risk during laparoscopic surgery. In children without cardiovascular problems, the rise in blood pressure remains below the critical threshold. The analysis of heart rate variability hasn't revealed any significant reaction in the functioning of the nervous system. A rise in the sympathetic activity of the nervous system is reflected in elevated serum endorphin levels during insufflation, but the changes don't indicate any significant endogenous stress reaction during the intervention. Our study demonstrated that the administration of sedatives and analgesics decreases the heart rate variability during surgery. This fall is evident in both the low frequency component, (LFV, 0.04 - 0.15 Hz) reflecting vasomotor activity and high frequency component (HFV, 0.15 - 0.40 Hz). The variability components rise substantially when the patient wakes up demonstrating the loss of effect of medications. The measured values are significantly lower in patients receiving both ropivacain and clonidine proving the higher quality of analgesia achieved by this drug combination. The deeper level of anaesthesia is also reflected in the measured serum endorphin concentrations, which were found to be significantly lower in those receiving combined therapy compared with ropivacaine alone analgesia. If proper anaesthesia and analgesia is provided, the value of both HFV and LFV are nearly constant during surgery, and this steady state level seems to be a good predictor of the sleeping time following the intervention. Children having lower variability values during surgery sleep longer in the postoperative period. Data show HFV and LFV to be a sensitive pain marker during surgery when the assessment of the depth (and quality) of anaesthesia would be very difficult otherwise.

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## MIKLÓS SZABÓ (2005)

### Iron metabolism and antioxidant defence in neonates and premature infants

Supervisor: Dr. Barna Vásárhelyi

The aim of our work was to investigate the association between iron metabolism and extracellular antioxidant capacity in human neonates. First, I tested the alteration of antioxidant capacity in blood specimens during the early postnatal adaptation. Simultaneously, I also measured parameters of iron metabolism. Our results indicate that extracellular antioxidant capacity of the neonate is increasing dramatically shortly after birth, indicating that neonates are able to improve their antioxidant defence mechanisms in spite of oxidative stress. Both primary and secondary antioxidant activities increase during the early postnatal period. Similar kinetics of serum iron and ferritin may be an explanation for the postnatal improvement of antioxidant capacity. Our results indicate that alterations of iron metabolism may play an important role in early postnatal adaptation to extrauterine oxidative conditions. In clinical studies I demonstrated that treatment with rhuEPO decreases the transfusion requirements of preterm infants with high morbidity. Treatment with rhuEPO is also protective against abnormal iron deposition in tissues. I observed a decreased tendency for BPD and ROP in preterm infants treated with rhuEPO. In spite of accelerated cellular iron consumption I could not, however, demonstrate any short-term impact of rhuEPO on the parameters of iron metabolism and antioxidant capacity. These results indicate that any effect of rhuEPO on antioxidant capacity may be anticipated after a longer treatment period. Possible impact of rhuEPO on the risk of BPD and ROP should be tested with meta-analysis of the results of large clinical trials.

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## KÁLMÁN TORY (2004)

### The occurrence and the pathomechanism of cardiovascular autonomic dysfunction in childhood renal failure

Supervisor: Dr. György Reusz

Autonomic dysfunction is an independent risk factor of cardiovascular mortality in end-stage renal disease. The aim was to determine the occurrence of autonomic dysfunction in children with chronic renal failure and to examine its pathomechanism. Mild uremic, dialysed, transplanted, hypertensive and healthy children and young adults were examined. Cardiovascular reflexes, heart rate variability and the effect of propranolol on heart rate variability were measured. On the basis of both cardiovas-

cular reflexes and heart rate variability, signs of autonomic dysfunction can be observed in childhood uremia. Its deterioration shows a direct relationship with that of glomerular filtration rate. In transplant patients marked improvement can be observed that supports the reversibility of uremic autonomic dysfunction. There are two mechanisms that seem to be related to the reduction of heart rate variability in chronic renal failure. The first is tachycardia whose degree is proportional to the loss of GFR, the second is anuria in end-stage renal disease. The improvement of  $\alpha$ -adrenergic blockade proved, that the decrease of heart rate variability after heart rate variability proportional to the increase of heart rate is due to increased sympathetic activity and not neuropathy. The role of neuropathy in the reduction of heart rate variability of anuric patients is suggested, however, its role cannot be excluded in patients with normal diuresis either. Since sympathetic overactivity is present in end-stage renal disease and can reduce heart rate variability, diagnosing autonomic neuropathy on the basis of low heart rate variability alone is unreliable. Sympathetic overactivity plays an important role in the reduction of heart rate variability in chronic renal failure. Therefore, prospective studies evaluating the effect of sympatholytics on cardiac mortality in chronic renal failure are proposed to be performed.

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**ANDRÁS TRESZL (2005)**

## The role of cytokine polymorphisms in the development of perinatal complications in children with very low birth weight

*Supervisor: Dr. Barna Vásárhelyi*

*Introduction:* Preterm neonates have an increased predisposition for the development of complications because of immaturity. The most common perinatal complications in neonates with very low birth weight (birth weight < 1500 gram, VLBW) are sepsis, acute renal failure (ARF), necrotizing enterocolitis (NEC) and bronchopulmonary dysplasia (BPD). Lately, it has been shown that functional imbalances of the inflammatory system play a crucial role in the pathogenesis of the diseases above and in the development of the subsequent complications. The cytokine cascade plays a central role in the regulation of the effectivity and intensity of the immune response. Based on the research carried out in the last several years it is now evident that the function of the cytokine cascade is disturbed in children with very low birth weight, partly because of the immaturity of this population, because of the therapy applied, and partly because of the infections. *Aims:* The aim of our study was to investigate the role of functional polymorphisms of the cytokine cascade in the development of perinatal complications in VLBW neonates. *Methods:* DNA was extracted from blood spots taken for metabolic screening. Allele specific primers and restriction endonucleases were used to study the polymorphisms. *Results:* The measured, IL-6 of IL-10 alleles did not differ between ARF $\beta$ , IL-1 $\alpha$  frequencies of TNF- and non-ARF groups and corresponded to those in healthy reference populations. The presence of at least one polymorphic allele at both the TNF- $\alpha$  and IL-6/IL-10 however, was more often found in ARF than in non-ARF babies (26% vs. 6%). The prevalence of the studied genetic variants did not differ between septic and healthy groups. Prevalence of alleles investigated was similar to frequencies measured in healthy populations. The prevalence and number of carriers of IL-4 receptor alpha chain mutant variants were lower in the NEC group. The presence of sepsis, bronchopulmonary dysplasia, and heart failure were identified as independent risk factors for the development of NEC. The adjusted odds ratio for NEC was 0.37 (95% confidence interval: 0.13 to 0.97) in the case of IL-4 -308A allele  $\alpha$ receptor alpha chain mutant variant. The carrier state of the TNF- was associated with a 40-hour longer period of mechanical ventilation and with an additional 36 hours of oxygen supplementation on average.

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**RITA UJHELYI (2004)**

## The bone mineral density, the bone acquisition, and the liver disease in children with cystic fibrosis

*Supervisor: Dr. András Arató*

In my study, I investigated the bone metabolism, the liver disease in patients with cystic fibrosis. I detected decreased bone mineral density in CF patients in good general condition, despite the fact that they had never received systemic steroid therapy. I found no differences in the serum levels of calcium and 25-OH vitamin D and the calcium intake of patients was adequate. The examinations repeated 2 years after the initial examination yielded similar results. Delayed onset of puberty was found in our patients, while boys had lower levels of testosterone. In the subgroup of children I proved decreased bone formation. The parameters of bone metabolism were within the normal range in the subgroup of adolescents. The values of the adult subgroup showed increased bone turnover. The decreased BMD value of the lumbar spine in mothers suggests the effect of the dysfunctional CF gene, as these mothers are necessarily heterozygous. A positive correlation was demonstrated between mothers and their children, this again implying the influence of genetic factors on bone development. Twenty one percent of our patients had liver disease. Two thirds of patients who had had neonatal meconium ileus developed liver disease. In our study population, the development of micro gall bladder and sludge was seen more frequently in patients with CF, while bile stones were seen less frequently than in healthy children. The total serum levels bile acids in CF patients without liver diseases were in the normal range, while CF patients with liver diseases, receiving no treatment, and CF patients with liver diseases receiving ursodeoxycholic acid had higher serum levels of bile acids. The levels of liver enzymes of our CF patients normalised soon after the initiation of ursodeoxycholic acid therapy, despite the fact that the ultrasonographic findings progressed in the majority of cases. The liver synthesis did not deteriorate no liver failure or varix haemorrhage developed. The two main conclusions of the study are as follows: 1. Based on the results of the bone density assays, increased emphasis should be accorded to nutrition and physical exercise, vitamin D-3 supplementation, in addition to forceful antibiotic therapy, in order to improve the general condition of our patients, 2. In order to ensure prompt diagnosis of liver diseases, the serum levels of bile acids should be measured semi-annually – as the serum bile acid elevation may be an early sign of liver impairment. In case of elevated levels of bile acids, close monitoring of liver enzymes and ultrasonographic examinations are recommended, and the timely initiation of ursodeoxycholic acid therapy may prevent or at least delay the progression of liver impairment.

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ÁDÁM VANNAY (2005)

## Synthesis and role of vascular endothelial growth factor in different hypoxic conditions

Supervisor: Dr. András Szabó

Vascular endothelial growth factor (VEGF) is a homodimeric polipeptid which posses different isoforms (VEGF121, VEGF145, VEGF165, VEGF189, VEGF206). VEGF plays a crucial role in the differentiation, proliferation and migration of the endothel cells and therefore in angiogenesis. VEGF also regulates multiple endothelial cell functions, such as synthesis of nitric oxide (NO) and prostacyclin (PGI<sub>2</sub>), proliferation of vascular smooth muscle cell and apoptosis. We investigated the regulation of VEGF synthesis and its role in different ischemic diseases. In our animal experiments we analysed the changes in VEGF synthesis in a rat model of ischemia/reperfusion (I/R) induced acute renal failure. Moreover, we developed a new real time RT-PCR method to measure the mRNA expression of different VEGF izoforms. In our human study we investigated the association of functional promoter polymorphisms of VEGF with the risk of proliferativ retinopathy (ROP) of very low birht weight infants (VLBW). In rats with I/R induced acute renal failure the increased VEGF protein levels but not mRNA expression suggesting that during renal I/R injury VEGF synthesis - distinct from other organs - is primarily regulated at a post-transcriptional level. Histamine, ranitidine and DHEA pretreatment of the rats resulted in distinct changes in VEGF mRNA expression and protein levels in the postischemic kidneys further supporting the importance of posttranscriptional mechanisms in the regulation of VEGF synthesis. Our real time RT-PCR system, irrespectively of each other, is able to detect the different VEGF izoforms.

We observed increased prevalence of VEGF +405C allele and VEGF -460TT/+405CC haplotype in VLBW infants between treated with or without cryotherapy/photocoagulation due to risk of proliferative ROP. In conclusion our data suggest that ischemic kidney has a unique regulation of VEGF synthesis. Our data emphasize the importance of the posttranscriptional regulation of VEGF synthesis in the postischemic rat kidney. Our findings also suggest that testing of VEGF SNPs would provide valuable information for the risk assessment of ROP in VLBW infants.

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GÁBOR VERES (2004)

## Expression of cytokines, adhesion molecules and proliferation markers in food allergy and in celiac disease

Supervisor: Dr. András Arató

The pathomechanism of food-protein induced enteropathy and of celiac disease is not well known. My work was based on the analysis of cytokines, adhesion molecules and proliferation markers in the small intestinal mucosa of patients with food allergy and celiac disease by means of immunohistochemistry and in situ hybridisation. Increased expression of IFN- $\gamma$  found in the lamina propria of children with food allergy indicating its role in immune activation. Despite the normal structure, higher rate of cryptal proliferation, increased density of T cells and HLA-DR staining were observed. It is of interest that lymphonodular hyperplasia was a characteristic finding in the duodenal bulb of children with food allergy which suggesting, with the increment of intraepithelial  $\gamma/\delta$  T cells, the loss of oral tolerance. Like in children, signs for immune activation (higher crypt prolifera-

tion rate, increased density of HLA-DR and CD4 positive T cells) in the normal duodenal mucosa of adult patients homing with food allergy were shown. Increased expression of ICAM-1 and  $\alpha 4\beta 7$  receptor positive cells suggest their role in the pathomechanism of food and IL-4 containing cells in the duodenal lamina propria. Higher numbers of IFN- $\gamma$  positive cells in the duodenal lamina propria of children with freshly diagnosed celiac disease using in situ hybridisation were detected. The prevalence of celiac disease among children with type 1 diabetes mellitus was 11.7%. In antiendomysium positive children, without symptoms and signs, and with normal villus structure, higher numbers of  $\gamma/\delta$  T cells were found indicating latent celiac disease. Pattern of cytokines, adhesion molecules and other markers could help to understand the pathomechanism of immune mediated disorders like food allergy, and could attract diagnostic and therapeutic consequences in the near future.

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## 2/4. PROGRAM

### GASTROENTEROLOGY

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The importance of gastroenterological diseases is increasing, and because of their frequency and complications, they are considered as one of the most menacing group of diseases. This fact is confirmed by the out-patient and in-patient numeric data, as well as morbidity and mortality indices. Gastroenterology has developed such a discipline that combines the knowledge of different specialties. It includes the pathophysiological data resulted from basic research, the results of clinical research and observations. The research of the gastrointestinal diseases can be done by different approaches and different methodological possibilities. This interdisciplinary topic offers unprecedented opportunities for scientific research. The achievements of last one and half decade resulted relevant changes in several aspects of gastroenterology, and the conventional understanding of development, progression and treatment of diseases had been changed. Despite of the undoubted results several questions need to be answered and new questions are appearing. Gastroenterological diseases can be the topic of wide-spread researches that fit to the scientific basis of public health priorities.

#### Sub-programs

Experimental pharmacological, immunopharmacological and clinical pharmacological research of the gastrointestinal mucosal protection  
Novel factors in the development of gastrointestinal mucosal lesions  
Clinical aspects of bowel diseases and their connection to the endocrine- and immunoregulation  
Pediatric gastroenterology: immunohistological, metabolic- and clinical researches

#### Supervisors

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Research of the pathogenesis, complications and therapeutic possibilities of chronic hepatic diseases	Ferenc SZALAY
Pathology and therapy of chronic pancreatic diseases (chronic pancreatitis and pancreatic cancer), regarding programmed cell death	Béla SZENDE
Gastroenterological surgery	Lajos FLAUTNER
Pathophysiology of the gastrointestinal tract	László ROSIVALL
The role of altered immunoregulation in chronic inflammatory diseases of gastrointestinal tract	Györgyi MÚZES, Zsolt TULASSAY

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András Taller	pt	László Harsányi
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a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

## LÁSZLÓ BENE (2004)

### New approaches for diagnosis and pathogenesis of inflammatory bowel disease

*Supervisor: Dr. Zsolt Tulassay*

In these clinical studies we compared and statically analyzed the clinical conditions and complete serum test of Inflammatory Bowel Disease's (IBD) patients visited our outpatient clinic randomly. Partially the results provided additional data to the comprehensive understanding of the pathomechanism of the IBD, and our findings could be useful in everyday practice. During the heat shock proteins examination we recorded significantly lower HSP65 antibody titer in the patients with inflammatory bowel disease compared to healthy controls. The antibody titer values in two classical subgroups (Crohn's Disease - CD, Ulcerative Colitis - UC) of patients showed insignificant difference. Comparing these results with the clinical features we found that in CD the antibody titer changes correlated to the clinical activity and the length of the disorder, while in UC did not. The significance of heat shock proteins comes from the fact that these proteins show a great phylogenetic homology. Namely the antigen structure of bacterial HSP65 and the human bowel epithel cell HSP60 are very similar due to the fact they belongs to the same heat shock protein group. In certain conditions the HSP65 immunodominant protein of many bowel bacteria may induce autoimmune process. The differences experienced between the patients' groups may confirm the previous theory that the role of bowel bacteria is not the same in CD and in UC. Nevertheless the induction of the disease may be rather infectious in CD and more autoimmune origin in UC. The results gained during the study of the complement proteins also underline this theory. We measured significantly higher values of C3 and C1-INH in CD than in UC. The higher C1-INH value means increased risk for CD Odds Ratio (OR): 1,95. In the scope of the earlier reported results the measured complement value differences could suggest that in CD the complement activation is bacterial origin and occurs via an alternative pathway. Hence in UC the autoantibody against bowel epithel could trigger the activation cascade via classical pathway. Results of the complement studies suggest that the C1-INH is a good indicator of the disease activity and its efficiency almost similar to C reactive protein (CRP) used in everyday clinical practice. Additionally C1-INH acute phase protein serum test is inexpensive and a reproducible with high accuracy. The laboratory methods measuring CRP and C3 together resulted in similar predictive value differentiating the CD and UC as the presently recommended ANCA/ASCA method in everyday practice. Undoubtedly, among of new therapeutic approaches the anti TNF2 treatment gave the most favorable results. The results with TNF2 allele were interesting due to its role in the production TNF2. In this study we found that the TNF2 allele occurred significantly more rarely in-patients with IBD than in the control group. These results may have significant roles in the IBD pathomechanism, namely the relationship of Th1/TH2 balance and the bowel bacteria, nevertheless the correlation of the results of this finding fascinating to in the scope of the anti-TNF treatment of the diseases. Moreover, according to previous experiences the anti-TNF treatment is only effective in CD, despite there wasn't significant statistic difference in the occurrence of TNF2 allele between the two patients' groups. The earlier studies related to the role of histamine reported that the number of mastocytes in secretion of the histamine are significantly raised in inflamed bowel. These observations initiated new therapeutic approaches such as the application of the mastocyte stabiliser ketotifen in active inflammation. In the animal experiment the effect of histamine deficiency in the bowel inflammation was examined which was induced by oral administration of dextran sulfate. Significantly depressed IL-10 expression was fund in the bowels of the histamine deficient mice us-

ing immunohistochemical method. This result coincided with the previous findings in other organs in histamine knock - out mice. Despite the anticipation in the knock - out mice model a not significant and clearly milder inflammation was found. Hence the bacterial bowel flora of the wild and the histamine deficient mice model were significantly different. According to many previous published data and the results of this study also underlined that in IBD the genetic background, inflammatory cytokines and bowel bacteria are significantly correlate in the progress of the disease. Nevertheless no publication was found in the reviewed literature could justify the decrease of IL-10 may have an effect on the composition of bowel bacterial flora. In the experiments was previously summarized in this study first time resulted in a conclusion; the histamine does not only play a role as a biogenous amin and inflammatory cytokine, but it has also an effect on the IL-10 metabolism. Further examinations have to be performed to interpret the results. Both the clinical and experimental results support that bacteria and bacterial antigens have the significant role in instigation and in the clinical course of IBD and draw attention to factors, which are less examined, such as the heat shock proteins and histamine.

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## ATTILA BURSICS (2004)

### Functionality before and after coloproctologic operations

Supervisor: Dr. Lajos Flautner

In my dissertation I present my work done on the field of functional changes observed in certain coloproctological diseases and also on the functional effects of treatment on the anorectal function. I describe the functional examination methods and also the methods for analysis of the anatomical basis of functionality. I touch six main topics. First I describe the functional changes occurring during the development and after the treatment of haemorrhoids in a prospective study. I observe that these changes are the consequence rather than the cause of haemorrhoidal disease. In addition I introduce a new operation (DGHAL) for haemorrhoidal disease. I prove that the main advantage of DGHAL procedure compared to conventional scissors haemorrhoidectomy is the minimalisation of the operative trauma to the anal canal. This reduces the postoperative pain, shortens the hospital stay and also the time to be on sick leave. I compare the early and late results of DGHAL with closed scissors haemorrhoidectomy in a prospective study. Thereafter I describe and assess our operative tactics for rectal prolaps and for the removal of villous tumors of the distal rectum. I performed a retrospective study on our results of restorative proctocolectomy on FAP and UC patients. I prove that the operation is safe, and favorable early and late functional results can be achieved. At last I describe our results in surgery for fecal incontinence. I demonstrate that reconstruction of the external anal sphincter can be done with excellent results on patients suffering from direct sphincter trauma. Anterior levatoroplasty for pudendal nerve neuropathy in perineal descent can also be done with reasonable results on selected patients.

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JUDIT GERVAIN (2003)

## The serological and molecular biological diagnostics of chronic viral Hepatitis B, C and C viruses

*Supervisor: Dr. Zsolt Tulassay*

Screening of blood donors for hepatitis C virus infection, further investigation and interferon (IFN) treatment of those infected and of patients with B and Delta viral hepatitis started in 1992 in Hungary. In our Virus Serology Laboratory, we have been carrying out the specific, sensitive and standardised serological and molecular biological tests which are necessary to the differentiation and adequate therapy of the different diseases appearing in the clinical picture of chronic viral hepatitis and are available for each Hepatology Centres since then. Over and above these routine methods, we pioneered in analysing the correlation between the changes in patients' anti-HCV IgM and HCV-RNA levels and the success of IFN therapy in 1994. We found that persistent presence of IgM antibodies is a predictive factor for negative treatment outcome. We demonstrated that basal viral titre is a major determining component of therapy outcome. We have been working on the analysis of the type distribution of HCV since 1995 and of its subtype distribution since 1999. Based on a representative cohort of more than 600 patients, we filled in the gap in the national and international literature by determining that 91.5% of the Hungarian population with chronic C hepatitis is infected by HCV-1b, the most resistant and the least recovery-prone subtype. These results confirm the high dosage, long-term antiviral treatment of Hungarian patients. Two years ago we introduced the IVth generation of anti-HCV tests for the examination of diagnostically problematic patients with suspected HCV infection. The test has 99.8% specificity and it is sensitive for the different subtypes of HCV. During the last two years, we analysed the genetic structure of the NS5A domain of HCV-1b isolated from the samples of Hungarian patients. We found that the 'Hungarian quasi species' of HCV-1b differs from HCV-J, the internationally accepted prototype. We also investigated the relationship between the mutations in the RNA protein kinasebinding region (PKR-BR) of the virus and the treatment outcome. We confirmed a positive correlation between the mutations within the Interferon-Sensitivity Determining Region of PKR-BR (aa 2209-2274) and the sensitivity to IFN therapy. Based on international results, lamivudine treatment of patients with chronic hepatitis B has been successfully applied during the past two years in Hungary. We worked out the method of detecting therapy-resistant mutants of the B and C domains within the HBpol gene by combining polymerase chain reaction and reverse hybridisation. This way, modification of the therapy became possible in the case of 'clinical breakthrough' generated by these mutants. In my Ph.D. Thesis, I summarised my work in the field of the serological and molecular biological diagnosis of chronic viral hepatitis and my researches which greatly contributed with new Hungarian results to the epidemiology and therapy of viral hepatitis B and C. The aim of my investigations was to determine the characteristics of the hepatitis viruses in Hungary and to examine the different markers with treatment outcome predictive values.

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**DALMA HEGEDŰS (2004)**

## Extrahepatic manifestations of Wilson disease

Supervisor: Dr. Ferenc Szalay

In summary, we found that osteoporosis is very common in young WD patients, most likely secondary to increased osteoclast activity. We also found early dysfunction of peripheral sensory neurons compared to the control group, and decreased heart rate variability only in patients with neurological symptoms, who therefore have increased risk for cardiovascular morbidity. The temperament and character profile deviated from healthy individuals on many scales, and psychiatric symptoms were very common in Wilson patients. We also found a 10% prevalence of attempted suicide among the patients. Nociceptin level was also significantly elevated. In this study we pinpoint various unsolved questions about the disease, to which we attempted to provide some answers. However, the mysteries about this disease has not all been solved, rather, further questions are raised. We strongly believe that there are many more interesting and exciting aspects and mechanisms related to the disease, waiting to be explored.

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**MÁRK JUHÁSZ (2004)**

## Evaluation of oncogenes and tumour suppressor genes in gastric and pancreatic cancer

Supervisor: Dr. Zsolt Tulassay

Gastric and pancreatic cancers are still a major cause of cancer-related deaths. Both cancers are frequently diagnosed in advanced stages resulting in a poor prognosis. Consequently, there is an immense need for markers that provide early diagnosis, reliable prognosis, and might serve as potential therapeutical targets. We focused on these properties as we selected caveolin-1, serum soluble E-cadherin, and carbonic anhydrase IX to be the subject of our research projects. The expression of caveolin-1 is downregulated in gastric cancer as compared to the normal gastric mucosa and more frequent in well-differentiated tumours than in poorly-differentiated and metastatic gastric cancers. Caveolin-1 is likely to play a tumour suppressor role in gastric cancer. Serum levels of soluble E-cadherin are significantly higher in gastric cancer patients as compared to control subjects, and in intestinal-type gastric cancer as compared to diffuse-type gastric cancer, respectively. Tumour progression exhibits a positive correlation with the serum levels of intestinal-type gastric cancer and a negative correlation with those of diffuse-type gastric cancer. Soluble E-cadherin concentrations should therefore be interpreted along with Laurén classification and thus might serve as a prognostic marker in intestinal-type gastric cancer. The expression of carbonic anhydrase IX is present in only a subset of pancreatic cancers and independent of clinicopathological parameters, p53 expression and microvessel density. Incubation with acetazolamide led to a significant inhibition of cell proliferation in pancreatic cancer cell lines suggesting that application of carbonic anhydrase inhibitors might be a potential future therapeutical approach in pancreatic cancers expressing carbonic anhydrase IX.

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## LÁSZLÓ LAKATOS (2004)

# Epidemiological and genetical investigations in inflammatory bowel disease

Supervisor: Dr. Zsolt Tulassay

We report our experience during a 25-year follow-up of patients with inflammatory bowel diseases (IBD) in Veszprem County. Examinations in chronic diseases require a long-term follow-up of clinical data in a large number of patients. Our aim was to determine the epidemiology and phenotype of the disease and to compare the results with data from the literature. We investigated the presence or absence of extraintestinal manifestations and their association with the location and behaviour of the disease. Further we determined the frequency of common NOD2/CARD15 mutations in Hungarian Crohn's patients in a multicenter study and investigated phenotype-genotype associations.

We observed a 6-fold elevation in the incidence of ulcerative colitis (UC: 1.66-11.01) during 25-year follow-up, that of Crohn's disease (CD: 0.41-4.68) rose more than eleven-fold and a 3-fold elevation was observed in the prevalence of both diseases between 1991 and 2001. Incidence and prevalence were in the range previously reported in high incidence Western countries. Male to female ratio was around equal; we observed one peak onset in UC in the 31-40-year olds, in CD in the 21-30-year olds. The UC/CD ratio of incidence decreased from 4:1 to 2:1. Urban residency moderately increased the risk of IBD. Smoking decreased the risk for UC by 75%, while it almost doubled the risk for CD. In contrast to Western European countries location of UC did not change in the last 25 years, proctitis became slightly more prevalent, while the percentage of pancolitis decreased. In CD colon was affected in two-third of the patients and there seemed to be a shift from ileal to colonic location during the observed period. Complication (stricturing, penetrating) developed in 70% of the cases.

Extraintestinal manifestations (EIMs) were found in 20 % of the patients in concordance with previous data from Western Countries (UC: 15.0%, CD: 36.6%). EIMs were more prevalent in women, age at presentation did not affect the likelihood of EIM. In UC there was an increased tendency of EIM in patients having a more extensive disease. In CD location did not affect the prevalence of EIMs, however they were more prevalent in patients with stricturing and penetrating disease. Non-alcoholic steatohepatitis (NASH) was found in 10% of UC and 20% of CD patients. Primary sclerosing cholangitis was found in 3 % of the patients.

The prevalence of familial IBD, especially in CD, was common in this IBD population, than in the normal population. Familial disease developed at a younger age, particularly in affected parent-child families, and was associated with a more severe course of the disease and with a higher number of complications. The rate of the three common mutations of NOD2/CARD15 was in the range reported in European white populations (29.6% vs. 11.3%, OR=3.3, p=0.0007). SNP8 (R702W, 11.6% vs. 4.3%, OR= 5.9, p=0.0026) and SNP13 (3020insC, 9.1% vs. 2.6%, OR=7.6, p=0.004) mutations were significantly more common in IBD compared to controls, while G908R mutation was uncommon in Hungarian Crohn's patients (4.9% vs. 4.3%). The presence of the mutation was associated with ileal but not with fibrostenosing disease and extraintestinal manifestations were less common in carriers of the mutation.

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## LÁSZLÓ ROMICS (2005)

### The role of Toll-like receptors (TLRs) and peroxisome proliferator activated receptors (PPARs) in endotoxin caused liver injury

Supervisor: Dr. Gyöngyi Szabó

Endotoxin injury in the liver has a central role in the pathophysiological mechanisms of various liver diseases. Increased LPS sensitivity has been described in alcoholic-, non-alcoholic steatohepatitis, as well as in fulminant hepatitis, however their molecular background is not known yet. LPS hypersensitivity results in increased production of proinflammatory cytokines, causes extensive necrosis in the liver and leads to higher mortality in experimental animals. A balance between pro- and anti-inflammatory activation is fundamental in steatohepatitis. Mortality of septic patients significantly increases with fulminant hepatitis. TNF $\alpha$  plays a central role in these pathomechanisms. TLR4 and its co-receptor, MD-2 has a crucial role in LPS recognition. The peroxisome proliferator activated receptors (PPAR) and the S-adenosylmethionine (SAME) are major components in the anti-inflammatory machinery of the liver. Our data indicate that increased LPS sensitivity in steatohepatitis and in fulminant hepatitis is reflected by the upregulation of MD-2, but not that of TLR4. PPAR activation is decreased during the progression of steatosis to steatohepatitis. Further, TLR2 activation does not result in the development of fulminant hepatitis. In vitro, SAME treatment augmented the anti-inflammatory interleukin 10 production. Moreover, stimulation with acute alcohol inhibited not only the LPS caused, but the interleukin 1 and tumor necrosis factor alpha induced nuclear factor  $\kappa$ B activation, as well. In conclusion, upregulation of MD-2 and inhibition of the intracellular PPAR and SAME activity have a fundamental role in the pathogenesis of LPS caused liver injury. Therefore, these molecules might represent a future target in the pharmacotherapy of steatohepatitis and fulminant hepatitis.

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## ÁGNES RUZSOVICS (2005)

### Recent possibilities of detection of helicobacter pylori infection from biopsy tissue samples in chronic gastritis diseases

Supervisor: Dr. Zsolt Tulassay

The polymerase chain reaction (PCR) is of great importance in the diagnosis of *H. pylori* infection, because attention has been recently adverted to the knowledge of *H. pylori* genotypes by the fact, that its association with role in outcome of cell damage and change of cell kinetic parameters of mucosa. Comparison of genotypes with histology, endoscopy, and non DNA-based techniques (immunohistochemistry, culture, urea breath test, immunoblot) and quantitative evaluation of *H. pylori* have not been performed yet, neither in diagnostic work nor in scientific research. The aim of this work

was the development of in situ qualitative-quantitative detection method of *H. pylori* by traditional and real-time PCR assay. Moreover, was observed change of cell kinetic parameters of gastric mucosa (proliferation, expression of epidermal growth factor receptor (EGFR) and intestinal mucin antigens) influenced by the presence of *H. pylori* or its different genotypes in adult and child samples. During our clinical research we prepared data of 232 patients according to different respect. We compared results of adult patients with 40 children's data used same examination. We found a significant difference in *H. pylori* density on more areas of the same stomach by quantitative FRET-PCR assay based on the determination of ureA gene of *H. pylori*, which may open a new perspective for appraisal of today *H. pylori*-associated diseases. Our observation suggests that the real-time PCR assay is the more sensitive method than traditional techniques compared with results of our DNA-based diagnostic technique and biopsy-based methods. Our data suggest that increased cell proliferation is not a *H. pylori*-associated response of the host to cell damage. EGFR is a better immunohistochemical marker than proliferation activity for the detection of altered gastric epithelial cell function. We suppose that p53 and small intestinal mucin production of epithelium are early event in the multistep process of gastric metaplastic transformation. Detection of monoclonal antibodies expression is a valuable marker for epithelial cell differentiation represents a novel approach to the definition of malignancy risk.

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LÁSZLÓ SCHANDL (2003)

## Expression of CDC25A, CDC25B and C-MYC in gastric cancer and in chronic gastritis before and after *Helicobacter pylori* eradication therapy

Supervisor: Dr. László Prónai

Despite a dramatic reduction in incidence and mortality rates, gastric cancer is the second most frequent cause of cancer-related death world-wide. According to the Lauren's classification, gastric carcinoma can be divided in two distinct types: the intestinal type and the diffuse type that can be separated by characteristic histological features. Gastric cancer is the end result of long-term process accompanied by a sequence of molecular changes that include increased cell proliferation and alteration in apoptosis. Cell proliferation is essential in normal cell life and turnover. Tight regulation of cell cycle progression is essential for the maintenance of genomic integrity. Orderly progression of the cell cycle is regulated by timed activation and deactivation of cyclin dependent kinases (CDKs), and by proteolysis of cyclins. CDC25 phosphatases play key roles in the control of cell cycle progression by activating cyclin-dependent kinases. CDC25 is a multigene family, comprising CDC25A, CDC25B and CDC25C. CDC25A is required to entry into S phase, while CDC25B seems to be the mitotic starter phosphatase. Alterations in the function of CDC25A and CDC25B phosphatases by overexpression might promote oncogenic transformation and it has been suggested that CDC25A and CDC25B (but not CDC25C) are potential oncogenes. Raf1, a kinase that is activated by RAS, phosphorylates and activates CDC25 in vivo, which suggest a possible synergism between RAS and CDC25 phosphatases. Several studies demonstrated the overexpression of CDC25A and CDC25B in various primary human tumors supporting a key role of these phosphatases in tumorigenesis. Due to the close association of CDC25B immunostaining with advanced stage, deep invasion and nodal metastasis, CDC25B has been suggested to predict malignant behavior of gastric carcinoma. The transcription factor and proto-oncogene c-Myc and oncogenic RAS cooperate in causing malignant transformation. The proto-oncogene c-Myc has been shown to play key role in growth control, differentiation and apoptosis. Furthermore it has been proposed that CDC25B can be induced by Myc, although

to a lesser extent than CDC25A. Overexpression of CDC25A, CDC25B and c-Myc was detected in various primary human cancers. However there are conflicting data regarding the overexpression of c-Myc in gastric cancer and the association between overexpression of c-Myc and that of CDC25A and CDC25B in human cancers. *Helicobacter pylori* infection is a crucial factor for development of gastric cancer. The pathogenesis of *H. pylori*-related gastric cancer consist of a multistep process, initiated by *H. pylori*-induced chronic gastritis and followed by the development of gastric epithelial atrophy, intestinal metaplasia, dysplasia and eventually, carcinoma. To date little is known about CDC25 and c-Myc expression in early steps of gastric carcinogenesis. Using immunohistochemistry we evaluated the expression of c-Myc as well as that of CDC25A and CDC25B phosphatases in gastric cancer. We investigated the potential interaction of CDC25A and CDC25B with c-Myc and evaluated their relation to histotype and grade of tumors. We evaluated the expression of CDC25A, CDC25B and that of c-Myc also in chronic gastritis with and without *H. pylori* infection. Furthermore we investigated the potential correlation of expression of the CDC25 phosphatases and c-Myc with presence of *Helicobacter pylori*. Finally we investigated the expression of CDC25A, CDC25B and c-Myc one year after eradication therapy.

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**ANDRÁS TALLER (2003)**

## Technique and importance of percutaneous endoscopic gastrostomy in cases of cancer in the head and neck region

*Supervisor: Dr. László Harsányi*

Cancer in the head and neck region causes malnutrition. Undernourishment of these patients is multifactorial; dysphagia, bad life style, alcohol and nicotine consumption play a role. Furthermore complex "tumour therapy" (radio- and/or chemotherapy or surgery) destroys nutritional state. Therefore artificial nutrition is necessary. The enteral route should be preferred in cases with normal gut function. If dysphagia is present nutrients must be delivered via tubes. Head and neck cancer (HNC) patients might need short or long term artificial nutrition. Mainly the percutaneous radiologically inserted gastrostomy tubes are preferred for them. In the perioperative period nasogastric tubes are used. Since 1980, percutaneous endoscopic gastrostomy (PEG) is accepted worldwide. Published experiences are based on mainly non-homogenous groups of several hundred patients who got almost only long term nutritional support. In Hungary PEG is underused. On HNC patients PEG is unsuccessful in 7-10% of the cases. Oesophagogastro-duodenoscopy (OGD) might be impossible because of stenosis/occlusion of the oesophageal inlet or partial trismus. The first of my aims was to analyse and solve the endoscopical technical problems concerning PEG placement on HNC patients. A further aim was to analyse experiences of PEG feeding on these homogenous and distinguished group of patients. Questions examined: 1. Are there endoscopical routes with which PEG placement becomes possible also on HNC patients? 2. Does these alternative routes result a success rate similar to the non-endoscopical procedures? 3. Is PEG feeding advisable for HNC patients also in the perioperative period? 4. Possible routes for removing a PEG.

With experiences on 446 PEG fed HNC patients we tried to give new data and suggestions. With this work we would like to contribute to a general and ethically justified use of that advantageous endoscopic therapy. Further aims were to analyse mucosal lesions and risk factors present at the time of PEG placement. We examined the effect of glutamine and arginine supplemented immunonutrition, too. 1. We examined the prevalence of *Helicobacter pylori* in that high risk group of patients. 2. We examined the role of radio- and/or chemotherapy. 3. We examined the factors which may affect the integrity of small bowel mucosa. 4. We examined the applicability of enteral immunonutrition. We published at first on alternate endoscopic routes that allows PEG placement in almost 100 per cent of

HNC patients as well. It has been established that PEG is advantageous to nasogastric tube also in the perioperative period. We ascertained that beside *Helicobacter pylori* radiochemotherapy plays a role in the development of mucosal lesions of the upper gastrointestinal tract. We have verified that mucosal lesions disappear after PEG feeding. Finally, it has been established that effective enteral immunonutrition is possible only with at least daily five or six time bolus feeding.

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ZSUZSA UNGER (2004)

## **Helicobacter pylori induced cell kinetics changes in premalignant lesions of the stomach**

*Supervisor: Dr. László Prónai*

*Background:* Based on its possible effect in gastric carcinogenesis, *H. pylori* was clarified as I. class carcinogen in 1994. The exact pathomechanism leading to cancer, however is still not cleared and the role in upsetting the balance of gastric epithelium is not well documented. Results are controversial about the effect of the bacterium on EGFR expression. It is not known how *H. pylori* influences the p53 mutation. Although the eradication of *H. pylori* reverses most of the molecular changes, there is still no consensus which patients need *H. pylori* eradication. *Aims:* 1) To investigate the role of *H. pylori* infection on gastric epithelial cell proliferation and apoptosis. 2) To evaluate the influence of *H. pylori* on EGFR and p53 expression. 3) To evaluate the effect of *H. pylori* eradication therapy on these parameters. The study also summarizes the current literature. *Patients and methods:* Antral biopsies were taken from 121 patients who underwent routine upper endoscopy (60 men, 61 women, mean age 58,5 years). Biopsies were fixed in formalin and embedded in paraffin. Patients were classified into four histological groups: (1) as normal epithelium, (2) gastritis, without intestinal metaplasia, (3) gastritis, with intestinal metaplasia and (4) carcinoma. Based on presence/absence of *H. pylori* the gastritis cases were classified into subgroups. Cell proliferation was measured by PCNA and AgNOR techniques, apoptosis was measured by TUNEL reaction and to evaluate the EGFR and p53 expression immunohistochemical method was used. *Results:* *H. pylori* did not cause significant change in cell proliferation. In gastritis cases - in the absence of intestinal metaplasia - *H. pylori* significantly increased apoptosis (0,013+0,001 vs. 0,029+0,012). Expression of EGFR was significantly lower in cases when intestinal metaplasia was present (48,30+23,70 vs. 32,80+30,40). In the group of gastritis with intestinal metaplasia the p53 index increased both in *H. pylori* negative (18,33+19,65 vs. 68,50+28,96) and in *H. pylori* positive (35,55+31,16 vs. 70,16+22,54) cases. *H. pylori* eradication reversed these parameters close to the normal. *Conclusions:* The results suggest that the hyperproliferation in *H. pylori* infection is not caused by the bacterium itself, but it is rather a non-specific reaction to inflammation. *H. pylori* can increase the apoptosis only in the absence of intestinal metaplasia. EGFR expression during *H. pylori* infection is decreased only if intestinal metaplasia is present. Mutation of p53 is an early event in gastric carcinogenesis. *H. pylori* eradication normalises altered cell kinetics, thus it is suggested in all cases with intestinal metaplasia.

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The aim of this Ph.D. Program is to select open minded, self-supporting applicants who are able to acquire the knowledge of complex understanding of theoretical and clinical state of the art in the field, and are able to use this knowledge in education and research. Therefore, the Program prepares for the two different but not unconnected directions: it focuses on the better understanding of physiological and pathological processes in the oral cavity, as well as on the development and application of new therapeutic methods in all branches of dentistry.

*Sub-programs*

Alterations of the different tissues and kinetic changes in developmental and musculoskeletal disorders

Clinico-pathological studies of diseases of the oral cavity

Physiology and pathology of blood flow regulation in the oral structures: circulatory disorders and measures for the therapy

Experimental oral biology

Application of autologous tissues and biocompatible materials in restoration of maxillofacial and oral structures

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a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

**BATBAYAR BAYARCHIMEG (2005)**

## Changes of the different neuropeptide containing nerve elements in the Sjögren's syndrome and diabetes mellitus

*Supervisor: Dr. Tivadar Zelles*

It has been suggested by many authors that changes in the neuropeptide containing nerve elements might have role in the pathogenesis of autoimmune and inflammatory diseases. Therefore, the main aim of our examination was to obtain morphological data regarding that these elements might have a role in the pathogenesis of oral inflammatory diseases. Our results: The number and localization of neuropeptide containing nerve fibres were similar in both normal labial and lingual minor salivary glands. We have found VIP, SP, NPY and GAL IR neurons in the root of the rat's tongue. Double staining has demonstrated that SP IR neurons do not show co-localisation with VIP, suggesting that these cells might be intrinsic sensory neurons involved in the intralingual reflex. The number of GAL, VIP, TH IR nerve fibres increased and SP, NPY IR nerve fibres decreased in the minor salivary glands of SS patients with sensory neuropathy. The number of IR nerve fibres decreased in the one week duration of diabetes, but after four weeks duration of diabetes their number significantly ( $p < 0.05$ ) increased.

After immediate insulin treatment the changes of nerve fibres has been prevented. However, after delayed administration of it, the number of neuropeptide containing nerve terminals was further enhanced. In the early stage of inflammation the number of inflammatory cells also increased and some of them became IR for SP and NPY. The number of mast cell – SP IR nerve fibres contacts more significantly ( $p < 0.001$ ) increased in the 4 weeks duration of diabetes. On the basis of literature and our results we suggest, that the breakup in the balance of neuropeptides might have a role in the pathogenesis of SS and diabetes mellitus. Some of the neuropeptides acting as neurogenic component of inflammation can have effects on the functions (activation) of immunocompetent cells involving the formation of lymphocytic infiltration. The neuropeptides released from nerve terminals and immunocompetent cells might have a direct effect on the tissue cells and other immunocells releasing different inflammatory mediators causing degeneration, regeneration and proliferation as well as they might have a role in the pathogenesis of different diseases in the oral cavity (SS, diabetes mellitus).

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## IVÁN IVÁNYI (2003)

### The effects of adhesive materials on the pulpal microcirculation

Supervisor: Dr. Ida Nyárasdy

The pulpal effects of the application of adhesive filling technology have not been clearly understood, however, there are data available which indicates to the early toxicity of the filling materials. Thus, the aim of our experiments was to evaluate the acute effects of the adhesive materials on the pulpal circulation. The experiments were carried out on the lower incisors of anesthetized Sprague Dawley male rats, employing laser Doppler flowmetry and vitalmicroscopy.

The results show that the application of the bonding material adjacent to the pulp will bring an immediate effect (vasodilatation and enhanced local blood flow), however, it is not accompanied by an irreversible change (stasis) within the dental circulation. It was shown that conditioning of the dentin according to the manufacturer's recommendations, does not lead to significant pulpal circulatory changes. Nonetheless, prolonged conditioning can bear serious effects on the pulp. It was documented that there are no significant differences between acetone-containing and acetone-free bond materials with respect to their effects on the pulpal circulation. For the first time, it was shown that the chemical contents of the bonding material cause vasodilatation when used at the clinically applied concentrations, however, the application of greater concentrations will lead to irreversible adverse effects. The role of substance P in pulpal basal microcirculation was shown; and for the first time, it was documented that substance P is involved in the acute pulpal response to bondmaterial constituents. In summary it is concluded, that the proper application of clinically used bondmaterials does not cause acute serious irreversible changes in the dental circulation. However, the improper application of these materials can lead to serious pathology of the pulp.

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ÁRPÁD JOÓB-FANCSALY (2004)

## Examinations of dental implants surface morphology

Supervisor: Dr. Tamás Divinyi

The author has conducted studies of the surface morphology of dental implants under *in vitro* and *in vivo* conditions. In these studies surfaces treated with Nd-glass laser had a priority. Comparative analyses were conducted *in vitro* using a scanning electron microscope, and microgeometric elements produced by various surface treatment methods were described. The chemical structure of the uppermost layer of the surface of individual implants was analysed by way of photoelectron microscopy. The nanometer layer of the surface was also examined by AFM analysis. The author's research has proved that the surface can be made free of contamination by forming surface morphology by laser and has drawn conclusions in a retrospective way concerning the effect of nanometer structures on osseointegration. *In vivo* it was demonstrated that a roughened surface has a better capacity to induce osseointegration, than a smooth one. Out of the various surface types studied, those treated with a laser showed the most favourable results in an animal experimental model. As a result of these surface research studies, the author could prove that micrometer elements on the surface of dental implants have a vital role in forming the implant – bone interface and microgeometric structures can have a bone-forming effect. An attempt was also made to explore the possibilities of creating a perfect surface morphology.

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KATALIN KÁROLYHÁZY (2005)

## Oral health of patients with epilepsy: an epidemiological study. Guidelines of dental treatment

Supervisor: Dr. Pál Fejérdy

While epilepsy is a heterogenous disease with varying clinical symptoms, we set up a new classification system with 4 subgroups, as a guideline for dental treatment. We took into consideration the type and frequency of seizures, the effectiveness of antiepileptic drugs, and the mental state of epilepsy patients. The statistical datas of our epidemiological study about oral health of epilepsy patients illustrates worse conditions in almost all aspects comparing with those of the age and sex matched control group. Employment rate and socio-economic background were worse compared to the control group, and to the WHO datas. The gnostical and mnestic functions of them are declining as a consequence of brain hypoxia. Therefore they take less care about dental care and oral hygiene, so the preventive dental treatments are surpassed. They get into higher prosthetic class because of treatment options that are simple (like extractions), and they become completely edentulous sooner, worsening their quality of life. We might call the attention of the caring neurologist for this important circumstance. Dentists like to choose treatment options that are simple, because of the patients noncompliance. The periodontal status was found to be worse in patients with epilepsy caused by the overloading of the remaining teeth, bad oral hygiene, and side effects of drugs. There was no gingival hyperplasia found in the epilepsy group, because phenytoin was suppressed by modern antiepileptic drugs. The functional evaluation of prosthetic appliances was slightly according to desirable guidelines both in the epilepsy and in the control group as well. We have got a lot of dental work to do both in the epilepsy and in the control group as well.

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**BARBARA KISPÉLYI (2005)**

## **Effect of dentinal sealers on inner diameter of rat's pulpal arterioles - a vitalmicroscopic study**

*Supervisor: Dr. Ida Nyárasdy*

Temporary crowns with an improper marginal adaptation allow invasion of bacteria towards the pulp through exposed dentine tubules. At the border of dentine and pulp, bacterial products detrimental to the pulp may activate sensory nerve fibres, stimulate immune system and cause pain and pulpal inflammation. By means of sealing open dentine tubules and chemical stump protection, penetration of bacteria and dentine sensitivity can be reduced. Stump protection agents applied to prepared dentine surface close to pulp will diffuse through the thin layer of dentine and may easily affect pulpal physiology, and thus blood circulation of the pulp. Since biocompatibility tests are essential for safe use of dental materials, our research studied acute vascular effects of stump protective sealers by examining pulpal circulation of teeth of rats using vital-microscopic method. We tested one respected member of each group of chemical stump protection agents with different effect mechanisms. For sealers with halogene light polymerisation, we also examined any possible pulpal changes caused by polymerising light – without sealer application – since we studied only actual effects of sealers. Among sealers tested, only strong, one-step, self-etch primer caused irreversible pulpal changes. Among sealers implying reversible vasodilating effects, the slightest effects were caused by total-etch bond without acidification, while the most expressed by the same saler with acidification. Polymerising light caused no significant changes in vascular diameter either during 40- or 80-second exposition periods. The increase in blood flow caused by chemical stump protection applied to dentine surface exposed by stump preparation exerts a defensive, „flushing” effect. Our second series of examinations studied the role of vasodilating nitric oxide in vasodilation caused by stump protection. Our studies show that NO plays a significant role in both maintaining basal blood flow and pulpal response to bond material.

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**KRISZTINA KORDÁS (2004)**

## **The effect of phytohaemagglutinin and deramciclane on gastric and pancreatic secretion**

*Supervisor: Dr. Gábor Varga*

*Background:* Cholecystokinin (CCK) plays an important role in the regulation of gastrointestinal tract, mainly in physiological processes of stomach and pancreas. CCK is regulated by feed back mechanism and this mechanism can be modulated by different compounds such as kidney bean lectin phytohaemagglutinin (PHA) and 5-HT<sub>2A/2C</sub> receptor antagonist deramciclane. We observed the effect of PHA and deramciclane in gastric and pancreatic secretion in vivo. *Methods:* Male Wistar

rats were surgically prepared with chronic gastric, duodenal and pancreatic cannulas. The treatments were administered through jugular vein catheter. Gastric and pancreatic juice were collected in conscious or anaesthetised rats. Gastric acid and pepsin secretion were measured in gastric juice samples but amylase and trypsin secretion in pancreatic juice samples. In deramciclanc experiments the weight of pancreas was determined and amylase secretion was measured from the homogenate of the organ. To measure gastric emptying phenol red was injected through the gastric cannula. *Results:* PHA inhibited basal and stimulated gastric acid but not pepsin secretion. In contrast, PHA stimulated the pancreatic secretion but PHA was not as effective as bile-pancreatic juice diversion alone. CCK-A receptor antagonist devazepide abolished this stimulatory effect of PHA. Furthermore, we found that PHA could stimulate the pancreatic amylase and trypsin secretion under halothane but not urethane anaesthesia. On the other hand, deramciclanc inhibited the stimulatory effect of bile-pancreatic juice diversion, but this inhibition was not observed after exogenous CCK administration. Deramciclanc inhibited the stimulatory effect of proteinase camostatine in pancreatic weight. Gastric emptying was observed during Intralipid infusion. Intragastrically administered deramciclanc significantly inhibited the delay induced by Intralipid. While CCK-A receptor antagonist lorglumide abolished the effect of exogenous CCK, deramciclanc did not modify it. *Conclusion:* PHA has diverse effect on gastric and pancreatic secretion mediated by CCK. 5-HT<sub>2A/2C</sub> receptor antagonist deramciclanc modulates pancreatic enzyme secretion, pancreatic growth and gastric emptying involving the release of endogenous CCK.

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**KATALIN KOVÁCS (2005)**

## **Effects of platelet rich plasma on the ossification induced by the synthetic bone-substitute material, $\beta$ -tricalcium phosphate. (A comparative study on bone formation in dogs)**

*Supervisor: Dr. György Acsády*

During the past decade, the development of dental implantation and parodontology has increased the need in oral and maxillofacial surgery for the substitution of lost bone. Various substances of human or animal origin or synthetic materials may be used to fill bone defects. However, none of the methods employed at present are perfect. In consequence of the fear of bovine spongiform encephalitis, nowadays there is a reluctance to utilise materials of bovine origin. This may also be the reason why interest in synthetic bone substitutes has increased so significantly in recent years. One of the synthetic materials is phase pure  $\beta$ -tricalcium phosphate ( $\beta$ -TCP). Animal experiments and clinical results have revealed that  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) has the advantages that neither  $\beta$ -TCP itself nor its breakdown products are toxic, and it does not contain viruses, prions, or other proteins. It is tissue-friendly and its transformation is not accompanied by inflammatory symptoms. Platelet rich plasma (PRP) can promote ossification through its various factors. The literature is lacking data demonstrating the benefits of a combination therapy with PRP and synthetic bone-substitutes. Therefore, the objective of the present animal experiment was defined as follows:  $\beta$ -TCP was implanted in a specific part of the maxilla of a beagle dog, while in another area, located symmetrically on the other side, PRP mixed with  $\beta$ -TCP was applied. This method provided an opportunity to undertake clinical, radiological and histological examinations under identical circumstances and to determine possible differences in the ossification process. The clinical examination showed that, after a healing time of 12 weeks, the bone defects were completely filled up with newly formed bone of similar hardness both in  $\beta$ -TCP and in  $\beta$ -TCP/thrombocyte suspension.  $\beta$ -TCP particles could still be recognised after 12 weeks in the histological examination, but the quality of the new tissue proved to be good. In

every defect, histological and histomorphometrical examinations demonstrated a picture corresponding fundamentally to bone tissue. In cases of b-TCP+PRP grafts, the b-TCP particles in the sections exhibited a greater degree of transformation than without PRP. b-TCP+PRP expressed a greater level of activity in computer tomography examinations.

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**KRISZTINA MÁRTON (2005)**

## **Clinical investigation of the major and the minor salivary glands in patients with autoimmune diseases (Sjögren's syndrome and idiopathic inflammatory myopathies) and in patients wearing complete dentures**

*Supervisor: Dr. Pál Fejérdy*

Objective of this study was to describe the oral properties of Sjögren's syndrome (SS) and idiopathic inflammatory myopathies (IIM) compared to healthy controls. Particular attention was given to the determination of palatal salivary flow rate and the subjective problems associated with denture wearing in SS patients. A further aim was to compare the unstimulated whole (UWS) and palatal (PS) saliva flow rates of patients wearing complete dentures and patients with SS and to test whether xerostomia or hyposalivation has a negative influence on maxillary complete denture stability. A third aim was to determine the influence of new complete dentures on UWS and PS flow rates in healthy individuals. Subjects and Methods: In the SS and the IIM groups after the determination of their general health state a questionnaire was employed to obtain and record the subject's symptoms. Clinical assessments were made of their oral mucosal, dental and periodontal and denture status. Sialometric tests were performed to determine the flow rates of unstimulated whole (WS) and in the SS-group: palatal saliva (PS). In the IIM-group: light- and electron microscopic analysis of the symptoms of capillary abnormalities or signs of focal infiltration in labial biopsy specimens were carried out; for comparison with healthy controls. The masticatory force (MF) and the handgripping force were measured with a specially developed device. All participants in the third study were questioned about possible subjective oral complaints (xerostomia or instability of the dentures), through use of a standardized questionnaire. UWS and PS flow rates of the healthy subjects (controls) and of the SS-patients were measured. In the fourth part of the study, new complete dentures were fabricated for healthy patients. Flow rates of UWS and PS were measured before and seven days after the insertion in order to compare data with their pre-fabrication values. According to the results, that dental and periodontal status correlate with the possible autoantibody positivity, authors can state that the oral health status of patients both with SS and IIM might be considerably related to the general autoimmune process, and no more than partly to the hyposalivation. SS patients' chief complaint is glossodynia, their most frequent mucosal alteration is the erythema of the hard palate. As it is indicated by the results of this study, IIM patients have significant gingival oedema, and teleangiectasia. Dysphagia either because of xerostomia or macroglossia, or secondary to the oesophageal dysmotility, weakness of the masticatory muscles makes eating difficult in IIM. Obtained data strongly suggest that the reduction in the thickness of the palatal film in SS patients is due to a decrease in the volume of the mixed, whole saliva, not to the viscous palatal saliva. Remaining palatal mucous saliva flow can help to stabilize the maxillary complete denture in patients with hyposalivation. Neither unstimulated whole nor palatal saliva flow rate are influenced by the placement of new dentures

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ÁKOS KÁROLY NAGY (2004)

## Biologically active salivary proteins, functional studies of the epidermal growth factor

Supervisor: Dr. Tivadar Zelles

Saliva and its constituents carry out a number of biological functions that are keys in maintaining oral and systematic health. The component proteins are produced and released into the saliva by the acinar and ductal cells. Two of these proteins were involved in our experiments. We examined the changes of salivary amylase in serum and parotid tissue during pharmacological and physiological stimulation. The other experiments showed reduced oral wound healing in the NOD mouse model for the 1 type autoimmune diabetes and its reversal by epidermal growth hormone supplementation. In the first experiments rats received pilocarpine injection or were fed after fasting. The changes in serum and parotid tissue amylase levels were monitored also in freely fed animals. In the second experiments NOD mouse were used, a model for type 1 diabetes. We examined how reduced concentrations of EGF in the saliva, after onset of diabetes, affect the oral wound healing. Our data showed that pilocarpine stimulation and feeding of fasted animals resulted in a significant decrease in tissue activity and increase in serum amylase activity according to previous investigators. During the spontaneous food intake parotid amylase level decreased and in parallel serum amylase level increased. Our study is the first to show that both parotid and serum amylase level is controlled by spontaneous food intake in rats. The antiparallel changes can be attributed to food-stimulated activation of neuronal and hormonal pathways leading to the discharge of parotid amylase into the saliva and also to the elevation of the enzyme activity in serum. NOD and control mice were given a cutaneous punch and allowed to undergo normal healing. With diabetes onset and a reduction in saliva derived growth factor levels, the rate of wound healing was reduced compared to control. Addition of exogenous EGF to the drinking water did not accelerate the rate of healing in control. However, diabetic mice exhibited accelerated wound healing similar to healthy mice. In conclusion, we provided further evidence that saliva-derived EGF is an important protagonist in oral wound healing. Furthermore, metabolic disorders, such as diabetes, reduce the level of EGF in saliva with the consequence that the normal wound repair is negatively affected.

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**GÁBOR SZALMAY (2004)**

## **Effect of bioactive substances on epithelial ion and bicarbonate secretion**

*Supervisor: Dr. Gábor Varga*

*Background:* The role of the upper gastrointestinal tract is chopping, passing and digesting of the meal. The function of the secretory epithelium of salivary glands, stomach and pancreas is very important in this process. The juice secreted by the organs mentioned above set the pH to the optimal level for the activation of digestive enzymes. We observed the peptiderg regulation of pancreatic bicarbonate and fluid secretion and the effect of lectins on gastric acid secretion. *Methods:* Microfluorometry and videomicroscopy for pancreatic secretion and in vivo experiments for observation of gastric acid secretion. *Results and conclusion:* The pancreatic duct cell pHi remains remarkably constant, during the transition from the unstimulated state to maximal HCO<sub>3</sub><sup>-</sup> secretion. However, when HCO<sub>3</sub><sup>-</sup> entry is blocked by the application of transport inhibitors, the continuing efflux of HCO<sub>3</sub><sup>-</sup> across the luminal membrane leads to a fall in pHi as a result of the net loss of base from the cell. The initial rate of fall in pHi is therefore an index of instantaneous HCO<sub>3</sub><sup>-</sup> efflux. CCK, bombesin, secretin, VIP and PACAP evoke a concentration-dependent increase in acidification. The stimulatory effect of CCK was completely inhibited by the CCK1 receptor antagonist, but was unaffected by the CCK2 receptor antagonist. In combination with a maximal dose of CCK, JMV-180 (an agonist at the high-affinity sites but an antagonist at the low-affinity sites) caused a partial inhibition of HCO<sub>3</sub><sup>-</sup> secretion, while at a mid-range CCK concentration it had neither stimulatory nor inhibitory effects. The GRP receptor antagonist completely abolished the stimulatory effect of bombesin while the NMB-type receptor antagonist had no inhibitory effect. The adenylate-cyclase inhibitor totally blocked the stimulatory effect of secretin. Our results clearly demonstrate the existence of CCK1 receptors, GRP-type bombesin receptors and secretin receptors in pancreatic ductal tissue, and that occupation of all these receptors evoke both HCO<sub>3</sub><sup>-</sup> and fluid secretion. In our in vivo experiments pentagastrin and histamine stimulated the acid secretion of the stomach. Phaseolus lectin, PHA blocked the basal, pentagastrin-evoked and histamine-evoked gastric acid secretion. These results suggest that lectins are able to inhibit the gastric acid and pepsin secretion.

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**JÁNOS VÁG (2003)**

## **Role of nitric oxide and angiotensin II in the regulation of submandibular gland blood flow**

*Supervisor: Dr. Árpád Fazekas*

The effect of NO on the vascular tone has been demonstrated in several organs, but no data are available on the possible blood flow regulatory action of NO in the salivary glands of animals with altered salt balance. The role of the angiotensin II (Ang II) has been documented in the perfusion of the salivary glands, but the relationship between NO and the renin-angiotensin system is not yet investigated in this respect. In the present studies we have investigated the role of the NO and AT1 subtype of angiotensin II receptors in the control of the blood flow in the anaesthetised rat submandibular gland (SMG). To this end we blocked NO production by Nw-nitro-L-arginine-methyl-ester (L-NAME). The role of the AT1 receptors was studied by means of an elective receptor blocker (candesartan), or by the alterations of the activity of the renin-angiotensin system (Na<sup>+</sup> load or Na<sup>+</sup> depletion). Blood flow to the SMG was measured by both laser Doppler flowmetry and by the Rb ac-

cumulation technique. The data obtained with the two methods well correlated. Blood pressure and the vascular tone of the SMG increased markedly under the effect of L-NAME, whereas both parameters decreased after candesartan treatment. The low Na<sup>+</sup> diet did not influence, whereas a high Na<sup>+</sup> diet potently enhanced the perfusion in the SMG. The vascular tone enhancing effect of L-NAME was markedly increased after the blockade of the AT<sub>1</sub> receptors, at the same time, the various Na<sup>+</sup> diets did not have influence on it. In response to the inhibition of NO production cardiac output decreased. On the basis of our results we suggest that the hypertension elicits a reflex (baroreceptor mediated) bradycardia that occurs primarily due to the lowered sympathetic activity on the heart. Under the effect of unilateral common carotid artery occlusion the perfusion of the SMG decreased moderately (by 16%) in conjunction with a marked fall (by 40%) in the vascular tone. The prior inhibition of NO production mitigated these responses. On the basis of our results we conclude that both NO and circulating Ang II play an essential role in the adjustment of the vascular tone of the SMG. During AT<sub>1</sub> blockade the production/effect of NO is enhanced. Shifts of balance in the Na<sup>+</sup> intake of the organism in either direction did not influence the effect of NO in the SMG. Under conditions of decreased perfusion pressure locally activated NO-independent vasodilator mechanisms play a crucial role in the preservation of blood flow to the SMG.

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- Vág J, Hably Cs, Bartha J Inhibition of beta-1 receptor but not vagotomy can abolish the L-NAME evoked bradycardia in anaesthetized rat. *Physiol Res*.
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**PÉTER WINDISCH (2004)**

## Complex analysis of periodontal regenerative procedures

*Supervisor: Dr. György Szabó*

Different treatment modalities have been used during the past decades to reform the tooth's supporting tissues which have been lost following periodontal disease. Both guided tissue regeneration (GTR) and the treatment with the enamel matrix proteins derivative (EMD) result in significant gains of clinical attachment and new hard tissue formation but there is very limited information about the mechanism of healing following these procedures and the extent to which the routine clinical data and radiographs reflect the "true" regeneration process. This situation prompted us to perform 9 clinical and histological (immuno-histochemical) studies to assess the clinical impact of these regenerative procedures, the mechanisms according to which they work and the follow-up methods used to assess the extent of the healing process. All adult patients enrolled in the clinical studies were referred to the Department of Periodontology, Semmelweis University for special treatment of advanced periodontitis. The complementary animal histological studies were carried out in Aarhus, Royal Dental College, Denmark. Based on our clinical results we found that both GTR (with non-absorbable and bioabsorbable membranes) and EMD regenerative procedures result in almost similar therapeutic outcome without any adverse reaction. The clinical improvements achieved in the first few months following surgery can be maintained throughout two or more years. Clinical, radiographic and histometric data show different projections of the healing process following regenerative surgery. Both CAL gain and radiographic data (BD) correlate (to varying extent) with the amount of new cementum formed as the result of regenerative therapy. The CAL gain is also linearly related to the new bone but does not provide a reliable estimate of the amount of actual new bone. Radiographic data fail to correlate with histological findings. The formation of new cementum and new bone are closely interrelated as reflected in the high correlation between these variables. Our histological results, however, showed that the GTR and EMD procedures affect different pathways in the healing process. In GTR-treated defects new bone and new attachment develop simultaneously. In defects treated with EMD primarily new cementum with inserting fibres is formed with less pronounced generation of new bone. The new cementum was shown to be predominantly of a cellular character regardless of the regenerative procedure used. Our histological studies also suggested a close relationship between cementum, periodontal ligament and

oxytalan fibres. Expression of cytokeratins demonstrated that the exclusion of the epithelial cells from the wound area is one of the most critical condition for achieving periodontal regeneration. The epithelial rests of Malassez islet cells were found not to be included in de novo formed periodontal ligament. The vimentin expression patterns clearly showed that a regenerative therapy can be successful only in the presence of an intact PDL with a certain amount of progenitor cells. Our histological data indicated that the neoformation of cementum and bone probably are unrelated phenomena.

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## 2/6. PROGRAM

### CLINICAL HAEMATOLOGY

#### *Coordinator*

**Lídia SRÉTER**

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Studies about the prognostic factors and complex therapies of haematologic disorders can contribute to more efficient treatment of this patient group. The role of infectious agents and environmental factors in the etiology of malignant lymphomas is a very interesting and up to date field of research (e.g.: post-transplantation). We will study the diagnostic use of the methods of modern molecular biology and its efficiency in the follow-up control of our patients, too. The pathogenesis of the thrombosis in malignancies and the frequency of the cytokine gene-polymorphism and p53 mutations and their therapeutic importance. The pathophysiological bases of the plasma cell-dyscrasies and their therapeutic implications are also part of our research. The bone marrow transplantation was a breakthrough in the therapy of the malignant diseases. The hemopoietic stem cell transplantation can be a model of the immunotherapy, and it may be studied well. The connection of the specific immunological state after transplantation with the complications of the transplantation, with the relapse of the disease and with the survival is still subject of investigation even nowadays. The course of the development of the immunological tolerance after allogeneic transplantation is still unknown either. The investigation of the haematologic disorders can be the subject of the research, which belongs to the scientific bases of the public health priorities because of the high frequency of oncohaematologic disorders.

#### *Sub-programs*

**Pathogenesis, diagnosis, clinico-pharmacology treatment and side-effects of oncohaematologic diseases**

#### *Topics*

The role of infectious agents and environmental factors in the etiology of malignant lymphomas

The role of growth factors and their receptors/coreceptors in the proliferation and apoptosis of lymphoma cells

#### *Supervisors*

Lídia SRÉTER

Lídia SRÉTER

László KOPPER,  
Anna SEBESTYÉN

<i>Sub-programs</i>		<i>Supervisors</i>
Proliferative and metabolic alterations of the human mononuclear cell compartment in malignant haematologic diseases		Béla MOLNÁR
Functional changes of the lymphoid cell repertoire in lymphoproliferative diseases		Györgyi MÚZES
Allogeneic and autolog bone marrow transplantation followed by hepatic injury: the role of the toxic and the immunological factors in proliferative diseases		Tamás MASSZI, Lídia SRÉTER
Investigation of the cardioprotective effect of cardioxane		András VERECKEI
Potential role of neutrophil granulocytes in thrombosis of oncologic diseases		Raymund MACHOVICH
<b>Frequency of cytokine gene-polymorphism and P53 mutations in malignant lymphomas and their therapeutic significance</b>		Judit DEMETER
<i>Topics</i>		
The role of polymorphism of cytokine-genes in the pathogenesis of malignant haematologic diseases		Judit DEMETER
Frequency of p53 mutations in hairy cell leukemia and its role in therapeutic answer after treatment with purine nucleoside analogues		Judit DEMETER
<b>Pathophysiology of plasma cell dyscrasia and their connection with the therapeutic possibility</b>		Gábor TARKOVÁCS
Etiology and pathogenesis of myeloma multiplex		Gábor TARKOVÁCS
Therapy of myeloma multiplex		Gábor TARKOVÁCS
<b>Bone marrow transplantation as an alternative treatment of malignant haematologic diseases</b>		Katalin PÁLÓCZI
Treatment of chronic myeloid leukemia (CML) with hematopoietic stem cell transplantation		Katalin PÁLÓCZI
Study of the development of chimerism and its relation to the success of bone-marrow transplantation		Katalin PÁLÓCZI
Investigation of the immunogenetic factors and mechanisms influencing the development of acute graft versus host disease		Éva GYÓDI, Katalin RAJCSY
Study of the autoimmune pathogenesis of chronic graft versus host disease		Kata MIKLÓS
Purging of bone marrow and blood stem cells from malignant cells: study of surviving normal and malignant stem cell populations		Ferenc UHER
Study of the structure of the stem cell population after cytostatic treatment (in murine experiments and in haematological disorders).		Ferenc UHER
Stem cell investigations: reproducibility and plasticity of stem cells		Ferenc UHER
<b><i>Ph.D. students</i></b>		<b><i>Supervisors</i></b>
Valéria Dudics	pt	Ferenc Uher
Zoltán Gasztonyi	pt	Lídia Sréter
Luca Kormos	pt (a)	Katalin Pálóczi
Adrienn Mohl	ft	Lídia Sréter, Tamás Masszi
Albertné Suhajda	pt	Ferenc Uher
<b><i>Ph.D. candidates</i></b>		<b><i>Supervisors</i></b>
Virág Vas	ft	Katalin Pálóczi
<b><i>Ph.D. graduates</i></b>		<b><i>Supervisors</i></b>
Judit Jánosi	pt	Gábor Tarkovacs
Olga Ujhelly	ft	Katalin Pálóczi

a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

JUDIT JÁNOSI (2005)

## Apoptosis and a characteristic marker (CD 138) in multiple myeloma

*Supervisor: Dr. Gábor Tarkovác*

The limited success of cancer chemotherapy calls for new and better drugs with more relevance to the regulation of tumor growth and progression. Apoptosis is a critical process in normal B-cell function, and in many cases the abnormalities in the apoptotic pathways contribute to the pathogenesis of B-cell malignancies. This scenario has particular relevance in multiple myeloma (MM).

In the present study we analysed the effect of mevastatin - a novel inhibitor of HMG-COA reductase, the rate-limiting enzyme of the mevalonate pathway - on U266 human myeloma cells. Apoptosis induced by mevastatin was associated with increased caspase activity and depolarisation of mitochondrial membrane. Expression of BCL-2 mRNA and protein was down-regulated, with no change in BAX or BCLxL protein production. The mitochondrial program was supported by caspase-8 and cleaved-BID activity. None of the antibodies neutralizing death-ligand/death-receptor pathway – TRAIL-R2Fc, anti-TNF- $\alpha$ , anti FASL (NOK-1) - influenced the mevastatin-induced apoptosis. Mevastatin also stimulated shedding of syndecan-1 from the surface of myeloma cells.

Syndecans are a family of cell surface proteoglycans. In the bone marrow of multiple myeloma patients syndecan-1 is expressed only on the surface of malignant plasma cells. In this study we analyzed the serum level of shed syndecan-1 in three groups of patients with multiple myeloma, solitary plasmocytoma, and monoclonal gammopathy of undetermined significance. Patients with multiple myeloma showed a significantly higher median serum syndecan-1 level than patients with plasmocytoma or monoclonal gammopathy of undetermined significance. Statistically significant differences were also observed among Salmon-Durie subgroups of 50 patients suffering from multiple myeloma. In addition to these findings a statistical correlation with other independent prognostic factors such as serum beta2 –microglobulin level, monoclonal immunoglobulin concentration, and bone marrow plasma cell count could also be noted. A significant decrease in median serum syndecan level was observed in patients who responded to chemotherapy, whereas no change in the median syndecan-1 level could be observed in nonresponders. Our findings confirm the observation that high serum soluble syndecan-1 level is associated with a more advanced disease stage and is a strong independent indicator of poor prognosis. A diminished serum syndecan-1 reading as a result of chemotherapy may be a good indicator of favorable response to antitumor treatment.

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- Jánosi J, Sebestyén A, Mikala G, Németh J, Kiss Z, Vályi-Nagy I (2004) Soluble syndecan-1 levels in different plasma cell dyscrasias and different stages of multiple myeloma. *Haematol* 89:370-371.
- Jánosi J, Sebestyén A, Mikala G, Peto M, Jákó J, Domján G, Németh J, Kis Z, Kopper L, Vályi-Nagy I (2005) Prognostic value of soluble syndecan-1 (CD 138) in myeloma multiplex (In Hungarian). *Orv Hetil* 146:165-168.

OLGA UJHELLY (2005)

## Application of a human multidrog transporter (ABCG2) as selectable marker in gene therapy

*Supervisor: Dr. Katalin Pálóczi*

Stem cell-based gene therapy is often unsuccessful because of the relatively low number of genetically modified cells with repopulating capabilities. To provide a selective advantage to the modified cells we applied the human ABCG2 protein, a resident xenobiotic transporter in stem cells, as a selectable marker. This protein is active as a homodimer, and its relatively small cDNA is an advantage in gene therapy applications. In the present study the gene therapy application of a mutant form of ABCG2 (R482G) was investigated.

ABCG2 variants were expressed in haematopoietic stem cells alone or co-expressed with the therapeutic gene (gp91phox) of X-linked Chronic Granulomatous Disease (X-CGD) by an efficient retroviral transduction system. Transgene expression was determined by Western blotting, immunohistochemistry and flow cytometry analysis. To estimate the multidrug resistance phenotype, functional assays of ABCG2 were performed. The differentiation of the transduced cells was followed by in vitro clonogen and in vivo mouse transplantation experiments.

High proportion of transgene positive cells could be detected in the ABCG2 transduced cells, where the mutant ABCG2 protein selectively protected the cells against clinically applicable cytostatic drugs as mitoxantrone (MX) and doxorubicin. Expression of the gp91phox protein in human gp91phox knock out hematopoietic progenitor cells corrected the mutation responsible for X-CGD after MX selection. Overexpression of ABCG2 did not affect hematopoietic cell maturation both in vitro and in vivo. We suggest that the mutant ABCG2 protein is an ideal candidate for human stem cell protection and for use as a selectable marker in gene therapy.

- Ujhelly O, Özvegy Cs, Várady G, Cervenak J, Homolya L, Grez M, Scheffer G, Roos D, Bates SE, Váradi A, Sarkadi B, Németh K (2003) Application of a human multidrug transporter (ABCG2) variant as selectable marker in gene transfer to progenitor cells. *Hum Gene Ther* 14:403-412.
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- Ozvegy-Laczka C, Varady G, Koblos G, Ujhelly O, Cervenak J, Schuetz JD, Sorrentino BP, Koomen GJ, Váradi A, Nemet K, Sarkadi B (2005) Function-dependent conformational changes of the ABCG2 multidrug transporter modify its interaction with a monoclonal antibody on the cell surface. *J Biol Chem* 280:4219-4227.

## 2/7. PROGRAM

### CLINICAL ENDOCRINOLOGY AND ITS EXPERIMENTAL ASPECTS

#### *Coordinator*

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This doctoral program is aimed at the investigation of various disorders of the endocrine system, further the role of hormones in cardiac diseases and in the pathogenesis of osteoporosis. PhD students interested in these subjects are welcome to this programme, where clinical investigation as well as basic science research is possible.

#### *Sub-programs*

##### **Ph.D. students**

Péter Bedőcs	ft
Csilla Jakab	pt (a)
Balázs Sárman	ft (a)
Réka Skoumal	ft (a)
Erzsébet Tarkó	pt

##### **Ph.D. candidates**

Szilvia Mészáros	ft
Ildikó Pósa	na

#### *Supervisors*

##### **Supervisors**

Miklós Tóth
Károly Rác
Miklós Tóth
Miklós Tóth
István Szabó

##### **Supervisors**

Csaba Horváth
Miklós Tóth

**Ph.D. graduates**

Éva Bajnok	ft
Zsuzsa Bencsik	na
Erika Hubina	pt
Zoltán Lakó-Futó	ft
Tamás Solymosi	na
Erzsébet Toldy (Salamonné)	na
Dóra Török	ft
Edit Ágnes Tóth	na

**Supervisors**

Péter Lakatos
István Szabolcs
Miklós Góth
Rudolf de Châtel
István Szabolcs
L. Gábor Kovács
János Sólyom
Csaba Horváth

a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

**ÉVA BAJNOK (2005)**

## The role of candidate gene polymorphisms in postmenopausal- and thyroid hormone-stimulated bone loss

*Supervisor: Dr. Péter Lakatos*

Osteoporosis (OP) is one of the most common chronic disorders; the disabilities that result from osteoporotic fractures (OPF) worldwide have an enormous impact on the health of individuals, societies and economies. Genetic factors together with environmental factors play an important role in regulating the development of osteoporosis as well as contributing to the susceptibility of osteoporotic fractures. Our aims were to analyze some genetic factors that affected osteoporosis permitting early detection of individuals who are at risk for the disease, and allowing for early initiation of preventive therapy. We have studied the role of certain gene polymorphisms of proteins having biological effects on bone metabolism in postmenopausal and thyroid hormone stimulated bone loss. We have also looked at the possible functional contributions of these genes to the pathogenesis of hyperthyroidism in toxic adenoma (TA), as one of the major group responsible for secondary osteoporosis. Our results demonstrate that COL1A1 gene G1245T (Sp1) and CaSR gene A986S polymorphisms might cause a predisposition to postmenopausal osteoporosis and might also be a prognostic marker of the disease. We could not prove the direct clinical significance of these gene variants on bone fracture. Based on our observation we conclude that IL-1RN VNTR polymorphism may not play an essential role in the determination of BMD in postmenopausal osteoporosis; however, our results support a hypothesis that it may influence the bone fracture risk, independent of BMD. This is the first study reporting the possible functional contribution of ER alpha gene XbaI and IGF-I gene CA repeat polymorphism in the pathogenesis of toxic adenoma. Based on our data VDR gene BsmI- and IL-1RN gene VNTR polymorphisms do not appear to have an impact on the development of TA. Our results also raise the possibility of the contribution of this microsatellite repeat variant of IGF-I gene in bone loss as the consequence of TA. We have not confirmed the role of ER alpha XbaI-, VDR BsmI- and IL-1RN gene VNTR polymorphisms in predicting low BMD caused by toxic adenoma.

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**ZSUZSA BENCSEK (2005)****Suprarenal incidentalomas: histological heterogeneity, measurement of dehydroepiandrosterone sulfate and radiocholesterol scintigraphy in the diagnostic work up, and follow up of the incidentalomas***Supervisor: Dr. István Szabolcs*

In the dissertation are summarized data of three articles about patients with incidentally detected adrenal tumor. In the first article (1) we reported data on heterogeneity of adrenal incidentalomas (63 cases), in the second article (2) we presented data on DHEA-S measurements in nonselected cases of suprarenal incidentalomas (84 patients), in the third article (3) we studied the diagnostic value of radiocholesterol scintigraphy and its correlation to subclinical hypercortisolism (41 patients). Ultrasound, CT, in selected cases adrenal scintigraphy and screening laboratory tests for excess cortical and medullary hormone products were performed. Conclusions: 1.1. Suprarenal incidentalomas are not a single pathological entity; they may be benign or malignant. The size of the tumors indicated by CT is often smaller than the pathological size. 1.2. Suprarenal incidentalomas often show subtle hormonal activity. Cortical adenomas with subtle hormonal overproduction should be operated, irrespective of their size. 1.3. Tumors >30 mm should be operated, but smaller ones can be followed-up, they rarely show progressive growth. 1.4. Based on our results, tumor size <30 mm excludes malignancy. 2.1. In nonselected cases of suprarenal incidentaloma a suppressed DHEA-S level is not a good predictor to detect a benign adrenal mass or cortical origin of the tumor. 2.2. In nonselected cases of adrenal incidentalomas a suppressed DHEA-S level is not a good predictor of hormonal activity. DHEA-S measurement may be valuable only after having ascertained the cortical origin and benign feature of the tumor. 3.1. Unilateral semiquantitative radiocholesterol uptake of an adrenal incidentaloma in combination with low serum DHEA-S concentration seems to be the most sensitive marker of subtle cortisol excess in the evaluation of suprarenal incidentalomas. 3.2. The combination of unilateral radiocholesterol uptake and low DHEA-S is strongly indicative of SH in adrenal incidentalomas. 3.3. Radiocholesterol scanning is a safe method to prove the cortical origin of suprarenal incidentaloma and should be used to exclude metastasis in selected patients with non-conclusive CT/MRI findings.

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- Bencsik Zs, Szabolcs I, Kovács Zs, Ferencz A, Vörös A, Kaszás I, Bor K, Gönczi J, Góth M, Kovács I, Dohán O, Szilágyi G (1996) *Low dehydroepiandrosterone sulfate (DHEA-S) level is not a good predictor of hormonal activity in nonselected patients with incidentally detected adrenal tumours. J Clin Endocrinol Metab 81:1726-1729.*
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**ERIKA HUBINA (2004)****Experimental and clinical investigations in diseases with pathological growth hormone secretion***Supervisor: Dr. Miklós Góth*

The intracellular mechanism becomes more and more important in the investigation of the regulation of growth hormone (GH) secretion. The effect of somatostatin (SS) was enhanced on somatotropinoma cells, GH secretion paradoxically increased after pituitary adenylate-cyclase polypeptide pretreatment in our *in vitro* study. Acromegaly is usually caused by a GH-secreting adenoma of the pituitary gland but it has been postulated that a reduction of the hypothalamic SS-activity could also play a role in the excess of GH secretion. The thyrotrop hormone responsiveness to

acute glucose ingestion was preserved, even increased. This observation did not support the concept that the SS-response decreases in acromegaly. In addition to insulin-like growth factor (IGF)-I, IGF binding protein (BP)-3 measurement is used for the diagnosis of acromegaly. We conclude that the IGFBP-3 measurement has low sensitivity and it is unable to measure the activity of acromegaly. The anatomical changes in the bone system are the most common symptoms of the acromegaly. Patients both with active and inactive acromegaly have preserved BMD and it did not differ between the two groups. Patients with inactive acromegaly had higher undercarboxylated osteocalcin (ucOC) than in active disease. The ucOC is a marker of bone quality. GH seems to have a positive effect on the carboxylation process.

GH deficiency in adults. GH increases the peripheral conversion of thyroxin to triiodothyronin. It inhibits the enzyme, which converts cortisone to cortisol, and stimulates the activity of several enzymes which participate in the synthesis of sex steroids. We studied the impact of GH replacement on the other pituitary hormone substitution and the need for dose adjustments in the therapies. A small but clinically significant number of our patients (12%) required dose adjustment, therefore the monitoring of pituitary hormone axes is advisable after the commencement of GH replacement and not only in patients previously treated with pituitary radiotherapy. We investigated the effect of GH replacement on the carboxylation of OC. GH has a beneficial action on carboxylation (as we have seen it in acromegaly). The estimation of the fracture risk would be more accurate by parallel measurement of the bone mineral density and the carboxylation product of OC. The physiological differences between genders should be taken into consideration in the optimizing the GH replacement. We found an age- and gender depending GH effect on bone metabolism, a gender depending action on body composition and an age- and gender independent influence on carbohydrate metabolism.

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## ZOLTÁN LAKÓ-FUTÓ (2005)

### Role of intra- and extracardiac factors in the early phase of cardiac hypertrophy

Supervisor: Dr. Rudolf de Châtel

Cardiovascular disease remains as the leading cause death throughout the industrialized nations of the world. Central to this statistic is our current inability to effectively repair or otherwise reverse severe forms of cardiac dysfunction and pathologic remodelling, that characterizes a failing heart. In response to various stimuli, diverse pathophysiologic conditions or genetic mutations the myocardium undergoes a hypertrophic growth phase as a compensatory measure aimed at maintaining cardiac output. The aim of the present study was to characterize the role of the distinct cardiac and extra cardiac factors mediating the activation of cardiac natriuretic peptide gene expression in the hypertrophied heart as well as the auto regulation of the tissue renin-angiotensin system the main operator on hypertrophic growth in vivo. Angiotensin (AngII) ( $33\mu\text{kg/h}$ ), the AT<sub>2</sub>-R antagonist PD123319 ( $1,25\text{mg/kg/h}$ ); the angiotensin II type 1 receptor (AT<sub>1</sub>-R) antagonist Losartan ( $400\mu\text{kg/h}$ ); AngII+PD123319 or AngII+Losartan was infused via subcutaneously implanted osmotic mini pumps in male SD-rats for 12 or 72 hours. The role of extra cardiac such as adrenal factors on the regulation of cardiac natriuretic peptide synthesis was studied by using normal and adrenalectomized rats. Ang II-induced increase in mean arterial pressure, left ventricular weight/body weight ratio and elevation of skeletal  $\alpha$ -actin and  $\beta$ -myosin heavy chain mRNA levels were not altered by PD123319. In contrast, AT<sub>2</sub>-R blockade resulted in a marked increase in the gene expression of c-fos, endothelin-1 and insulin-like growth factor-1 in Ang II-induced hypertension. In parallel, Ang II-stimulated mRNA and protein expression of atrial natriuretic peptide were significantly augmented by AT<sub>2</sub>-R blockade. Moreover, PD123319 markedly increased the synthesis of B-type natriuretic peptide. Furthermore, the expression of vas-

cular endothelial growth factor and fibroblast growth factor-1 were down regulated by Ang II only in the presence of AT2-R blockade. Adrenalectomy either abolished (ANP) or blunted (BNP) the early activation of ventricular gene expression by Ang II, however, left hemodynamical parameters unchanged. Our results provide evidence that AT2-R plays a functional role in the cardiac hypertrophic process in vivo by selectively regulating the expression of growth-promoting and growth-inhibiting factors. Further, results also indicate that activation of ventricular gene expression of ANP and perhaps BNP as well by Ang II has components independent from hemodynamic changes and left ventricular hypertrophy, and requires factors derived from the adrenals.

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## TAMÁS SOLYMOSSI (2004)

### Clinical and morphological methods to improve the diagnostic accuracy of thyroid nodular goiter

Supervisor: Dr. István Szabolcs

Based on metaanalysis of 45 publications the sensitivity of thyroid fine needle aspiration cytology (FNAC) is 91.6%, the specificity is 73.2%, the diagnostic accuracy is 78.5% and the positive predictive value is 58.1%. 4.4% of all patients underwent FNAC had advantage while 3.2% of the patients underwent on unnecessary operation because of FNAC. The size and shape of the nucleus are of limited diagnostic value in the thyroid considering the great overlap between benign and malignant lesions. Based on the specific nuclear atypia the accuracy of FNAC in diagnosing papillary cancer is very good except for oxyphilic lesions. Papillarization is not a sign of malignancy. Parameters determined by the AgNOR-method reflecting the proliferative activity of the cells can distinguish benign from malignant lesions in consecutively operated patients. Testing the method in patients with suspicious FNAC the overlap between benign and malignant lesions is greater, but the mean AgNOR area may share even practical value: we did not find any cancers of follicular origin with an AgNOR area less than 5  $\mu\text{m}^2$ . The ultrasonography (US) applied routinely improves significantly the accuracy of FNAC. The sensitivity increases from 76 to 92%, the specificity increases from 65 to 87%, and the diagnostic accuracy increases from 66 to 88%. The main cause of this improvement is the decrease of unnecessary operation in patients with follicular proliferation without significant atypia and in those with the differential diagnostic difficulty arising in lymphocytic thyroiditis. In these cases we advised US follow up instead of immediate surgery. It seems an effective (the number of surgeries decreased with 50%) and safe method. The ratio of multinodular goiters, that of patients with nonpalpable nodules, the ratio of nondiagnostic FNACs was significantly higher while the ratio of malignancy was significantly lower in iodine deficient (ID) compared with iodine sufficient (IS) regions (59.6% vs. 49.6%, and 47.6% vs. 37.3%, and 8.8% vs. 5.1%, and 1.2% vs. 2.3%, ID vs. IS, respectively). The sensitivity, specificity and diagnostic accuracy of FNAC was not significantly different in ID and IS regions, while the ratio of malignancy and that of carcinoma: adenoma was lower in ID than in IS region. The PPV was significantly worse in ID region (36/106, i.e. 34% vs. 21/36, i.e. 58%,  $P=0.001$ ), because the accuracy of FNAC was significantly lower in adenomas than in carcinomas.

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**ERZSÉBET TOLDY (SALAMONNÉ) (2005)**

## Hormone measurements: diagnostic errors related to preanalytical factors

*Supervisor: Dr. L. Gábor Kovács*

The special preanalytical and analytical factors have their impacts on the clinical evaluation of hormonal findings. Due to the specific features of the immunoassay technique (cross-reactivity of the antibodies, specificity, technology-dependent sensitivity limits, the matrix effect, etc.), bare numbers alone on the laboratory report might be misleading. In many instances, the numeric result is mutually influenced by the serum concentration of the analyte, and by the ambient substances in the patient serum. The main aim of this thesis was to investigate and quantify the power of preanalytical influences on hormone (immunoassay) measurements in the two most common endocrine diseases, such as thyroid dysfunctions and hyperprolactinemia. Thyroid parameters were measured in 2981 patient sera, whereas pathologically elevated prolactin levels were analyzed in 1051 sera. The algorithm, based on sensitive TSH measurement, resulted in 66 % reduction of thyroid test requests and in a higher quality of test reporting. Furthermore, it has been confirmed that free thyroid hormones (fT4 and fT3) are more reliable test parameters than the indirect (calculated) free hormone indices (fT4i, fT3i), which are misleading in a very high percentage (40 %) of hyperthyroid patients on methimazole therapy. Opposite to the common view about patients with elevated TBG levels (pregnant women or those taking oral contraceptives), fT4 levels significantly decreased. This new observation is confirmed with three widely, accepted methods. In patients with low serum albumin (liver cirrhosis), the binding capacity correlated well with the clinical stage of the disease. The frequency of low T3 syndrome also depends on the analytical method. In sera with low albumin and/or low IgG levels, the concentrations of the two proteins were manipulated in vitro by addition of exogenous proteins to the tubes. The bias of fT4 and fT3 exhibited positive correlations with the resulting protein concentrations. In this respect, the effect of albumin was more pronounced than that of IgG.

Macroprolactin (MPRL) was present in 21-23 % of sera with > 700 mU/L prolactin levels. Morphological abnormalities in pituitary imaging in the patients with dominant form of circulating biologically active monomeric prolactin (true hyperprolactinemia, tHPRL) were, also significantly more frequent (in 22-35 % of the tHPRL patients compared to the 5-10 % in MPRL patients). Leading clinical symptoms of hyperprolactinemia were also much more frequent in the tHPRL patients. It was a new finding that in 15-28 % of the female patients with high prolactin levels, both MPRL and tHPRL occurred simultaneously. Likewise, it has never been observed earlier that the prevalence of MPRL increases in women with advancing age. Summarizing, the results support the view that the measurement of sensitive TSH may compensate for the relative uncertainty of peripheral thyroid hormone assays. The frequency of both T3 hyperthyroidism and the low T3 syndrome may depend on the analytical method used by the laboratory. The fT4 level is decreased not only in pregnancy but also in women taking oral contraceptives may raise the possibility of a laboratory artifact in patients with high TBG. We have also described that free thyroid hormone levels depend on serum albumin and IgG level. This finding supports the view that pathological thyroid hormone levels in seriously ill patients may derive not only from a physiological counter-regulation due to the inhibition of anabolic pathway but may also be evaluated as a laboratory artifact. Screening for macroprolactinemia may help avoid unnecessary and expensive radiological investigations and bromocriptine therapy. However, since MPRL and tHPRL may occur simultaneously, the concept of "free prolactin" should be introduced and interpreted for the clinicians.

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**DÓRA TÖRÖK (2005)**

## Current diagnostic considerations in congenital adrenal hyperplasia from birth until puberty

Supervisor: Dr. János Sólyom

- *Congenital adrenal hyperplasia (CAH) is a potentially life-threatening adrenal disorder. In the past twenty years in several countries neonatal screening for CAH was introduced. This changed the diagnostic practice of CAH, therefore the classical diagnostic methods should be reevaluated. There is a lack of information about the false negativity rate of the neonatal CAH screening. We determined the false negativity rate by means of retrospective screening of stored neonatal blood spot samples. We found that prolonged storage, e.g. a decade, decreased the 17-hidroxiprogesterone (17-OHP) by some 2-3% yearly. If the samples were autoclaved, the original 17-OHP concentration can be assessed by an additional mathematical correction step with acceptable precision. Stored blood spot samples, autoclaved or not, are therefore appropriate candidates for retrospective population studies based on 17-OHP measurements. In the retrospective screening blood spot 17-OHP concentrations were measured and analyzed exactly the same way as in the neonatal screening programs. We found that patients with the life-threatening salt-wasting form were all identified by the screening, on the contrary, one third of the simple virilizer patients, i.e. 10% of all CAH patients were not identified by the screening. In Hungary CAH is not included in the neonatal mass screening program. Blood spot 17-OHP measurement is available for patients suspected to have CAH based on clinical signs and symptoms. We reviewed our past twenty years experience with this method. We found that all patients with the salt-wasting form were identified, however, the salt wasting crisis in boys cannot be prevented without a mass screening. Patients are diagnosed remarkably later without screening, which is unwelcome for the long-term outcome. Incidence of CAH in Hungary without mass screening was found to be 1:15 000, which is very similar to those found in Europe by the neonatal mass screening programs. The results of the blood spot 17-OHP measurements should be verified by at least measurement of 17-OHP in the serum. One of the most popular tests for verification is ACTH-stimulation test. By retrospective analysis of 280 ACTH-stimulation tests we found that in most of the cases this method differentiates clearly the CAH patients from the healthy population, however, a minority of the cases with moderately elevated 17-OHP response to ACTH should be further investigated by more sensitive and specific methods.*
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**EDIT ÁGNES TÓTH (2005)**

## The male osteoporosis-pathogenesis and therapy

Supervisor: Dr. Csaba Horváth

The objective of this study was to examine the appearance of osteoporosis in men. My new establishments to conclude the results are as follows: 1. There is a little population-based data concerning Hungarian prevalence rate of male osteoporosis. According to our results and international observations the incidence of male osteoporosis must be higher than we thought earlier. 2. Chronic back pain is frequently caused by osteoporosis in men. 3. Excessive alcohol consumption highly contributes to bone loss among Hungarian men. 4. Beside the older age the low body weight are considered to be risk factors for bone loss in men. 5. We do not have exact data about the prevalence of idiopathic and

secondary osteoporosis in men, but idiopathic male osteoporosis seems to be more common among middle-aged men in our study. 6. Between the secondary causes of osteoporosis the alcohol-induced male osteoporosis might be more frequent in Hungary. 7. Total testosterone and estrogen levels are not correlated to the decrease of BMD at any skeletal sites. 8. The age related decrease in adrenal androgens may contribute to the involutional bone loss in men. 9. The decrease of serum 25-hydroxycholecalciferol is also very common among men. 10. Over age 65 in men, vitamin D deficiency induces secondary hyperparathyroidism, which causes bone loss. 11. Bone fracture is a common symptom of osteoporosis in men. It is more frequent in secondary osteoporosis, however more serious fractures develop in idiopathic osteoporosis. 12. The modified Minne method is an accurate and sensitive method, which can also be used to estimate vertebral deformities in men. 13. Vertebral deformities are very common cause of idiopathic osteoporosis that can be established by an objective method. 14. Beside bone mass, so-called "non-mass" properties have role on development of vertebral deformities. 15. The SOS, which is one of the QUS parameters, gives further BMD-independent information on bone and that is able to predict the risk of vertebral fractures in men. 16. Calcitonin treatment induces bone mineral acquisition in men with idiopathic osteoporosis. 17. The risk of having further vertebral fractures can be reduced by 30% on calcitonin treatment in idiopathic osteoporosis. 18. Active vitamin D treatment can be used as an alternative of antiresorptive therapy in idiopathic male osteoporosis, especially in relative vitamin D deficiency.

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## 2/8. PROGRAM

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### PHYSIOLOGY AND PATHOLOGY OF THE MUSCULOSKELETAL SYSTEM

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The Ph.D. Program is designed for medical doctors who wish to be specialized in basic science and clinical research of musculoskeletal medicine, orthopaedics, trauma surgery and rheumatology. Our aims are: to provide medical and science based students with comprehensive knowledge in the field of orthopaedics and trauma surgery, and rheumatology, and surgery of the bone and soft tissue tumors, to provide suitable environment for clinical or biomechanical laboratory based research projects, to enable students for the use of laboratory techniques such as classical histology, immunohistochemistry, collagen typisation and to train students in modern biomechanical laboratory techniques, as gait analysis.

#### *Sub-programs*

Alterations of the different tissues and kinetic changes in developmental and musculoskeletal disorders  
 Biomechanical examinations of the musculoskeletal system  
 Gait analyses of the movements in musculoskeletal diseases

#### *Supervisors*

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**PÉTER GERGELY (2004)****The role of mitochondrial and intracellular biochemical parameters in systemic lupus erythematosus**

*Supervisor: Dr. Gyula Poór*

Peripheral blood lymphocytes (PBL) from systemic lupus erythematosus (SLE) patients exhibit increased spontaneous and diminished activation-induced apoptosis. In my thesis I tested the hypothesis that key biochemical checkpoints play an important role in the abnormal signaling process in SLE. The significance of these parameters, i. e. mitochondrial transmembrane potential ( $\Delta\Psi_m$ ), reactive oxygen intermediate (ROI) levels, intracellular pH (pHi), ATP and glutathion levels in apoptosis has recently been identified.  $\Delta\Psi_m$ , ROI production and pHi were elevated in lymphocytes from SLE patients compared to controls including healthy donors and patients with rheumatoid arthritis. Intracellular glutathion contents were diminished in SLE suggesting increased utilization of reducing equivalents.  $H_2O_2$ , a precursor of ROIs, increased  $\Delta\Psi_m$  and caused apoptosis in normal PBL. In contrast,  $H_2O_2$ -induced apoptosis and elevation of  $\Delta\Psi_m$  were diminished, particularly in T cells, and necrosis was increased instead in SLE. Intracellular ATP content and ATP/ADP ratio, which had earlier been shown to determine the form of cell death, were reduced and correlated with  $\Delta\Psi_m$  elevation in SLE. As a model, CD3/CD28 co-stimulation led to a transient elevation of the  $\Delta\Psi_m$  followed by ATP depletion and sensitization of normal PBLs to  $H_2O_2$ -induced necrosis. Oligomycin, an inhibitor of  $F_0F_1$ -ATP-ase, had similar effects. CD3/CD28-induced T cell activation in SLE was also blunted as measured by diminished increase in pHi,  $\Delta\Psi_m$ , and ROI production. IL-10, a cytokine known to play an important role in the pathogenesis of SLE, led to a transient elevation of  $\Delta\Psi_m$  but caused no apoptosis in normal PBL. In contrast, IL-10 induced ROI production and apoptosis in SLE lymphocytes without affecting  $\Delta\Psi_m$ . IL-10 blockade with neutralizing antibodies or antagonistic IL-12 normalized baseline and CD3/CD28-induced changes in ROI production and pHi with no impact on  $\Delta\Psi_m$  of lupus PBL. This work concludes that mitochondrial hyperpolarization, increased ROI production, intracellular alkalization and ATP depletion as well as altered IL-10 responsiveness play a crucial role in defective T cell activation and apoptosis in SLE. These findings may not only contribute to the better understanding of the pathogenesis of SLE but may also represent novel targets of pharmacologic intervention.

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PÉTER CSERHÁTI (2005)

## Further development of osteosynthesis in medial femoral neck fractures by repeated prospective international epidemiological surveys

Supervisor: Dr. Károly Fekete

Since 1988 the author has been one of the members in the team, studying issues of hip fractures from 1953 onwards in the National Institute of Traumatology and Oxyology. He performed retrospective analysis of primary treatment and comparison with the patients' state of health 2-8 years later in 122 injured, conservatively treated for non-displaced femoral neck fracture, and 125 patients treated with osteosynthesis in 1985-90. These data showed the advantages of surgery: the length of immobilization and in-hospital stay was shorter, and there were no cases of secondary displacement.

In 1990 he participated in the prospective comparison of primary treatment and 4 months rehabilitation of 583 Swedish and 754 Hungarian patients with hip fractures, within the 'Multicenter Hip Fracture Study'. He also assessed the state of the latter 1 and 5 years after the injury. Their data demonstrated insufficiency of rehabilitation in Hungary, and resulted in changes of treatment of femoral neck fractures: nailing was exchanged with the more advantageous percutaneous technique. In 1993-94 assessments of 489 patients, operated with the new technique and their 3-4 year follow-up were made. Percutaneous fixation with cannulated screws proved to be more advantageous compared to nailing with exposure in respect of the length of hospital stay, mortality and late functional state. In view of the less significant than expected decrease of the rate of wound haematoma and infections as well as the increase of the number of redisplacements, after performing etiological assessment they made several changes in the instrumentation and technique.

In 1997-98, based on the treatment and 4 months follow-up of 5064 hip fractures, registered in 10 countries within the 'Standardized Audit of Hip Fractures in Europe', several observations were presented about the relationship between the social structures and the outcome. The rate of complications, observed after 240 internal fixations with cannulated screws significantly decreased as compared to former years. In 1997 internal fixation with cannulated screws, designed in Budapest, was introduced at the Hannover Clinic of Traumatology. Increase in the number of redisplacements and non-union cases after 205 operations can be explained with the difficulties of changes in the practice after arthroplasties. The very low rate of wound complications and early mortality depended on the immediate operation. In view of the good results the method was introduced also in another German institute in 1998.

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The subject of pulmonology comprises diseases of major public health importance, ie. chronic obstructive lung disease (affecting 3-5% of adult population), bronchial asthma (200 000 patients in Hungary), lung cancer and tuberculosis. This Program offers research opportunities and advanced training for physicians interested in pulmonological science. The 8 sub-programs cover the major areas of respiratory research and students will acquire specialized clinical skills, physiology, microbiology, biochemistry and molecular biology laboratory experience. In addition, the Program offers comprehensive courses in clinical pulmonology and basic science.

**Sub-programs**

Pulmonary allergic diseases

Pathomechanism of chronic obstructive lung disease

Regulation of bronchial and vascular tone in pulmonological diseases

Infections of the lower respiratory tract

Allergic pulmonary diseases II. Role of inheritance, food and physical stress

Lung cancer

Clinical pathology and experimental study of pneumoconiosis

Examination of respiratory disturbances in neurological disorders

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a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

**ZOLTÁN BÁRTFAI (2003)**

## **New methods for primary recovery of *Mycobacterium tuberculosis* and determination of rifampin resistance based on culture and nucleic acid amplification**

*Supervisor: Dr. Ákos Somoskövi*

Tuberculosis continues to be a great challenge all over the world. The cause of recent epidemic is multifactorial, including economic and social factors, synergism between the human immunodeficiency virus and *Mycobacterium tuberculosis* and spreading of multidrug resistant tuberculosis, what emerged as an important problem by the end of the XX. century. For these reasons the present recommendations of Center for Disease Control and Prevention should be kept in mind, it states that the complex isolation, identification and further resistance examinations of *Mycobacterium tuberculosis* should be performed within 2-3 weeks (isolation, identification) and 2-5 weeks (resistance tests) after sample taking. Routine use of new liquid media and the more rapid and accurate detection of antituberculous – especially rifampicin–resistance – are essential factors for putting these recommendations into daily clinical practice. Using two different liquid media and one conventional solid medium, a total of 57 mycobacterial isolates (*Mycobacterium tuberculosis*, n = 55; nontuberculous mycobacteria, n = 2) were recovered from 377 clinical specimens. The rates of recovery of *Mycobacterium tuberculosis* were 96,4% with the BACTEC MGIT 960 liquid medium, 92,7% with BACTEC 12B liquid medium, and 81,8% with the Löwenstein-Jensen medium. The mean time to detection of *Mycobacterium tuberculosis* in smear-positive specimens was 12,6 days for BACTEC MGIT 960 medium, 13,8 days for BACTEC 12B medium, and 20,1 days for Löwenstein-Jensen medium, and in smear-negative specimens it was 15,8 days for BACTEC MGIT 960 medium, 17,7 days for BACTEC 12B medium, and 42,2 days for Löwenstein-Jensen media, respectively. In conclusion, the nonradiometric, fully automated 7-ml BACTEC MGIT 960 system can be considered a viable alternative to semiautomated, radiometric BACTEC 460 TB system. Two regions of *rpoB* associated with rifampin resistance were sequenced in 29 rifampin-resistant (determined by the proportion method) isolates of *Mycobacterium tuberculosis* obtained from patients from three counties of Hungary. Of the 29 resistant strains, 27 had a mutation either the 81-bp region (26 strains) or the N-terminal region (1 strain), while the other 2 strains had no mutations in either region. The locations and frequencies of the mutations differed from those previously reported. The most common mutation in this study, D516V, was found in 38% of the Hungarian strains, a frequency 2 to 10 times higher than that found in studies from other countries. These same 29 isolates were also evaluated with the Inno-LiPA Rif. TB test (LiPA), a reverse hybridization assay for the rapid detection of rifampin resistance. Although LiPA detected the presence of an *rpoB* mutation in 26 of the resistant isolates, the type of mutation could not be determined in 4 isolates because the mutations present were not among those included on the LiPA strip. In addition, a silent mutation in one of the rifampin-susceptible control strains was interpreted as rifampin resistant by LiPA. These find-

ings demonstrate the importance of validating this rapid molecular test by comparison with DNA sequence results in each geographic location before incorporating the test into routine diagnostic work.

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## SZUZSANNA CSOMA (2005)

### Non-invasive assessment of chronic airway inflammation in childhood asthma and primary ciliary dyskinesia

Supervisor: Dr. Ildikó Horváth

Our results reflect that the levels of cys-LT are elevated in exhaled breath condensate collected from asthmatic patients with mild and moderate-to severe persistent disease treated with inhaled steroid. There was no significant increase in the levels of cys-LT in mild intermittent asthma. These results support the role of cys-LT in chronic airway inflammation in pediatric asthma, especially in more severe disease which require regular and in some cases high dose antiinflammatory therapy. The role for LTB4 in the pathomechanism of asthma is less known. According to our results LTB4 may be supposed to take part in the airway inflammation in patients with mild and moderate to severe asthma. Steroids decrease the intensity of airway inflammation in asthma and may also influence its composition enhancing the role of neutrophils and LTB4.

Our results supported the previous finding that exhaled NO may not reflect the total scale of inflammatory processes in asthma. Symptoms, physical examination and lung function used in daily routine for controlling asthmatic patients do not always provide appropriate information about intensity of airway inflammation. Our results demonstrated that NO metabolites in exhaled breath condensate are not decreased in PCD compared to healthy control subjects despite the lower levels of exhaled NO. On the basis of these findings it may be speculated that defects of NOS activity and increased NO metabolism may take place in PCD leading to decreased exhaled NO levels.

Studies with potent NOS inhibitors and NO donors and further investigation of NO biochemistry in the airways using non-invasive methods may make clear the role of NOS and NO in the pathomechanisms of PCD and may also lead to discover new possibilities in the therapy of this disease. Such a low levels of exhaled and nasal NO are not seen in patients with any other conditions. The measurement of exhaled and nasal NO might be used as a screening procedure to detect PCD among patients with recurrent chest infection. The diagnosis of PCD would then be confirmed by specific diagnostic tests. Regular treatment and controls by pulmonologists and ear-nose-throat specialists may prevent irreversible damage of upper and lower airways.

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- Csoma Zs, Kharitonov SA, Donnelly LE, Balint B, Bush A, Wilson NM, Barnes PJ (2003) *Nitrogen oxide metabolites are not reduced in exhaled breath condensate of patients with primary ciliary dyskinesia. Chest 124:633-638.*
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PÁL DEMETER (2004)

## The study on relationship between obstructive sleep apnea, gastroesophageal reflux disease and daytime somnolence

*Supervisor: Dr. Pál Magyar*

There is an increasing mass of evidence for a link between the Obstructive Sleep Apnea (OSA) and Gastroesophageal Reflux Disease (GERD). On the other hand, the investigations focusing on the relationship between sleep related breathing disorders and GERD have indicated the adverse effect of nocturnal reflux events on the sleep structure. In this respect some epidemiological studies have suggested a relationship between GERD and daytime sleepiness. The aims of this study were follows: 1. to assess the relationship between severity of GERD and apnea-hypopnea index as an indicator of severity of OSA, 2. to assess severity of GERD and its relation to intensity of snoring in patient with primary snoring, 3. to assess the relationship between severity of gastroesophageal reflux disease and Epworth sleepiness scale as an indicator of daytime somnolence in GERD patients. All patients underwent upper panendoscopy. 1.-2. underwent apnea monitoring during night and were asked about typical reflux symptoms. In the 3rd group the GERD patients were asked about daytime sleepiness using Epworth Sleepiness Scale. Our study revealed that a positive correlation could be found between severity of GERD and OSA in patients with OSA compared with primary snorers. Severity of GERD was connected with intensity of snoring in primary snorers. Hence we found that severity of GERD influences daytime somnolence.

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GÁBOR HORVÁTH (2004)

## Rapid, nongenomic glucocorticoid actions on vascular smooth muscle cells in the bronchial circulation

*Supervisor: Dr. Endre Vastag*

The main nervous control of the tracheobronchial circulation is provided by the sympathetic nervous system. Sympathetic nerves release norepinephrine (NE) thereby causing vasoconstriction through activation of  $\lambda$ -adrenoreceptors. This signal is partially regulated by the glucocorticoid (GS)-sensitive, extraneuronal catecholamine uptake. Uptake inhibition by inhaled GSs could increase NE concentration at  $\lambda$ -adrenoreceptors and might be responsible for the acute bronchial vasoconstriction caused by inhaled GSs in vivo. Therefore, using cell and molecular biological techniques, we investigated the extraneuronal uptake of NE in our experiments.

We showed that a plasma membrane-associated transporter is responsible for NE uptake into vascular smooth muscle cells. We identified this transporter, namely the extraneuronal monoamine transporter (EMT), by demonstrating its function and mRNA expression. Our further experiments confirmed that GSs have an acute, nongenomic inhibitory effect on EMT. We also showed that systemic ovalbumin (OVA) sensitization ('atopy model') downregulated NE uptake and transporter mRNA expression in vascular smooth muscle. Downregulation shown here could be responsible for the increased bronchial vasoconstrictor responsiveness to inhaled GSs and  $\lambda$ -adrenergic agonists seen in asthmatic subjects. Further studies demonstrated mRNA expression and functional role of EMT in freshly isolated human bronchial arterial SMCs. We also showed that GS action on EMT is a nongenomic steroid action and it is

likely mediated through the activation of specific K<sup>+</sup> channels. Furthermore, we showed that various GSs commonly used in clinical practice (e.g., methylprednisolone and budesonide) acutely interfere with NE uptake. These GS actions in the bronchial circulation could consequently increase NE concentration at its receptor sites, and thus cause vasoconstriction and decrease blood flow.

We believe that our studies characterized an important blood flow regulatory mechanism and identified new therapeutic targets for GSs, although we do not claim that these studies conclusively established a causal relationship of NE uptake inhibition by inhaled GSs and bronchial vasoconstriction. The acute vascular effects of inhaled GSs in the airway have biological and clinical significance and our experiments provided information on these effects.

- Horvath G, Torbati A, Conner GE, Salathe M, Wanner A (2002) Systemic ovalbumin sensitization downregulates norepinephrine uptake by rabbit aortic smooth muscle cells. *Am J Respir Cell Mol Biol* 27:746-751.
- Horvath G, Lieb T, Conner GE, Salathe M, Wanner A (2001) Steroid sensitivity of norepinephrine uptake by human bronchial arterial and rabbit aortic smooth muscle cells. *Am J Respir Cell Mol Biol* 25:500-506.
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**ATTILA KISS (2005)**

## The role of exogen proteases in Th 2 cell differentiation and in the pathogenesis of asthma

Supervisor: Dr. Pál Magyar

Asthma is a chronic disorder of the airways that is characterized by reversible airflow obstruction, eosinophil airway inflammation, persistent airway hyperreactivity and airway remodeling. Defining the immune pathologic basis of allergic inflammation is paramount to understand the unique properties of respiratory allergens which initiate and sustain the allergic inflammation what underlies pathologic processes such as airway obstruction or mucus overproduction. As the innate immune system recognizes conserved molecular patterns by pattern recognition receptors to identify the type of invaders and launch the proper adaptive defense mechanism against intracellular pathogen, there might be other still unknown molecular patterns in allergens, which can be recognized by the host immunity. In order to this we scrutinized different allergens, which have been used in experimental asthma model and those, which play pathologic roles in human allergy and asthma. We have found that all of these allergens contain active proteases and amylases, which have been previously suggested to play pathologic role in human allergy and asthma. The aim of our study was to show whether active amylases or protease play any role in initiating allergic airway inflammation. Using *A. fumigatus*, *A. oryzae* antigens and specific enzyme inhibitors, we have shown that protease activity accounts for the intrinsic allergenic activity of these allergens. Further we have shown that a non-allergic antigen, in combination with protease, is sufficient to bypass airway tolerance induction and initiate allergic lung inflammation. Understanding the role of exogen proteases in allergic lung disease may leads us to establish new strategy in disease prevention and to develop new therapy.

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ÁKOS LANTOS (2004)

## The role of invasive specimen sampling methods, histology and bacteriologic technics in the rapid diagnosis of pulmonary and pleural tuberculosis

Supervisor: Dr. Ákos Somoskövi

Tuberculosis is still one of the most important public health problem in the world. Smear negative pulmonary infiltrate or patients with pleuritis tuberculosa often represent a differential diagnostic dilemma which requires the application of different invasive diagnostic methods. Using special preparation histologic diagnosis from lung and pleura specimens can be obtained within 24 hours. Between 1999 and 2003 lung biopsy was performed via bronchoscopy in 381 individuals and histology identified 22 cases with tuberculosis. In the same period of time pleura biopsy of 422 patients revealed 18 tuberculosis cases. In a portion of these patients the pathogen *M. tuberculosis* could also be isolated by growth detection. All of the pleuritis tbc cases was diagnosed with Ramel-needle biopsy and there was no need to perform pleuroscopy to prove pleuritis tuberculosa.

Using Bactec MGIT 960 and Bactec 12B liquid media and the conventional LJ solid medium, a total of 57 mycobacterial isolates were recovered. In summary, the fully automated, no radiometric MGIT 960 has been shown to be a better alternative to the radiometric Bactec 460 TB for the rapid and reliable laboratory diagnosis of tuberculosis.

Recently, in the Budapest Zoological Garden a Siberian tiger was diagnosed with tuberculosis. With application of a panel of molecular and conventional identification methods the isolated acid fast bacterium from the animal was identified as *M. bovis* subsp. *caprae*. Using this case we show that the differentiation within the *M. tuberculosis* complex using our rapid identification panel is also indispensable for individual patient treatment and for epidemiological purposes.

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- Somoskövi A, Kodmon C, Lantos A, Bartfai Z, Tamasi L, Fuzy J, Magyar P (2000) *Comparison of recoveries of mycobacterium tuberculosis using the automated BACTEC MGIT 960 system, the BACTEC 460 TB system, and Lowenstein-Jensen medium.* *J Clin Microbiol* 38:2395-2397.
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JUDIT MESTER (2004)

## Tuberculosis in Hungary - an overview based upon traditional and molecular epidemiologic methods

Supervisor: Dr. Ákos Somoskövi

Tuberculosis notification rates in Hungary decreased continuously from the 1950s (490/100.000 population) until 1990 (34/100.000 population), an 11,1% average decrease annually. However, between 1990 and 1996 notification rate increased by on average 1,3% annually. It then decreased again, and dropped below the level seen in 1990 until 2002 (30/100.000 population). In provide to collect information on individual risk factors, drug-resistance and the adequacy of the anti-tuberculosis treatment, a national tuberculosis surveillance system was implemented in Hungary in 1996. The still considerably high proportion of previously treated patients and the high proportion (20-25%) of resistant cases in this patient group need more effort to decrease the number of these cases. In order to increase the number of bacteriologically confirmed tuberculosis diagnoses and the number of isolates from previously not treated patients that are tested for drug susceptibility, the mandates for such testing should be strengthened. External and internal quality control events for

mycobacteriological diagnostic methods are also needed. Because the high number of mis/over-diagnosed tuberculosis cases, there is a need for a more comprehensive and broader cohort analysis to uncover the proportion of false-negative or false-positive cases. The numbers of cases among patient groups with relatively high risk factors indicates the need for development of more aggressive case finding measures for this population. The starting anti-tuberculous therapy should still be based on a four-drug regimen, in line with the existing national recommendation.

The new methods in mycobacteriology have significantly reduced the time required to isolate and identify *M. tuberculosis* complex (MTBC) and detect drug-resistant isolates. Using the new liquid medium for isolation in conjunction with the molecular assays provides the correct information on bacteriological diagnoses weeks earlier than conventional "gold standard" methods results became available. The routine application of these methods is an excellent approach to shorten the turn-around time for rapid identification and drug susceptibility testing of the MTBC, therefore the clinician can start adequate anti-tuberculous therapy in time.

In our study we used nucleic acid amplification method to detect drug-susceptibility of strains. Our findings suggest that in East Hungary less common or novel mutations of the *rpoB* gene occur more frequently, which mutations are different from other geographical regions. It seems to be important to validate molecular tests for detection of rifampin (RMP) resistance using DNA sequencing analysis and thus determining the frequencies of particular mutations in the test region before introducing the assay into routine clinical practice. The properly implicated and used molecular assays for RMP susceptibility testing could be helpful for clinicians in early detection of multidrug-resistant (MDR) cases. Our results demonstrated also epidemiological information for clinicians, too. The relatively high rate of less common mutations in the three studied counties suggested, that resistance of RMP and MDR did not develop by transmission but local selection of strains from patients with no compliance.

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## LÁSZLÓ RÓKUSZ (2005)

### Infections of febrile neutropenic patients in malignant hematological diseases

Supervisor: Dr. Ferenc Rozgonyi

At the I Department of Medicine (Oncohematology) of the Central Military Hospital of Hungarian Defense Forces we performed a study together with Oncohematology Team in two periods from the 1995 to 1997 and from 1998 to 2001, to analyse infections among patients with febrile neutropenia following chemotherapy. The aims of the study were: 1. To survey the frequency and distribution of microbiologically and clinically defined infections in febrile neutropenic patients following chemotherapy in two study periods. 2. To examine susceptibility patterns of antibiotics, used in the therapeutic protocols. 3. To survey the frequency of pneumonia and its role in the mortality in two study periods. 4. To examine in vitro and in vivo activities of ciprofloxacin and levofloxacin in Extended-Spectrum  $\beta$ -Lactamase producing *Klebsiella pneumoniae* in an experimental animal model.

Conclusions: 1. During the 132 febrile neutropenic episodes following chemotherapy we observed microbiologically documented infections in 50,8% and clinically documented infections in 21,2% of cases. There were 47 bacteremias and 2 fungemias. In the case of bacteremia we verified Gram-positive organisms in 70,2%. 2. Isolates of coagulase-negative staphylococci from blood stream infections were resistant to oxacillin. We have not found glycopeptide resistant strains yet. In one case we found an imipenem/cilastatin resistant *Pseudomonas aeruginosa* strain. Among those antibiotics used in the current antimicrobial protocols we have not found multidrug resistance to the

Gram-negative strains. 3. In our study among 132 febrile neutropenic episodes following chemotherapy we observed pneumonia in 26 cases (19,7% of documented infections) and in 5 cases played a role in the lethal outcome. 4. Based on our experiment the excellent activities of ciprofloxacin and levofloxacin in vitro and in vivo seem to be promising for the treatment of serious infections due to members of the family Enterobacteriaceae producing an extended-spectrum  $\beta$ -lactamase.

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**JUDIT SÁRADY (2004)**

## **Heme oxygenase-1 mediated cytoprotection in acute lung injury**

*Supervisor: Dr. Ildikó Horváth*

Heme oxygenase-1 (HO-1) is an inducible microsomal enzyme that degrades the heme molecule into equivalent amounts of biliverdin, free iron and carbon monoxide (CO). It can be induced by heme and a battery of non-heme cellular stressors including lipopolysaccharide (LPS), hyperoxia, hypoxia and cytokines. HO-1 has been shown to exert cytoprotective effects including potent anti-inflammatory properties and therefore plays a vital function in maintaining cellular homeostasis. The mechanism however is still elusive.

The objective of this dissertation was to investigate the role of HO-1 in vitro and in vivo in models of LPS-induced acute lung injury. We hypothesized that one of the by-products, the gaseous molecule CO, was mediating HO-1 induced cytoprotection. To test this hypothesis, animals and cells were exposed to low concentrations ranging from 10-250 parts per million (ppm) of CO. CO effectively abrogated the inflammatory response, consistent with previous results observed with HO-1 and imparted a potent defense against lethal endotoxic shock. These anti-inflammatory effects were studied by measuring pro- and anti-inflammatory cytokine levels and examining end-organ damage. CO inhibited the pro-inflammatory cytokine, granulocyte macrophage-colony stimulating factor (GM-CSF) production in RAW 264.7 macrophages and reduced LPS-induced multi-organ failure by differentially modulating inducible nitric oxide synthase (iNOS) and nitric oxide (NO) expression as well as nuclear factor  $\kappa$ -B (NF- $\kappa$ B)-activation in vivo in the lung and liver which correlated in vitro in alveolar macrophages and hepatocytes.

Taken together this dissertation presents further evidence that CO, a product of HO-1, is cytoprotective mimicking that otherwise observed with HO-1. As an anti-inflammatory agent, CO at low concentrations may thus prove to be a beneficial therapeutic modality in the treatment of acute lung injury and other inflammatory disease states.

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ÉVA VIZI (2005)

## The role of the increasing endogenous adenosine level in exercise-induced bronchoconstriction

*Supervisor: Dr. Ildikó Horváth*

1. Our results indicate that exercise-induced increases in adenosine and its metabolites are significantly higher in patients with asthma than in healthy subjects. In patients with asthma, the increase in adenosine was related to the decrease in oxygen saturation, however, the degree of airway obstruction was related to the increase in adenosine.

2. The findings of this study show that adenosine measurement in exhaled breath condensate is reproducible and that adenosine concentration is elevated in steroid-naive asthmatic patients and in patients with worsening of asthma symptoms, than in healthy control or in stable asthmatic patients.

3. Our data showed that exercise-induced airway obstruction was associated with pronounced increase in adenosine concentration in exhaled breath condensate in asthmatic airways, but didn't change in normal subjects. The post-exercise changes in airway function and in adenosine concentration were related.

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### 2/10. PROGRAM

## OPHTHALMOLOGY: THE PHYSIOLOGY AND PATHOLOGY OF VISION

### *Coordinator*

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Vision is essential for human life, but not endangered by several frequent eye diseases, the treatment of which has not been completely established. The Ph.D. Program in Ophthalmology addresses the pathophysiology and pathology of the most important eye diseases. The research projects cover the diseases of the light refractive media of the eye, the biochemical and histological alterations important for vision, and the experimental models of the ocular alterations. Ocular blood flow and aqueous humour dynamics are especially important topics of the Ph.D. school. Glaucoma, one of the blinding eye diseases with the highest prevalence is investigated with special emphasis.

### *Sub-programs*

Diseases of the ocular refractive media

Disease of the macula lutea

Ocular fluid dynamics

### *Supervisors*

Ildikó SÜVEGES

György SALACZ

Piroska FOLLMANN

Ocular ultrasonography	János NÉMETH
Early diagnosis of glaucoma	János NÉMETH
Physiology/pathophysiology of the retina	Ágnes FARKAS
Ocular blood flow, apoptosis, neuroprotection	Gábor HOLLÓ

**Ph.D. students**

Béla Erdélyi	pt (a)
Mária Éva Ferencz	pt
Orsolya Fiedler	pt
Krisztina Hagyó	ft
Balázs Lesch	ft
János Nemes	pt
Mónika Popper	ft (a)
Miklós Schneider	pt (a)
Enikő Siklódi-Nemes	pt
Gábor Márk Somfai	ft (a)
Viktória Szabó	ft (a)
Ágnes Ildikó Takács	ft
Márta Tóth	ft
Balázs Varsányi	ft (a)
Rita Vámos	pt

**Supervisors**

János Németh
György Salacz
Ágnes Farkas
János Németh
Ágnes Farkas
György Salacz
Ildikó Süveges
Ildikó Süveges
Ildikó Süveges
György Salacz
Zoltán Zsolt Nagy
Ildikó Süveges
Gábor Holló
Ágnes Farkas
Ágnes Farkas

**Ph.D. candidates**

Árpád Bereczki	pt
Katalin Gombos	na
Andreas Katsanos	it
Miklós Resch	pt

**Supervisors**

Ildikó Süveges
György Salacz
Gábor Holló
Ildikó Süveges

**Ph.D. graduates**

György Szabolcs Barcsay	ft
Ágnes Füst	pt
Zsófia Intódy	ft
Ágnes Kerényi	na
Péter Kóthy	ft
Nóra Szentmáry	pt

**Supervisors**

János Németh
Ildikó Süveges
János Németh
Ildikó Süveges
Gábor Holló
Ildikó Süveges

a: absolutorium, ft: full-time, pt: part-time it: international, na: non-affiliated

**GYÖRGY SZABOLCS BARCSAY (2005)****Ultrasonic and optical morphometry of the eye; the effect of the parameters' scatter on the measurement accuracy**

*Supervisor: Dr. János Németh*

Improper emmetropization leads to refractive error, but we have observed signs of emmetropization in all refractive groups. We have made a new objective scheme to classify the refractive errors on the basis of axial length (Al) and corneal refractive power. Using this scheme we have determined within each group the percentage of eyes in which the error was caused by the deviation of Al, corneal refractive power, or both. We have found that – in contrast with the general opinion – in 83% of medium and highly myopic eyes the error is not caused by Al alone, but corneal refractive power also contributes to it.

A further effect of emmetropization is that the morphometric parameters scatter in a wide range even among eyes with the same refraction, which affects the precision of several measurement methods in clinical practice. This has to be considered during planimetry. Our results show that in case A1 is not taken into account during the correction of measured values, the error exceeds 5% in 7% of the eyes. Therefore, we think it necessary to measure A1 for planimetry.

The error resulting from the correction of measurements can be avoided in dynamic studies, when relative changes are followed. This is possible during studies of the changes of retinal branch vessel diameters with the new Retinal Vessel Analyzer (RVA). We have proven that recordings made with the near-infrared light of a Scanning Laser Ophthalmoscope can reliably be analyzed with the RVA. Using this method we have proven that the diameters of retinal branch vessels do not change relevantly during dark adaptation, thus it is sufficient to concentrate on flow velocities in similar studies. The natural scatter of central corneal thickness (CCT) affects the precision of Goldmann applanation tonometry. Based on our results we reached the conclusion that applanation tonometry readings cannot be directly corrected using CCT, although theoretically the parameter itself may be apt for it. However, it can only be proven by invasive studies, in contrast with the ordinary approach of the problem.

- Barcsay Gy, Seres A, Németh J (2003) *The diameters of the human retinal branch vessels do not change in darkness. Invest Ophthalmol Vis Sci 44:3115-3118.*
- Barcsay Gy, Nagy ZZs, Németh J (2003) *Distribution of axial, corneal and combined ametropia in a refractive surgery unit. Eur J Ophthalmol 13:739-744.*
- Rondeau MJ, Barcsay Gy, Silverman RH, Reinstejn DZ, Chabi A, Krishnamurthy R, Coleman TD, Coleman DJ (2004) *Very high frequency ultrasound biometry of the anterior and posterior chamber diameter. J Refract Surg 20:454-464.*

ÁGNES FÜST (2003)

## The effect of surface excimer laser treatment of the cornea and its environment - experimental and clinical study

Supervisor: Ildikó Süveges

The aim of my dissertation is the examination of the effect of the 193-nm excimer laser treatment on the cornea and on the composition of the tear film, which covers the cornea, and to estimate the results of certain types of refractive and therapeutic treatments. We found that the excimer laser gives rise to such physical changes which permits to remove tissue from the surface layers of the cornea in a well controlled way, and the environment does not suffer considerable damage. On the other hand, during the wound healing scar tissue develops, which later on influences the refractive properties of the cornea, and so the success of the treatment, too. The shape of the ablation and the type of the original refractive error also influence the result of the photorefractive keratectomy. In this way, although the laser treatment of the hyperopic patients reduced the refractive error in every case, the predictability and the stability were less than in the case of myopia. Our experiences with the phototherapeutic treatments show that the granular dystrophy can be treated effectively with excimer laser, and because of the danger of hyperopic shift, it is advisable to treat the minimum in one section. When the refractive or therapeutic treatment is carried out on a previously operated or injured cornea (radial keratotomy, corneal transplantation, alkali burn), generally there are more complications (multilayered endothelium, severe scar formation, corneal decompensation) than in cases of intact corneas. Changes in the protein secretion of the lacrimal gland were experienced as an acute side effect of the excimer laser corneal ablation.

- Füst Á, Németh J, Süveges I, Nagy ZZ, Bereczki Á (1998) *Excimer laser treatment of hyperopia. Acta Ophthalmol Scand 76:686-691.*
- Bor Zs, Hopp B, Rácz B, Szabó G, Márton Zs, Ratkay I, Mohay J, Süveges I, Füst Á (1993) *Physical problems of excimer laser cornea ablation. Opt Eng 32:2481-2486.*
- Füst Á, Veres A, Kiszkel P, Nagy ZZs, Cervenak L, Csákány B, Maka E, Süveges I, Grus FH (2003) *Changes in tear protein pattern after photorefractive keratectomy (PRK). Eur J Ophthalmol 13:525-531.*

ZSÓFIA INTÓDY (2003)

## Blocking the rhodopsin gene expression by triple helix forming oligonucleotides

Supervisor: Dr. János Németh

To explore the ability of triplex forming oligodeoxyribonucleotides (TFOs) to inhibit genes responsible for dominant genetic disorders, we used two TFOs to block expression of the human rhodopsin gene, which encodes a G-protein-coupled receptor involved in the blinding disorder autosomal dominant retinitis pigmentosa. First, an optimal system for studying rhodopsin expression in tissue culture cells was developed. A plasmid (pSRG) containing the human genomic rhodopsin gene fused to the GFP gene was constructed and transfected into cells. Detecting green fluorescence proved to be an easy and reliable way to follow the expression of the rhodopsin-containing protein. Psoralen-modified TFOs and UVA-irradiation were used to form photoadducts at two target sites in the pSRG plasmid, which was then transfected into HT1080 cells. Each TFO reduced rhodopsin-GFP expression 70-80%, whereas treatment with both reduced expression 90%. Expression levels of control genes on either the same plasmid or one co-transfected were not affected by the treatment. Mutations at one TFO's target eliminated its effect on transcription, without diminishing inhibition by the other TFO. Northern blots indicated that TFO-directed psoralen photoadducts blocked progression of RNA polymerase, resulting in truncated transcripts. Irradiation of cells after transfection with plasmid and psoralen-TFOs produced photoadducts inside the cells and also inhibited expression of rhodopsin-GFP. Inhibition of gene expression in human cell lines was not relieved over a 72-hour period, suggesting the TFO-induced psoralen lesions are not repaired on this time scale. However, gene expression recovered in both repair-proficient and repair-deficient Chinese Hamster Ovary (CHO) cells, demonstrating that the duration of such a transcriptional block can vary dramatically among cell lines. Further studies are needed to elucidate the mechanisms by which such DNA damage is handled in mammalian cells. The main conclusion of my experiments is that directing DNA damage with psoralen-TFOs is an efficient and specific means for blocking transcription from the human rhodopsin gene.

- Intódy Z, Perkins BD, Wilson JH, Wensel T G (2000) Blocking transcription of the human rhodopsin gene by triplex-mediated DNA photocrosslinking. *Nucleic Acids Res* 28:4283-4290.
- Sargent RG, Meservy JL, Perkins BD, Kilburn AE, Intódy Z, Adair GM, Nairn RS, Wilson JH (2000) Role of nucleotide excision repair gene ERCC1 in formation of recombination-dependent rearrangements in mammalian cells. *Nucleic Acids Res* 28:3771-3778.
- Vasquez KM, Marburger K, Intódy Z, Wilson JH (2001) Manipulating the mammalian genome by homologous recombination. *Proc Nat Acad Sci USA* 94:8403-8410.

ÁGNES KERÉNYI (2004)

## Surgical treatment of corneal diseases

Supervisor: Dr. Ildikó Süveges

Due to the problems encountered during the surgical treatment of corneal diseases, the aim of the study was to choose the most suitable method in the treatment of pterygia, to solve some methodological problems in penetrating keratoplasty (PKP) and to study the complement activation in the early postkeratoplasty period.

Pterygium surgery was combined with limbal-conjunctival autotransplantation, intraoperative mitomycin C application and amniotic membrane transplantation in three consecutive series of operations. The first two methods were found to be effective and safe on condition that certain pre-, intra- and postoperative measures were respected. The advantage of amniotic membrane grafts is in avoiding normal conjunctival areas and the risk induced by using antimetabolites.

Eccentric PKP sparing the central cornea was performed in cases of peripheral corneal disorders re-

quiring corneal grafting and not involving the central area of the cornea. Depending on the extent and shape of the disorder, the grafts were round or biconvex. Due to the tectonic effect and functional results, this method seems to be an option in some eyes with perforated peripheral corneal disorders.

Corneal topography indicate that peripheral, biconvex PKP may result in a regular central anterior corneal surface and thus a good visual acuity. Two suturing techniques during PKP were compared. Double diagonal running sutures were safe in wound apposition. Mean postoperative astigmatism and keratometry results after suture removal were comparable in the double running and single running suture groups. Increased concentration of C1rs-C1inh in several tear samples taken early after human PKP was demonstrated. This provides direct evidence that the classical pathway of complement may be activated in the early postoperative period after PKP.

- Kerényi Á, Nagy Gy, Veres A, Varga L, Füst Á, Nagymihály A, Czumbel N, Süveges I, Füst Gy (2002) C1r-C1s- C1inhibitor (C1rs-C1inh) Complex measurements in tears of patients before and after penetrating keratoplasty. *Curr Eye Res* 24:99-104.
- Kerényi Á, Süveges I (2003) Corneal topographic results after eccentric, biconvex penetrating keratoplasty. *J Cataract Refract Surg* 29:752-756.
- Farsang Cs, Debreczeni L, Kerényi A, Takács L (1979) Regulation of myocardial oxygen consumption by perfusion pressure in isolated fibrillating canine heart. *Pflügers Arch* 379:223-228.

## PÉTER KÓTHY (2004)

### Modern diagnostics and treatment of glaucoma

Supervisor: Dr. Gábor Holló

Glaucoma is an irreversible eye disease, which causes severe visual impairment. It cannot be healed, but in most cases early diagnosis and appropriate treatment prevents the significant visual damage. Using cutaneous cold provocation cold-induced retinal capillary perfusion changes were investigated in vasospastic control and capsular glaucoma patients with the scanning Doppler flowmetric Heidelberg Retina Flowmeter. The changes were similar in the two groups: retinal and optic nerve head flow decreased immediately. The flow reduction diminished due to warm bath of the hand, and tended to return to the baseline value.

Using the digital Retinal Vessel Analyser diameter changes of the human retinal arterioles were investigated for instillation of different IOP decreasing eye drops (betaxolol, brimonidine, brinzolamide, latanoprost and timolol). No change was observed for any of the tested eye drops. We investigated the measurement accuracy and reliability of the Ocuton A and S tonometers using Goldmann tonometry as gold standard. Accuracy was found satisfactory. Diurnal intraocular pressure (IOP) curve obtained with the Ocuton S and the Goldmann tonometers were different: the Ocuton tonometry underestimated the IOP in the night and at daybreak compared to the Goldmann tonometry.

Scanning laser tomographic HRT II was evaluated for cross-sectional glaucoma diagnostic purpose and mass screening. The sensitivity was relatively low, therefore the recent software cannot be considered for glaucoma screening. The instrument, however, may be useful in the diagnosis of difficult cases. IOP lowering efficacy and tolerance of two non-selective beta-receptor blocking eye drops (levobunolol and timolol) were compared in a crossover study. No significant difference was found in the glaucoma groups tested, which shows that the clinical value of the two medications is equal.

Latanoprost (a PGF<sub>2a</sub> analogue) medication was retrospectively evaluated for efficacy and tolerance. In the study population of one hundred randomly selected glaucoma patients introduction of the topical latanoprost in the medication resulted in 23 % further IOP reduction. This reduction, as well as visual functions remained stable during the follow-up. Tolerance of the drug was excellent.

- Kóthy P, Vargha P, Holló G (2001) Ocuton-S self tonometry vs. Goldmann tonometry; a diurnal comparison study. *Acta Ophthalmol Scand* 79:294-297.
- Kóthy P, Vargha P, Holló G (2003) Glaukomreihenuntersuchung mittels Heidelberg-Retina-Tomograph II. *Klin Monatsbl Augenheilkd* 220:540-544.
- Holló G, Kóthy P, Lakatos P, Vargha P (2002) Endothelin-A receptor antagonist BQ-485 protects against intraocular pressure spike induced by laser trabeculoplasty in the rabbit. *Ophthalmologica* 216:459-462.

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**NÓRA SZENTMÁRY (2005)**

## Examination of corneal dystrophies

*Supervisor: Dr. Ildikó Süveges*

In conformance with studies performed at the United States, Canada and Denmark, the most frequent indication at the 1st Department of Ophthalmology of the Semmelweis University for PK was pseudophakic / aphakic bullous keratopathy. Fuchs' dystrophy was the sixth, other corneal dystrophies were the eighth indication for penetrating keratoplasty.

In stromal corneal dystrophies penetrating keratoplasty is a safe procedure in improvement of visual acuity without early complications and may provide better best corrected visual acuity for the patients than phototherapeutic keratectomy. However, in patients with stromal corneal dystrophies with or without preceding phototherapeutic keratectomy there was no significant difference in keratometric, topographic and refractive cylinder, in keratometric and topographic central corneal power, in best corrected visual acuity, surface regularity index, surface asymmetry index and potential visual acuity mean values following second suture removal of penetrating keratoplasty (PK). Preceding phototherapeutic keratectomy does not appear to impair the outcome of subsequent penetrating keratoplasty in stromal corneal dystrophy patients.

Phototherapeutic keratectomy is a safe and effective procedure for relieving symptoms of recurrent erosions and improving visual acuity in patients with lattice dystrophy. Besides changes in the distribution and size of corneal deposits, increased apoptotic activity of the corneal cells may also form part of the progression of granular and lattice dystrophy; and this seems definitely to be a concomitant, and possibly a pathogenic, factor in macular and Fuchs' dystrophy. However, the pathways that are triggered to result in increased apoptotic cell death and the genetic factors still remain to be clarified in corneal dystrophy patients.

- Szentmáry N, Langenbucher A, Hafner A, Seitz B (2004) Impact of phototherapeutic keratectomy on the outcome of subsequent penetrating keratoplasty in patients with stromal corneal dystrophies. *Am J Ophthalmol* 137:301-307.
- Szentmáry N, Szende B, Süveges I (2005) Epithelial cell, keratocyte and endothelial cell apoptosis in Fuchs' dystrophy and in pseudophakic bullous keratopathy. *Eur J Ophthalmol* 15:17-22.
- Szentmáry N, Bausz M, Tóth J, Süveges I (2004) Indications of keratoplasty during eleven years in the I. Department of Ophthalmology (1992-2003) – In Hungarian. *Szemészet* 141:387-393.

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## 2/11. PROGRAM

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### RADIOTHERAPY

#### **Coordinator**

**Olga ÉSIK**

University of Pécs

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Tel. (3672)536 480

E-mail:

Accreditation of a Ph.D. Program in 2001 in radiotherapy was very important, as half of the radiotherapists, radiation physicists and radiobiologists with scientific degrees currently active in Hungarian radiotherapy will retire within 5 years. The Ph.D. Program includes the announcement of Ph.D. courses and the provision of scientific work for the Ph.D. students. Accreditation and initiation of Ph.D. Program in radiotherapy would promote the training of adequately-prepared personnel and the establishment of a critical, comprehensive view for further modernization of the training and research. The organized Ph.D. training will ensure the possibility for the forward advancement of the most talented young specialists. During the past 3 years, 8 Ph.D. students took part at the Program, and two of them have finished successfully her education.

*Sub-programs**Supervisors***Clinical radiotherapy**

Olga ÉSIK

The central issue of radiotherapy is the homogeneous or inhomogeneous delivery of a radiation dose, adequate for the quantity and localization of the viable tumour tissue, with simultaneous protection of the critical organs (sensitive to radiation). New, conformal external irradiation and brachytherapeutic techniques are required to increase the survival probabilities of the patients via decrease of the treatment-related morbidity. The most important principle of marketable health care is “qualitative patient care”, a prerequisite of which is application of the up-to-date principles of evidence-based medicine. Attainment of this goal requires the initiation of randomized studies, and the elaboration of consensus-based, efficient, national radiotherapeutic protocols relying on international experience and the results of Hungarian investigations.

Population-based and individual survival probability estimation in different tumours

Olga ÉSIK

The individualized treatment of cancer patients demands individual prognosis estimation. The primary requirement is a critical assessment of the clinical course of previously treated patients of a given histological type, i.e. establishment of a population-based survival function on the basis of data concerning the clinical status, molecular pathological/biological prognostic markers, treatment modalities and follow-up information. This makes possible the prognosis estimation of new patients with the same disease.

**Radiobiology**

Géza SÁFRÁNY

Initiation of the clinical radiobiological research profile is completely new territory, as it has only scanty Hungarian precedents. Collaboration should be built up with research groups which having worked successfully in different fields of this interdisciplinary branch of sciences. Urgent commencement of the radiobiological investigations is very important: radiation physics and radiotherapy planning supported by diagnostic imaging have almost reached the upper limit of their potential; accordingly, further improvement of the therapeutic results can be attained mainly by utilizing biological modalities. Initiation of the radiobiological education and research work is the primary need: only those radiotherapeutic departments can obtain European accreditation where a radiobiological section is functioning.

*Topics*

Genetic background of proliferation and progression in glioma tumours

Éva GÖMÖRI

Cytogenetic manifestation of individual radiation sensitivity

Sarlota GUNDY

Application of bioindicators /biodimeters in radiation therapy

Sarlota GUNDY

Markers of individual sensitivity to radiation or DNA-targeted drugs in cancer therapy

Katalin LUMNICZKY

Complex evaluation of the biological behaviour and prognosis of various tumours based on quantitation of their vascularization, mitotic activity, DNA-ploidy, immunohistochemical phenotype and metabolic characteristics

Péter MOLNÁR

Increasing the radiosensitivity of the tumours by means of gene therapy	Géza SÁFRÁNY
Measurement of individual radiosensitivity in cancer patients, identification of genes responsible for radiation sensitivity	Géza SÁFRÁNY
The late genetic effects of ionizing radiation	Géza SÁFRÁNY
ustright	

*Sub-programs*

*Supervisors*

**Radiation physics**

Judit FIDY

The patient-oriented research work (conformal forward and inverse radiation treatment planning, the quality assurance of the radiotherapeutic process, geometric and dosimetric verification of the radiation treatment fields, and *in vivo* dosimetry involving the use of modelling and measurements) should be in first place for radiation physics.

*Topics*

Combined application of photodynamic therapy and radiotherapy in the treatment of malignant diseases	Gabriella CSÍK
Study on the biological effects of ionizing/non-ionizing radiation by spectroscopic methods in cells/tissues and model systems	Pál GRÓF

*Sub-programs*

*Supervisors*

**Diagnostic imaging**

Lajos TRÓN

The most important input parameter for radiotherapy is the most precise determination possible of the viable tumorous tissue, as this exerts a fundamental influence on the probability of survival of the patients. From this respect, the simultaneous application of modern metabolic and functional (functional MRI, SPECT and PET) and also anatomical (CT and MRI) tomographic methods provides the most powerful tools, and these imaging modalities have therefore become an organic part of modern radiotherapy planning. At the same time, tomographic methods permit the identification of critical organs, ensuring their adequate protection during radiotherapy.

*Topics*

Optimizing tracer kinetic methods to safely analyze the biochemistry of normal tissues damaged through irradiation	László BALKAY
Tomographic methods in 3D radiotherapy planning MRI methods in radiotherapy planning	Ervin BERÉNYI
PET technique of investigating the kinetics of binding in adenosine receptors and their PET isotope-labelled ligands playing a role in control of the CNS and myocardium	Teréz MÁRIÁN
New prospects of MR imaging in oncology processes	Imre REPA
MR-controlled surgical interventions in oncological processes	Imre REPA
Investigations on tissue metabolism by using positron emission tomography	Olga ÉSIK

**Ph.D. students**

Ildikó Hegedűs	pt
Gergely Huszty	pt
Enikő Kis	pt (a)

**Supervisors**

Zsolt Tarján
Géza Sáfrány
Géza Sáfrány

András Polyák	pt (a)	Olga Ésik
Tünde Szatmári	pt (a)	Géza Sáfrány
<b>Ph.D. candiditates</b>		<b>Supervisors</b>
Zsuzsa Póti	pt	Olga Ésik
<b>Ph.D. graduates</b>		<b>Supervisors</b>
Erzsébet Lengyel	na	Olga Ésik
Barnabás Horváth	pt	Zsuzsa Schaff

a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

## ERZSÉBET LENGYEL (2004)

### Special problems in the treatment of the tumours of nasopharynx

Supervisor: Dr. Olga Ésik

*Introduction:* Radiotherapy is the standard treatment of the nasopharyngeal cancer, and the 5-year survival rate is reported to be 35-60%. 15-48 % of the patients have locally persistent, recurrent or new primary tumor in the previously irradiated volume or its vicinity. At present reirradiation is considered the most promising treatment of locally recurrent nasopharyngeal carcinoma. *Patients and methods:* Our patient group consists of 21 reirradiated nasopharyngeal cancers, 9 histologically rare types and 2 cases of long-standing radiogenic Lhermitte's sign. The locally progression-free, the cause-specific and distant metastasis-free survival were computed by the Kaplan-Meier method in patients reirradiated with full dose. The radiation induced side-effects were graded on the basis of the „Common Toxicity Criteria, Version 2.0”. Individual radiosensitivity was examined before reirradiation. The possibly mechanism of regeneration of the radiogenic injury of the spinal cord was investigated by Positron emission tomography (PET). *Results:* In our patient group with nasopharyngeal cancers the cause-specific survival, the locally progression-free and distant metastasis-free survival were 32%, 100%, and 38%. Severe, grade 3 or higher late toxicity has so far been observed in 3 patients. In the background of the development of Lhermitte's sign we found the case 1: higher radiosensitivity than in healthy controls and high biological effective dose (BED) of cervical spinal cord (103,8 Gy), while case 2 radiosensitivity and BED (94,8 Gy) were normal. PET demonstrated increased [18F]-fluorodeoxyglucose (FDG) accumulation and [5O]-butanol perfusion but negligible [11C]-methionin uptake in the irradiated spinal cord segments in both patients. The treatment of the rare histological type of tumor different from the patients with squamous cell carcinoma, the primary treatment is surgery (instead of lymphoma) and the radiotherapy is recommended with the postoperative and palliative intent. *Conclusion:* Retreatment of nasopharyngeal carcinoma with radiotherapy can result in long-term local control and survival in a substantial portion of patients, at the price of an acceptable morbidity. The clinical behaviour and the treatment of the rare types of nasopharyngeal tumors are different from the usually occurred nasopharyngeal cancers. PET-investigation may be useful in the examination of the patients with Lhermitte's sign and help to understand the regeneration mechanism of the spinal cord.

- Lengyel E, Somogyi A, Godény M, Szerdahelyi A, Németh Gy (2000) *Polymorphes Adenokarzinom des Nasopharynx mit niedrigem Malignitätsgrad. Fallbeschreibung und Literaturübersicht. Strahlenther Onkol 176:40-43.*
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- Ésik O, Csere T, Stefanits K, Szakáll Sz Jr., Lengyel Zs, Sáfrány G, Vönöczky K, Lengyel E, Olajos J, Bajzik G, Trón L (2003) *Increased metabolic activity in the spinal cord of patients with long-standing Lhermitte's sign. Report of two cases and review of the literature. Strahlenther Onkol 179:1-4.*

**2/12. PROGRAM****CLINICAL AND EXPERIMENTAL RESEARCH IN ANGIOLOGY****Coordinator****György ACSÁDY**

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The education of clinicians and researchers having profound scientific background is the basic aim of the postgraduate Program, offering a possibility of research work on organ failures due to vascular disorders and of application of the results in the therapeutic processes. The study of the pathogenesis of the diseases of ischemic origin using the latest techniques is also part of the Program.

*Sub-programs**Supervisors*

Chlamydia pneumoniae infection of vessels

Zsuzsa SCHAFF

Experimental investigation of the angiogenesis

György ACSÁDY

Homologous vascular graft implantation in the clinical practice

Attila NEMES

Laboratory diagnostic methods in studying the pathogenesis of aneurysm formation

István KARÁDY

i180Biochemical monitoring of cerebrospinal fluid during thoraco- and thoracoabdominal aortic surgery

Csaba DZSINICH

Endovascular treatment for aneurismal disorders

Kálmán HÜTTL

Relationship between inflammation and blood coagulation in patients with enhanced risk for atherosclerosis

Albert CSÁSZÁR

**Ph.D. students****Supervisors**

Al-Sieady Ali Mohsen

ft

György Acsády

Tamás Benkő

pt

Attila Nemes

Endre Kovács

pt

György Acsády

Krisztina Eszter Marosi

pt

Kálmán Hüttl

Zsófia Panna Patkó

ft

Albert Császár

Zoltán Pencz

pt (a)

Zsuzsa Schaff

Csanád Várallyay

ft

Kálmán Hüttl

Gábor Fónyad

pt

Kálmán Hüttl

Zoltán Szeberin

pt

György Acsády

**Ph.D. candidates****Supervisors**

Barnabás Galambos

pt

Attila Nemes

a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

**2/13. PROGRAM****HORMONAL REGULATORY SYSTEMS****Coordinator****Károly RÁCZ**

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There are numerous important interactions among the hormonal system and neural, immune and other regulatory mechanisms, by which hormones may influence the physiology or pathophysiology of organs or organ systems. The Program includes research projects dealing with interactions between hormones and other regulatory mechanisms, such as neuroendocrine regulation of thyroid and gonadal function, and regulation of pituitary and adrenal hormone secretion. Other research projects include studies on the molecular mechanisms of hormone sensitivity, pathomechanism of metabolic bone disorders associated with endocrine disorders, hormonal disturbances associated with inborn metabolic errors, and the development of microsensors for the detection of gene variants involved in hormonal regulation.

*Sub-programs*

Neurohormonal regulation of thyroid function

Neurohormonal interactions

Development and application of new molecular biologic methods for studying hormonal regulatory systems

Pathomechanism of endocrine disturbances in inborn metabolic disorders

Mechanism of metabolic bone disorders associated with endocrine diseases

Molecular mechanisms of glucocorticoid sensitivity

Interactions between hormonal regulatory mechanisms in disorders of the pituitary and adrenal glands

*Supervisors*

Csaba BALÁZS

Ida GERENDAI

Gábor HARSÁNYI

Ágnes SCHULER

Miklós TÓTH

Károly RÁCZ

Károly RÁCZ

**Ph.D. students**

György Barta pt

Rita Bertalan ft

Boyle Belema ft

László Fűtő pt

Péter Gergics ft

Ágnes Mondok ft

Ágnes Szappanos ft

Márta Sereg ft

Judit Tóke ft

**Supervisors**

Károly RácZ

Károly RácZ

Csaba BalázS

Károly RácZ

Károly RácZ

Károly RácZ

Miklós Tóth

Miklós Tóth

Miklós Tóth

**Ph.D. candidates**

Katalin Balogh ft

Judit Majnik ft

**Supervisors**

Károly RácZ

Károly RácZ

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**2/14. PROGRAM**


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**CLINICAL AND EXPERIMENTAL RESEARCH OF UROLOGICAL DISEASES**
**Coordinator**
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*Sub-programs*
*Supervisors*
**New diagnostic and therapeutic approaches of urological tumour**

Imre ROMICS

Incidence of urological cancers is increasing all around the world. Parallel to this new surgical, diagnostic and therapeutic approaches have been developed. The aim of this topic to evaluate the different methods of urological cancer treatments. Pharmaceutical and surgical treatment of the upper- and lower urinary tract tumours. Curative and palliative therapy of internal and external genitalia cancers

**Genitourinary disorders of inflammatory or igin**

László FARKAS

Genitourinary disorders of inflammatory origin produce the largest number of patients in the everyday practice of urology. The aim of this topic is the research of nosocomial infections occurring during institutional operations, serious, life threatening diseases of inflammatory origin and rare but exciting entity of interstitial cystitis. Nosocomial infections. Interstitial cystitis (IC)

**Congenital and acquired anomalies of the urological tract**

Zsolt KELEMEN

The main issues of this topic are the surgical and conservative treatments of non palpable testis, vesico ureteral reflux (VUR), obstructive uropathies, the primary and secondary healing of hypospadias and healing of urethral strictures iatrogenic and traumatic origin. Balanitis xerotica obliterans is an increasing incidence, unknown origin dermatologic disease on the penis which shows close correlation to penile carcinoma. Congenital anomalies of the urinary tract. Urethral surgery. Balanitis xerotica obliterans

**Diagnostic and therapeutic opportunities for problems of urine keeping and emptying**

Imre ROMICS

Physiology and pathophysiology of the upper- and lower urinary tract is relatively not developed field of urology, although many urological diseases could be interpreted with understanding of normal and abnormal function of it. The aim of this topic to research the function of the urinary tract using urodynamics. Upper urinary tract urodynamics. Lower urinary tract urodynamics

**Ethiopathogenesis and etiology of urinary tract stones, medical and surgical treatment of prevention**

Csaba TÓTH

Urinary stone disease is one of the “classic” chapters of urology, its treatment means a significant part of the daily urological health care. Development of the treatment has been fairly seen since the beginning of the XX. century, and exponentially accelerated during the last decades by introducing newer instruments. The aim of the topic is the summary of etiology, development, explanation of different treatment modalities, discussion of the actual knowledge of the prevention. The cause, the mechanism of formation and position of urinary stones. Medical, extracorporeal shock wave lithotripsy and surgical treatment of urinary stones

### Reconstructive urology

László PAJOR

The incidence of urooncological cases has been showing a significant increasing trend for the last decades. Therefore, the aim of this topic to highlight the minimally invasive but necessary reconstructive surgical procedure for severe urological diseases. Cystectomy for invasive bladder cancer. Intersex operations: male to female and female to male

#### *Ph.D. students*

Katalin Bedi ft  
Fares Mohammed Osman f

#### *Supervisors*

Imre Romics  
Emil Monos

#### *Ph.D. graduates*

András Kiss na  
Péter Nyirády na

#### *Supervisors*

Zsolt Kelemen  
Zsolt Kelemen

a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

## ANDRÁS KISS (2004)

### Treatment options for lichen sclerosus, hypospadias and their complications in paediatric urology practice

*Supervisor: Dr. Zsolt Kelemen*

In the general paediatric urology practice operation due to phimosis is the most frequent procedure. New research data show that penile lichen sclerosus (LS), also called as balanitis xerotica obliterans (BXO) may appear in childhood as phimosis and can cause severe complications. Although the importance of BXO is accepted, the precise incidence of childhood penile LS is not known and there are unmet needs in its treatment. Based on the above reasons, in the first part of my work I investigated the incidence, clinical and histological appearance of childhood BXO and studied the effect of local corticosteroid treatment in the care of this disorder. I demonstrated that in 40% of phimosis cases BXO forms the background and that local corticosteroid treatment effectively attenuates the clinical symptoms of the disease.

While the surgical treatment of phimosis is usually simple, surgical correction of hypospadias is one of the greatest challenges for paediatric urologists. Despite the several known surgical approaches, better functional and aesthetic results can only be achieved by continuous improvement of currently available methods or development of newer techniques. The most frequent complication of hypospadias is the urethral fistula. Therefore, in the second part of my studies I studied the surgical correction methods of the repair of hypospadias and its complications. By using and combining two known techniques I have developed a new method. Our modification uses the combination of the Mathieu and the Snodgrass methods using a deep midline incision of the urethral plate to help to form an anatomically normal looking urethral orifice and providing a good urethra calibre without tense suture lines. With our modification the risk for development of urethral strictures following repair is decreased because this techniques ensures better blood supply and the advantage of the

Mathieu-technique, that suture lines are not in one line above each other, remains, therefore the risk for development of fistula is lower. In the closure of big, recurrent urethral fistulas I have used free buccal mucosa graft, which has not been applied previously with this purpose. Based on our results its use to close recurrent large urethral fistulas is a good and safe therapeutic choice.

- Kiss A, Csontai Á, Pirót L, Nyirády P, Merksz M, Király L (2001) *The response of Balanitis Xerotica Obliterans to local steroid application compared with placebo in children.* *J Urol* 165:219-220.
- Kiss A, Nyirády P, Pirót L, Merksz M. *Combined use of perimeatal based flap urethroplasty (Mathieu) with midline incision or urethral plate in hypospadias repair.* *Eur J Pediatr Surg* 13:383-385.
- Kiss A, Csontai Á, Berényi M (1999) *Urinary bladder stone composed of xanthine in an infant boy.* *Urol Int* 63:4.

**PÉTER NYIRÁDY (2003)**

## **Experimental fetal urinary bladder outflow obstruction perturbs electromechanical properties of detrusor muscle**

*Supervisor: Dr. Zsolt Kelemen*

Congenital urinary bladder outflow obstruction (BOO) caused by posterior urethral valves is a common cause of end-stage renal failure in boys. We hypothesised that fetal BOO perturbs detrusor contractility and innervation and bladder storage volume-pressure relationships. Severe BOO was induced in male fetal sheep by placement of a urethral ring and urachal ligation midway through gestation, at 75 days. These fetuses were examined 30 days after surgery when urinary tract dilatation, enlarged urinary bladders and cystic renal dysplasia were documented. As assessed by force-frequency relationships, obstructed fetal bladder strips were significantly hypocontractile versus sham-operated controls in response to electrical field stimulation and specific agonists carbachol, KCl and  $\alpha$ - $\beta$  methylene-ATP. Hypocontractility was greater with nerve-mediated stimulation than with carbachol, suggesting denervation. Reduced innervation was confirmed by S100 and PGP 9.5 immunohistochemistry, and by measuring a significant reduction in PGP 9.5 protein expression using western blotting. In addition, ex vivo filling cystometry established that the obstructed fetal bladders were more compliant, with a larger capacity, more flaccid and retained stress relaxation. Hence, in response to severe experimental fetal BOO, the urinary bladder becomes hypocontractile, and this is associated with aberrant innervation.

- Nyirády P, Kiss A, Pirót L, Sárközy S, Bognár Zs, Csontai Á, Merksz M (2002) *Evaluation of 100 laparoscopic varicocele operations with preservation of testicular artery and ligation of collateral vein in children and adolescents.* *Eur Urol* 42:534-537.
- Nyirády P, Thiruchelvam N, Fry CH, Godley ML, Winyard PJD, Peebles DM, Woolf AS, Cuckow PM (2002) *Effects of in utero bladder outflow obstruction on fetal sheep detrusor contractility, compliance and innervation.* *J Urol* 168:1615-1620.
- Thiruchelvam N, Nyirády P, Peebles DM, Fry CH, Cuckow PM, Woolf AS (2003) *Urinary outflow obstruction increases apoptosis and deregulates Bcl-2 and Bax expression in the fetal ovine bladder.* *Am J Pathol* 162:1271-1282.

**2/15. PROGRAM****MOLECULAR GENETIC, PATHOLOGICAL AND CLINICAL ASPECTS OF METABOLIC DISEASES****Coordinator****Péter LAKATOS**

I. Department of Medicine

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The Program consists of 13 research sub-programs completed with appropriate theoretical courses for postgraduate students. Molecular as well as pathological and clinical aspects of different metabolic diseases are studied including metabolic bone diseases and disturbances of calcium metabolism and lipid metabolism, disorders in onco-hematology processes, endocrine glands, diabetes mellitus and vascular diseases. PhD students are working under the supervision of a qualified scientist but also participate in the work of the whole lab. Publication in peer-reviewed international journals is a requirement for a successful PhD thesis.

**Sub-programs****Supervisors**

Thyroid disorders and their effects on bone metabolism	Péter LAKATOS
Molecular genetics, pathomechanism, early diagnosis, prevention and therapy of chronic liver diseases	Margit ABONYI,
Regulation of endocrine functions of fat tissue and its relation to insulin resistance	Péter L. LAKATOS
Metabolic aspects of malign hematological disorders	Károly CSEH
Investigation of disorders associated with macro- and microvascular complications and risk factors of atherothrombotic vascular diseases	Judit DEMETER
The pathologic hypophysis: vlinical and experimental studies	Csaba FARSANG,
Ghrelin and the cell proliferation	Zoltán JÁRAI
The effect of the disorders of calcium and bone metabolism and their medications on mineral content, quality and mechanical properties of bone tissue	Miklós GÓTH
Gestational diabetes as precondition for type 2 diabetes and metabolic syndrome	Miklós GÓTH
Frequency of diabetes mellitus and its pathophysiologic aspects of its normoglycaemic treatment	Csaba HORVÁTH
Molecular mechanisms in bone metabolism	Zsuzsa KERÉNYI
Extraskeletal effects of genes and proteins essential in bone metabolism	Gyula TAMÁS
Epidemiology, pathogenesis and clinical aspects of thyroid cancers	Attila MOCSAI
Molecular genetics, pathomechanism and clinical aspects of metabolic bone diseases	Gabor SPEER
	István SZABOLCS
	Istvan TAKACS

**Ph.D. students**

Bernadett Balla  
 Bertalan Csaba Fekete  
 Petra Haller  
 Áron Lazáry  
 Eszter Madarász

ft  
 pt  
 pt  
 ft  
 ft

**Supervisors**

István Takács  
 Zoltán Járai  
 Péter Lakatos  
 István Takács  
 Zsuzsa Kerényi

El Hadj Othmane Taha

f

Zoltán Járαι  
Csaba Farsang**Ph.D. candidates**

Gyula Ádám Tabák

na

**Supervisors**

Gyula Tamás

a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

**2/16. PROGRAM****DERMATOLOGY AND VENEREOLGY****Coordinator****Sarolta KÁRPÁTI**Department of Dermatology-Venereology and  
Skin Oncology

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The Ph.D. program of the Department Dermatology-Venereology and Skin Oncology at Semmelweis University Ph.D. School aims to fill a gap in development of skin and venereal diseases that will provide support for scientific research, education and postdoctoral training of the specialty.

The foundation of this new doctoral program stems from a sub-program that belonged to the Molecular Medicine PhD School since 1998. Graduates from this sub-program have already started an independent research work. As a result of the scientific activity of the group, it gained accreditation as a joined research group of the Hungarian Academy of Sciences. The previous PhD program "Basics of Human Molecular Genetics and Genetic Diagnostics" under which our sub-program was listed could no longer harbor all that diverse clinical and research activities that include venereology, STD-related microbiology, genetics, dermatologic immunology and dermatologic oncology. Our department has accumulated a very extensive dermato-venereologic clinical data that requires further research that eventually will benefit to our patients in understanding their diseases and will result in providing better healthcare.

Our program is being transferred from the Molecular Medicine PhD School and will continue the research of monogenic inherited skin diseases. Our department is also the home of the National Epidermolysis Bullosa Center that carries out studies in the genetics of this severe inheritable group of diseases and has established successful prenatal diagnostics in the lethal forms. Diagnostics is now provided for more than 20 genes at the moment that are responsible for epidermolysis bullosa, Darier's disease and Hailey-Hailey disease and ichthyosis. The analysis of the genotype-phenotype relation as well as macro- and microalterations will give further insight into the pathophysiologic events in keratinocytes. Our facilities and expertise enable us to carry out clinical as well as basic science.

A close scientific partnership is reflected by the introduction of two co-program leaders on the field of stem cell research. The skin is largest organ in our body and also serves as the largest organ of our immune system. The skin is easily accessible and has great regenerating potential. The therapy of inherited, immunologic and all erosive skin diseases could benefit from a better understanding of the nature of *epidermal stem cells*. We wish to join the hot research area of stem cells with the tracking of stem cells of bone marrow transplanted recipient patients and with the use of an animal model. Our future aim, along with investigating skin differentiation and the dynamics of keratinocytes, is to explore the potential in gene therapy.

In the current situation with the closing of the National Institute for Dermato-Venereology the university clinic got the obligation to further care of STD patients in form of a state center for *STD diseases* with national coverage that is based on the previous expertise from the above mentioned institution. To this area of dermatology is given special attention in our PhD program. Beside the classical STD's, the altered immune reactions of HIV positive patients and opportunistic infections that fre-

quently occur among HIV positive individuals are being investigated. This program is strongly supported by a complete microbiological laboratory, including the National Mycology Reference Laboratory, which also belongs to the department. This activity provides a diagnostic background for rare infectious diseases and also is in the process of introducing novel molecular biology diagnostic tools that yield new research data and scientific achievements.

A long-lasting successful research activity on *autoimmune blistering skin diseases and gluten sensitive diseases*, like dermatitis herpetiformis and celiac disease, is well indicated by the fact, that in this field one PhD work had been completed, and two further PhD works are in progress. The large number of patients, the regular and careful study of circulating and tissue bound autoantibodies render good possibilities for the project. *Pharmacogenomics*, a new research area of the institute, is also based on the large number of patients with drug induced side effects on the skin. Within the planned biobanking, genetic and bioinformatic studies we started to elaborate material and data from patients with drug side effects. Our further goal is to focus to the predictivity of drug induced damages. Collecting data we plan to get important information about the pathomechanism of toxicodermas as well. With our *Pharmacogenomics* PhD course we join the molecular toxicology, bioinformatics and pharmacology as well.

Two years ago the Dermato-Venereology Clinic changed its name incorporating the *Skin Oncology* words as well, to underline the extended activity of the institute on the field. Scientific goal: the rapidly growing number of melanoma malignum forces us to organize extended preventive programs with organizing auto-investigation of the skin, and dermatological screening of the Hungarian population. The UV induced carcinogenesis, the development of skin tumors and its molecular biological background is also one of our ongoing studies. The Center of the Lymphoma Group of the Hungarian Dermatological Society is also in our clinic. Clinical, immunohistochemical, therapeutical and pathological features of cutaneous lymphomas will be worked up. Epidemiology would be part of different themes: incidence of STD diseases, skin tumours: melanoma, cutaneous lymphomas is planned to be evaluated.

#### *Sub-programs*

Pharmacogenomics: Molecular pathomechanism of drug induced skin diseases

Genetics of hereditary skin diseases

Effects of ultraviolet light in physiological and pathological processes of the skin, its role in the development of skin cancers

Screening and prevention program for early recognition of melanoma

Occurrence of cutaneous lymphoma: Incidence, prognostic and etiological factors, therapeutic options

STD. Dermatoinfectology

Retroviral infections: Microbes as pathogens and cofactors. Opportunistic infections

Allergic skin diseases

Autoimmune skin diseases: Clinics and immunopathology

Epidermal stem-cell research and therapeutic adoption

#### *Supervisors*

Sarolta KÁRPÁTI,

Gyöngyvér SOÓS

Sarolta KÁRPÁTI,

Márta CSIKÓS

Norbert WIKONKÁL

Beáta SOMLAI,

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Márta MARSCHALKÓ,

Katalin VERES

Attila HORVÁTH,

Sarolta KÁRPÁTI,

Katalin VERESS

József ONGRÁDI

Erzsébet TEMESVÁRI

Sarolta KÁRPÁTI,

Márta MARSCHALKÓ,

Miklós SÁRDY

Sarolta KÁRPÁTI,

Éva MEZEY

#### *Ph.D. candidates*

Péter Holló

na

Klaudia Preisz

na

na: non-affiliated

#### *Supervisors*

Márta Marschalkó

Sarolta Kárpáti

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### 3. PH.D. SCHOOL OF PHARMACEUTICAL AND PHARMACOLOGICAL SCIENCES

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*Head of School*

**Éva SZŐKE**

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The Ph.D. School of Pharmaceutical and Pharmacological Sciences focuses on two scientific disciplines:

- *Pharmacological research* is needed to select new active substances, to develop and use medicinal products. In addition, new scientific results and discovered relationships may help to understand functions of human living organism.

- *Pharmaceutical research* is related to drug research, development of drug delivery systems as well as it is a prerequisite to produce and apply pharmaceutical preparations. Although pharmaceutical science involves the knowledge of other disciplines (e.g. chemistry and medical science), but the evaluation of medicinal products requires specialised knowledge from the viewpoint of this interdisciplinary science.

The objective of the Ph.D. School is to train qualified experts with an internationally recognized scientific degree (Ph.D.) for pharmacological and pharmaceutical research. Scientific results of the above mentioned research topics will be summarized in doctoral thesis and research papers which will be published in international journals of high impact. Research topics provide students with theoretical and practical experience in different fields of pharmaceutical and pharmacological sciences. Special problems are covered by the research projects of the educational Program:

- *study of bioactive substances of plant origin in connection with phytochemical and biological evaluation as well as biotechnological production; pharmaceutical chemistry and analysis; design, manufacturing and biopharmaceutical evaluation of novel dosage forms; clinical pharmacy and pharmacoconomics; study of organic compounds with potential bioactivity; investigation of medical and pharmaceutical aspects of biology and environmental protection;*
- *pharmacodynamic investigations; pharmacokinetic and drug metabolism; influence on neurochemical transmission; study of neurodegenerative and neuroprotective mechanisms; cardiovascular pharmacological investigations; separation methods and their applications; study of drugs affecting on calcium and bone metabolism; human study of cytostatic drugs; role of iontransport mechanisms controlling neurochemical transmission.*

**3/1. PROGRAM****MODERN TRENDS IN PHARMACEUTICAL SCIENCES****Coordinator****István RÁCZ (at present: István ANTAL)**

Department of Pharmaceutics

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*Aim:* The scientific-education scope of the participating 15 institutes/departments provides the eligible professionals (pharmacists, medical doctors, chemists, biologists, physicists, chemical engineers) with a wide selection of topics in the fields of fundamental and specific drug-oriented research, including current problems of inorganic, organic, physical, analytical, bioinorganic, bioorganic, and coordination chemistry, biology, biophysics, biotechnology, botany, microbiology and virology. The specific topics of drug- and pharmaceutical research are drug design and synthesis, structure-activity relationships, mechanism of action of drugs, drug-receptor binding, isolation of active compounds from natural sources, drug metabolism, biochemical toxicology, relationships between physicochemical properties and biological function, pharmacognosy, elucidation of biosynthesis of natural compounds, pharmacokinetics, drug-drug interactions, transport mechanisms, biopharmaceutics, pharmaceutical technology, physical pharmacy, chemical pharmacy and social pharmacy.

*Sub-programs*

Production of bioactive compounds by biotechnological methods  
 Phytochemical and biological evaluation of bioactive substances of plant origin  
 Pharmaceutical chemistry and drug analysis  
 Design and preparation of modern dosage forms  
 Biopharmaceutical considerations of design and evaluation of pharmaceutical preparations  
 Pharmacoeconomics and clinical pharmacy  
 Study of potentially bioactive organic compounds  
 Researching effective drugs for chronic systemic diseases  
 Pharmaceutical considerations of biology and environmental protection

*Supervisors*

Éva SZŐKE  
 Éva LEMBERKOVICS  
 Béla NOSZÁL  
 István RÁCZ  
 Sylvia MARTON  
 Zoltán VINCZE  
 Péter MÁTYUS  
 Tamás TÖRÖK  
 Éva ÁDÁM

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 Angéla Benedek pt  
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 Balázs Blazics ft  
 Szabolcs Béni ft  
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 Orsolya Csernák ft (a)

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Viktória Vukics	ft	Ágnes Kéry
Péter Adolf Weinsheimer	it	Sándor Nagylucskay

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Hajnalka Hank	ft
Attila Sándor Halász*	ft
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András Zoltán Kakasy	ft
Zsuzsanna Kovács	na
Ákos Barnabás Rác	ft
Csaba Szántai-Kis	kkk
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\*Defended after Nov. 2005.

**Supervisors**

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Bálványos, István	ft
Zoltán Bozóky	na
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Imre László	ft
Kornélia Lovas	ft
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Lajos Barcza
Ottó Dobozy

a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated, kkk: Cooperation Research Center

PÁL GYÖRGY APÁTI (2004)

## Antioxidant constituents in *Solidago canadensis* L. and its traditional phytopharmaceuticals

Supervisor: Dr. Ágnes Kéry

Representatives of *Solidago* species have been used in European phytotherapy for centuries as a component of urological and antiphlogistical remedies. *Solidago canadensis* L. (Asteraceae) contains a wide range of active ingredients, such as flavonoids, saponins, hydroxycinnamates and mineral elements, which are responsible for its characteristic anti-inflammatory, spasmolytic and diuretic properties.

Quality control of collected plant material and determination of total flavonoid content of *Solidaginis herba* were performed according to the instructions of the X. German Pharmacopoea („A” = 35,42 mg g<sup>-1</sup>, „B” = 51,09 mg g<sup>-1</sup>), while different LC-MS technologies were applied to evaluate the exact phenoloid composition. Three flavonol aglycons (quercetin, kaempferol and isorhamnetin) connected to several sugar components (glucose, rhamnose, galactose and rutinose), caffeoyl-quinic acid and a caffeoyl-shikimic acid glycoside were identified in the samples. Incidence of acetyl-glycosidic flavonoids and absence of flavonoid galactosides and rhamnosides in the sample “B” together give support for the taxonomic recognition of varieties *Solidago canadensis* L. var. *canadensis* (“A”) and var. *scabra* (“B”).

Comparison of HPLC- and CE-methods was performed, and more sensitive HPLC method have been chosen to follow dissolution of characteristic polyphenols into traditionally applied phytopharmaceuticals (Infusum-, Decoctum-, Maceratum *solidaginis*, and Tinctura *solidaginis* 40-, 70-, 96% ethanol). Release of phenoloids into alcoholic extracts was affected mainly by solvent polarity, while application of aqueous techniques have resulted differences in flavonoids due to temperature and duration of extraction (1). Mineral element composition of *Solidaginis herba*, and its traditionally applied extracts were analysed by ICP-OES for Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, P, Pb, Ti and Zn content (2).

Biological activity of *Solidaginis herba* drug has been confirmed in a combined test method comparing to silibinin-dehydrosuccinate. Investigated samples were acted as primary and secondary antioxidants, and additionally membrane protecting activity of extracts has been proved. To estimate the contribution of polyphenolic compounds of extracts to enzymatic antioxidant defence system of human body glutathione S-transferase (GST) inducing activity of extracts was assayed. GST inducing ability of quercetin derivatives depends on the sugar moiety connected to the molecule, although inhibiting activity of the aglycon was observed (quercetin: -30,15%, quercitrin: 24,55%, rutin: 51,36%). Correlation of phytochemical characteristics and antioxidative properties of phytopharmaceuticals have been examined using the previously developed test method. H-donor activity and reducing power of extracts were mainly correlated with chlorogenic acid content, while chelating- and superoxide scavenging activity were correlated with rutin content of the samples. Hydroxyl radical scavenging- and membrane protecting activity were mainly influenced by both flavonol glycosides and hydroxycinnamates of the extracts (3).

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**ISTVÁN BÁLVÁNYOS (2003)**

## **Studies on the growth and secondary metabolite production of *Lobelia inflata* L. hairy root cultures**

*Supervisor: Dr. Éva Szóke*

*Lobelia inflata* L. contains pharmacologically important secondary metabolites. More than 20 piperidine alkaloids were detected in the herb. The main alkaloid is lobeline which has a stimulatory effect on the respiratory centre and it is applied in the cases of asthma, gas- and narcotic-poisoning. Lobeline is also used in anti-smoking preparations. Antidepressant effect of  $\alpha$ -amirin-palmitate isolated from leaves of *L. inflata* was published. Recently high quantity of polyacetylenes (lobetyol, lobetyolin and lobetyolinin) were determined in the plant. The growth and secondary metabolite production of genetically transformed and non-transformed cultures of *L. inflata* was studied to increase biomass formation and the amount of effective substances.

The organised cultures cultivated *in vitro* on solid medium containing total alkaloids are similar quantity to the intact plant. The alkaloid content of the plants cultivated in greenhouse increased in contradiction to the content of lobetyolin (the main polyacetylene compound). The survival of the plants cultivated in field was 100%. The alkaloid content in these plants decreased, but it was higher than in the *in vitro* cultures. On the other hand the amount of polyacetylenes increased greatly.

With the aim of increasing the secondary metabolite production genetically transformed cultures were produced. Hairy roots were obtained by direct infection of the sterile plants with *Agrobacterium rhizogenes* strain R1601. The cultures grew intensively and had good biosynthetic ability. The polyacetylene content of hairy root clones was prominent (about 3,5% lobetyolin and 1,5% lobetyolinin) and the total alkaloid content of the cultures was similar to the intact plant (but the lobelin content in the hairy roots was less than in the intact plants). Secondary metabolite production was increased by optimizing of the cultural conditions (macroelements, sucrose, growth regulators, precursor amino acids). The cultivation of the hairy roots in liquid medium, in bioreaktor is a further possibility to increase the amount of the active ingredients.

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**ZOLTÁN BOZÓKY (2004)**

## **Elaboration of separation and investigation methods of lipoprotein macromolecules for radiopharmaceutical applications**

*Supervisor: Dr. István Antal*

$^{99m}\text{Tc}$ (Technetium)-labelled lipoproteins can be used as a radiotracer because it acts as an intracellularly trapped ligand providing an scintigraphic measurement of lipoprotein uptake by tissues. Preparative density gradient centrifugation methods were applied for the isolation of the major lipoprotein density classes i.e. VLDL, IDL, LDL, HDL. Analytical ultracentrifugation methods were developed for the investigation of native and modified lipoprotein. We have prepared modified lipoproteins with chemical agents and with radioactive irradiations. Radiolabelling of lipoproteins with  $^{99m}\text{Tc}$  was performed using sodium dithionite as a reducing agent. The radiolabelling of Low-Density Lipoproteins were tested in animal models of atherosclerotic and tumors. Atherosclerosis was induced in rabbits by a hypercholesterolemic diet. Gamma camera *in vivo* scintigraphy of

rabbits revealed visible signal corresponding to atherosclerotic plaques of aorta (mainly in the aortic arch) and carotid arteries. After oxidative modification of lipoproteins can be increased the scintigraphic detection of atherosclerotic plaques. In nude mice developed human tumor cells and in dogs spontaneous developed tumors were detected on the basis of  $^{99m}\text{Tc}$  labelled LDL with Gamma camera. Liposomes can be prepared with similar features to lipoproteins.

In the future we should like to investigate radiolabelled modified or recombinant lipoprotein fractions and liposomes which are better for early lesion detection of atherosclerosis and of tumor cells and for the targeted delivery of drugs to various tissues.

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**ERIKA CZINNER (2003)**

## Bioactive compounds and antioxidant activity of *Helichrysum arenarium* (L.) Moench

Supervisor: Dr. Éva Lemberkovics

The inflorescence of *Helichrysum arenarium* (L.) Moench (*Helichrysi flos* syn. *Stoechados flos*) has long been known in herbal medicine in Europe for its choleric, diuretic, antiinflammatory and detoxifying activities. In course of our phytochemical studies organic bioactive compounds and the inorganic element content of *Helichrysi flos* drugs of different origin (from cultivation or from the commercial network) and their several extracts were examined. The polyphenol and flavonoid content in the drug (42.3–2.3 g kg<sup>-1</sup>; 3.7–12.7 g kg<sup>-1</sup>, resp.) and tea samples (720–1730 mgL<sup>-1</sup>; 26-91 mgL<sup>-1</sup>, resp.) were determined by a spectrophotometric method. Eight flavonoids (kaempferol, kaempferol-3-glucoside, apigenin, apigenin-7-glucoside, naringenin, naringenin-5-glucoside, naringenin-4'-glucoside, quercetin-3-glucoside) the chalcone derivative isosalipurposide and chlorogenic acid were identified in *H. flos* extracts by TLC, HPLC and HPLC-ESI-MS methods.

Composition of *H. flos* essential oil obtained by hydrodistillation was examined by GC and GC/MS, and 24 components were identified. The characteristic volatile constituents were: linalool, anethole, carvacrol and  $\alpha$ -muurolol; the oil contained aliphatic acids and their esters, further aromatic compounds in significant quantity, beside the typical volatile terpene components (1). On the basis of GC analysis, the main compound of *H. flos* fatty oil obtained by Soxhlet extraction was palmitic acid (C16:0) in commercial samples, and oleic acid (C18:1) in cultivated sample.

The concentration of 23 macro- and microelements was measured in crude drugs and their water extracts by ICP-AES. The cultivated drug sample from 1999 (Hungary) contained aluminum, chromium, copper, manganese and phosphorus in highest level, while the concentration of barium, calcium, iron, and zinc was highest in a commercial sample from 1998 (Poland). Our *in vitro* studies provides evidence of the fact that water extracts of *Helichrysi flos* exhibit significant antioxidant properties expressed by their H-donating ability, reducing power property and free radical scavenging activity depending on concentration (2). Our experiments proved that enzymatically induced lipid peroxidation was diminished by *Helichrysi flos* extracts dose dependently, these natural plant extracts were also able to influence the microsomal NADPH-cytochrome P-450 reductase activity in a dose-dependent manner (3).

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**BALÁZS ZOLTÁN HANKÓ (2005)**

## **Opportunities in pharmaceutical care in Hungary, in the case of type 2 diabetic patients**

*Supervisor: Dr. Zoltán Vincze*

Diabetes mellitus, especially type 2 diabetes which occurs usually over 35 years means greater and greater public health problem. In my surveys, I dealt with already diagnosed type 2 diabetic patients. They represent the target population of the third, most complex step of the pharmaceutical care programme. Within the framework of my Ph.D. thesis, I was the first who measured the therapy, the interest, the compliance and the quality of life of type 2 diabetic patients in Hungary. I also studied the opportunities of improving quality of therapy and care with the role of pharmacists.

The Hungarian therapeutic practice develops in accordance with international and national guidelines. The use of antidiabetics is dynamically growing which is incidental to the change of the emphasis of therapeutic groups. However, some out-of-date drugs are still in use. I measured the ratio of types of treatment. Thus, I recognized more data about the use of unreasonable combinations and about the use of insufficient and out-of-date cardiovascular drugs. Type 2 diabetic patients have great interest in pharmaceutical diabetes care programme. I discovered that the interests of patients with different general and health parameter are different.

Compliance of diabetic patients in relationship with life style and medication is very low in each field. 50-60% of the reasons of drug purchasing and drug taking non-compliance could be prevented with modifying the therapy and with giving advices. According to my study, EQ-5D quality of life questionnaire can be well used to forecast non-compliance. My own studies also confirmed the results of foreign surveys that type 2 diabetes lower the patients' quality of life. In the care of type 2 diabetic patients it is necessary to reach more development. In Hungary, it can be reasonable to involve pharmacists in definite areas. The results of this Ph.D. thesis could be the basis of the Hungarian protocol.

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**MÁRTA KRASZNI (2004)**

## **Introduction and application of conformer specific physico-chemical parameters in the characterization of biologically active compounds**

*Supervisor: Dr. Béla Noszál*

Drugs are required not only to be potent at a given receptor but also to have appropriate pharmacokinetic properties. The latter is related to different physico-chemical characteristics of the molecules such as solubility, basicity, lipophilicity *etc.* Therefore, study of these parameters is an important task in drug research. The aim of this work was to study how two physico-chemical parame-

ters, the basicity and partition coefficient are affected by the conformational changes of the molecules.

Conformer-specific basicities of two biologically active molecules, N-acetylcysteine and histamine have been determined by NMR-pH analysis. It was found that the carboxylate basicities of N-acetylcysteine rotamers differ to a small extent, but the conformer-specific thiolate basicities show larger variations. In histamine, the protonation of the imidazol group has significant effect on the rotamer-specific basicities. Differences between conformer-specific parameters can be explained in terms of the existence or lack of intramolecular interactions in the conformers.

Theoretical basis of the determination of conformer-specific partition coefficients has been elaborated and the experimental method of the determination has been presented for amphetamine in the chloroform/water solvent system. This method has been extended to the octanol/water system widely used in pharmaceuticals. Rotamer-specific octanol/water partition coefficients of amphetamine and clenbuterol have been measured. Lipophilicity of amphetamine rotamers showed only small variations, while rotamer-specific partition coefficients of clenbuterol differed significantly, which could be interpreted in terms of intramolecular interactions between the vicinal polar sites and the solvent-accessibility of the groups.

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**SZIDÓNIA T. KRISTÓ (2004)**

## Investigation of bioactive compounds from *Taraxacum officinale* and *Morus nigra*

*Supervisor: Dr. Ágnes Kéry*

The result of continuously expanding and detailed pharmacological researches of bioactive plant components press for new, accurate and efficient extraction and phytoanalytical methods to discover and identify the pharmacologically active components. They give, at the same time, new answers to some emerging problems about relations between chemical structure and pharmacological activity.

In the modern times, can be concluded, that some so far not bio-active considered components may influence the efficacy of medical plant extracts, or the use of them may suffer changes. To study from the active components of *Taraxacum officinale* and *Morus nigra*, the antiinflammatory, immunomodulator triterpenes and phytosterols, we have chosen the supercritical fluid extraction, which has been used until now mainly to obtain the volatile antiinflammatory components. We determined those optimal parameters, among them this kind of extraction method is the best way to obtain triterpene rich fractions. For quantity evaluation of extracts we developed a thin layer – densitometry method and demonstrated that the products obtained by supercritical fluid extraction are richer in triterpenes and phytosterols, than those obtained by Soxhlet extraction.

The lyophilized aqueous and methanolic extracts from *Taraxacum officinale* and *Morus nigra* have shown to possess antioxidant activity in *in vitro* models. To search for possible active components responsible for scavenging activity we used capillary electrophoresis, which was compared to HPLC. Identifying the flavonoid components from drugs, we postulated their topical use, based on their different pharmacological (chelating and superoxid scavenging) activity.

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**IMRE LÁSZLÓ (2005)**

## **Investigation of primary and secondary metabolism of *Datura innoxia* Mill. tissue cultures**

*Supervisor: Dr. Éva Szóke*

*Datura innoxia* Mill. contains several secondary metabolites with pharmacological and toxicological importance, including more than 30 tropane- and pirrolidin-base alkaloids. Total amount of these alkaloids varies between 0.05%-0.5% in different organs of the plant. Main alkaloids are scopolamine and hyoscyamine which have parasymphatholytic effect.

The aim of our experiments was to study different – genetically modified and non-transformed – in vitro cultures of *D. innoxia*. The growth (fresh and dry weight, dry matter content, growth value and pH of liquid culture media) and tropane alkaloid content (scopolamine, hyoscyamine and apatropine, using HPLC technique) of in vitro plants, callus tissues and genetically modified, so called hairy root cultures were investigated. The transferred character of hairy root tissues obtained by *Agrobacterium rhizogenes* A4 microinjection was demonstrated by using polymerase chain reaction (PCR) and opine detection (paper electrophoresis). We have established that the total alkaloid content of hairy root clones (#410, #411 and #415, 0.23% hyoscyamine and 0.21% scopolamine) reached that of the root of the in vivo plants.

Growth and alkaloid content of in vitro cultures has been effected by several circumstances. We studied the effects of culturing in light, the sucrose and Mg<sup>2+</sup> content of liquid MS basal medium on the growth, biomass production and tropane alkaloid content of the tissues. The presence of apoptotic processes in cells of different tissues (callus and root) accompanied with the shrinkage of the nuclei was investigated by TUNEL reaction. To study the relationship among abiotic stress, growth, alkaloid metabolism and measurable endogenous HCHO concentration different molecules affecting transmethylation reactions have been administered to the culture media (dimedone, semicarbazide, aminoguanidine and hidralazine, 1ppm-1000ppm) of tissues [1]. The endogenous HCHO concentration of callus (4.6µg-27.0µg) and hairy root cultures (345.0µg) was determined by OPLC and HPLC techniques as formaldemethone [2]. Following administration of dimedone and semicarbazide the apoptotic processes were investigated by TUNEL reaction [3].

The results obtained might help deepening our knowledge about the growth of *D. innoxia* in vitro cultures, tropane alkaloid metabolism, apoptotic processes in plant cells and the effect of abiotic stress (special chemical stress).

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**KORNÉLIA LOVAS (2003)**

## **Apligraf the role of economic evaluations in the reimbursement strategy in a case of a tissue-engineered product**

*Supervisor: Dr. Zoltán Vincze*

*Objective:* The aim of this thesis is to investigate the role of economic evaluation in supporting the reimbursement strategy of a tissue-engineered product. Introduction: Market access of medical products depends on the registration and reimbursement. Novel technologies are usually more expensive than alternative therapies because of the extreme cost of product development, therefore, health eco-

nomic arguments are crucial to gain reimbursement from authorities. *Methods:* Markov model was developed by the Erasmus University (Rotterdam) to demonstrate the cost-effectiveness of Apligraf® in various different countries. Country-specific cost-of illness studies were conducted in the Netherlands, Germany, Israel, Argentina and the US. Based upon the published literature, French, Swiss, Swedish, British and Belgian cost vectors were also used to populate the Erasmus model. Effectiveness defined by disease free period, avoided amputation, and treatment costs were calculated in both treatment arms for each individual country. *Results:* Efficacy data demonstrated the dominance of Apligraf® treatment, resulting in increased time spent in an ulcer-free state (24 %) and reduced incidence of amputation (63 %). Apligraf® treatment resulted in cost-savings at 12 months in each country, with the exception of Belgium. Because of technical challenges during registration, the company decided to withdraw the dossier for resubmission at a later date. *Conclusion:* The results obtained from the Markov model indicate that the use of Apligraf® yields a clear economic benefit when compared to the standard medical practice defined by good wound care. To generalise our experience with Apligraf® for all tissue-engineered products, we can conclude that the “quasi-nonregulation” of the registration procedure carries at least as many challenges as the uncertainty of getting reimbursement.

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MASSUD ALLAG. S. ANWAIR (2005)

## Synthesis, lipophilicity and antifungal properties of 3(2H)-pyridazinone derivatives

*Supervisor: Dr. Péter Mátyus*

We have prepared a series of pyridazinone derivatives, starting with dichloro-pyridazinone (37, 133 and 134), available from mucochloric acid. Due to their easy nucleophilic substitution and their biological activity, they were used as starting materials for the further synthesis of several 3(2H)-pyridazinone derivatives.

We have investigated antifungal properties of some known derivatives with the agar dilution method, which showed that 134 and 397 display a broad spectrum of activity against all the fungi tested, but 134 was the most active of this series against *Candida albicans* and *Cryptococcus neoformans*, and much more active than amphotericin B and Ketoconazole, particularly against *Trichophyton rubrum* and *Trichophyton mentagrophytes*, the two major ethiological agents of dermatomycoses. This compound also proved to be a moderate inhibitor of (1,3)- $\alpha$ -D-glucan synthase, a catalyst of one of the major polymers of the fungal cell wall, without any inhibitory activity against chitin synthase. Significant activities were also shown by other of these derivatives against dermatophytes, which is a contribution to the development of antifungal drugs. The best activity was observed in the presence of a 6-nitro group and/or halo atoms in the positions of the ring as in 134 and 397.

The lipophilicities of several pyridazinone derivatives were described by the log P<sub>exptl</sub> values determined experimentally with the standard shaking flask method, which revealed a higher lipophilicity of the 4-isomer than of the 5-isomer, which is in agreement with our previously published results, and confirmed that not only the type, but also the position of the substituent plays a significant role in the lipophilicity of the pyridazinone derivatives. log P calculated by methods based on quantum-chemical approaches, such as ScilogPultra, could reproduce the higher lipophilicity of the 4-isomer, while Qlog P and Ghose could not distinguish the regioisomers at all; Villar and 3DNET reproduced the observed log P. The log P values calculated with the KOWWIN program were very dif-

ferent from the experimentally determined log P data. This program could be run with the EVA option and the log P values were acceptable in terms of overall precision when they were calculated with the KOWWIN-EVA program based on structural relationships and the log P<sub>exptl</sub> value of one of the compounds used as starting material. To calculate log P values with the KOWWIN-EVA and 3DNET Computational methods, of new compounds careful selection should be made by using a set of structurally closely related compounds. The dipole moments of 4- and 5- amino regioisomers calculated with the semiempirical PM3 method indicate there is no direct correlation between dipole moments and lipophilicity. The most relevant structural parameters that playing important roles in the determination of the log P values of this class of compounds are the hydrogen bonding capacity and/or aromaticity. The different intra- and intermolecular hydrogen bonding capacities and the different solvation behavior of the regioisomers appear to contribute to the observed lipophilicity trend. From other studies of the electronic and X-ray structural features of regioisomers, we conclude that the values of single structural parameters such as bond length or bond orders are not necessarily translated into differences of log P values.

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## ÁGNES MÉSZÁROS (2003)

### Outcome analysis of the asthma therapy

Supervisor: Dr. Zoltán Vincze

The control of asthma means that people lead a high quality of life and in the majority of instances they do not feel compromised by the condition they have. Good disease management includes the rational use of financial resources as well. The objectives of this study were as follows: to identify actual patterns of use of medications, to identify the costs related to the asthma therapy and to evaluate patients quality of life (QoL), in terms of its relationship to non-asthmatics, to assess the relationship between the VAS and the SGRQ, to evaluate the potential influential factors on patients QoL, to measure the effectiveness of a newly developed patient education instrument. This study was the first, which measured the quality of life of asthmatics in Hungary. It was confirmed that asthma has a major impact on the patients QoL and so asthmatics experience several restrictions. The results suggest the reliable application of the Euro-QoL and SF-36 among asthmatics. The gained correlation rates between the VAS and the SGRQ provides the opportunity to use the VAS in every-day clinical practice instead of the SGRQ, and still gain important information. The findings point out that patients might have bad quality of life results but good FEV1 rates at the same time, as there was only a moderate correlation between the QoL data and FEV1. This suggests that, clinicians can no longer be confident if there are improvements in the clinical parameters that mean the patient has a better QoL. The obtained polynomial model was successfully applied to the analysis of patient's QoL and its influential factors. The results suggest that QoL may be used as a non-invasive patient monitoring system if measured regularly. The results indicate that it is necessary to regularly refresh asthma knowledge; to assess patients' self-management plans to achieve long-term effectiveness of asthma-management. The effectiveness of a newly developed education instrument was confirmed.

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JUDIT NAGY (2005)

## Preparation and evaluation of zinc sulphate matrices for the individual clinical therapy of Wilson's disease

Supervisor: Dr. Romana Zelko

Wilson's disease is a genetic disorder of copper transport resulting in the accumulation of copper in organs such as the liver and the brain, which leads to progressive hepatic and neurological damage. The prevention of severe permanent damage depends upon early recognition and diagnosis by the physician, followed by appropriate anticopper treatment. Zinc is now one of the recommended therapies for the long-term management of the disease. Zinc has shown clinical efficacy at doses of 50 mg three times daily in the stimulation of metallothionein synthesis and reduction of copper absorption. The mean plasma elimination half-lives of most highly water soluble drugs, like zinc sulphate, are relatively short (2-4.5 h), which necessitates several applications a day. Long-acting sustained and controlled release preparations make a once-a-day dose treatment possible, thus improving the patients compliance. The rate and extent of drug release from most controlled release wax matrices are influenced by the drug loading/embedding excipient ratio of the systems. The purpose of my thesis was to prepare and evaluate hydrophobic wax zinc sulphate matrices of different drug loadings for the therapy of Wilson's disease. Wax zinc sulphate matrices were prepared by hot melt technology. The drug release parameters, scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS) and diffuse reflectance spectroscopy of the samples were analysed. The drug release from matrices was tested by the rotating paddle method of USP and the dissolution data were analysed assuming different kinetic models. Both the dissolution rate and kinetic profile can be controlled by alteration the quantity of embedding material. Matrices of 75% zinc sulphate loadings showed steady state diffusion-controlled matrix release with good correlation in vitro. As a result of the steady state diffusion-controlled matrix release, the matrices containing 75% drug loadings were selected for the in vivo examinations. Good absorption of zinc sulphate from the gastrointestinal tract was proven by significant elevation of serum zinc level in patients with Wilson's disease. No side effect was registered and clinical symptoms of Wilson's disease remained as stable as they were during the previous D-penicillamin treatment. The abdominal discomfort complaints of patients treated previously zinc sulphate in powder form disappeared when the therapy was changed to wax matrices.

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NORBERT SZOBOSZLAI (2003)

## Determination of cadmium, lead, selenium and tin in human brain by graphite furnace atomic absorption spectrometry

Supervisor: Dr. Lajos Barcza

Trace elements play a key role in a variety of processes necessary for life. Various studies have shown that a definite correlation exists between trace element content and many common diseases. In the present study our first aim was to work out a graphite furnace atomic absorption spectrometry (GF-AAS) method to determine the Cd, Pb, Se and Sn concentrations at trace level in human brain samples. Our second goal was to study different digestion techniques, and to determine the normal concentrations of these elements in different brain regions. In the case of the Cd and the Pb simultaneous method was carried out. In the case of the selenium pd-nitrate was used as a chemical modi-

fier. The decrease of the background and the improvement of the shape of the atomic absorption peak were experienced using the <sup>2</sup>thermally pre-reduced<sup>2</sup> modifier (reduction of the Pd-nitrate to Pd on 1000 oC). The pre-treatment temperature was 1200 oC, the atomization temperature was 2100 oC. For Sn measuring instead of Ar, 95% Ar and 5% H<sub>2</sub> gas was used as an internal gas, applying 10 mg Pd + 3 mg Mg were recognized as the most appropriate techniques.

The average concentrations of the brain samples (dry weight): Cd: 80-300 ng/g, Pb: 100-500 ng/g, Se: 500-600 ng/g, Sn: 30-120 ng/g. The present results show a non-homogeneous distribution of the essential element (Se) in normal human brain. Corresponding regions in both hemispheres of one human brain show almost identical concentration for this element. In the case of toxic elements (Pb, Cd, Sn) an average value in different brain regions can not be established because of the high variability of individual data.

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## VIOLA TAMÁSI (2004)

### In vivo and in vitro induction of P450 enzymes in regenerating liver cells

Supervisor: Dr. Ottó Dobozy

The aim of our study was to investigate the basal activities and protein levels and inducibility of CYP1A, CYP2B, CYP2E1 and CYP3A enzymes during liver regeneration. From our in vivo studies it could be concluded that the activities and protein levels of the enzymes mentioned above decreased after partial hepatectomy. Dexamethasone treatment moderated the changes caused by liver regeneration. In in vitro studies we measured the inducibility of CYP1A, CYP2B, CYP2E1 and CYP3A enzymes. For measuring the inducibility of CYP2E1 from liver cell culture, the method of chlorzoxazone 6-hydroxylase assay in microsomes was adapted for monitoring CYP2E1 activity in hepatocyte culture.

In hepatocytes, isolated from regenerating liver, the activities and protein levels of CYP1A, CYP2E1 and CYP3A were investigated. Our in vitro studies proved that the inducibility of some P450 enzymes changed during regeneration. The inducibility of 3-methylcolantrene treated cells isolated from 3-day long regenerating liver was 3 times higher, than in hepatocytes from sham operated animals. In our experiments, the inducibility of CYP2E1 in imidazole treated hepatocytes, isolated from regenerating liver was the same as in imidazole treated cells from sham operated animals. The inducibility of CYP3A also change in cells from regenerating liver, it was 2-2.5 times higher in dexamethasone treated cells than in normal ones.

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### 3/2. PROGRAM

## EXPERIMENTAL AND CLINICAL PHARMACOLOGY

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*Aim:* The topic of pharmacokinetics becomes increasingly important for studying bioequivalence and planning modern drug preparations for optimal drug treatment. The study of the mechanism of drug action by pharmacodynamic tools aims at recognising new active substances and various opioid receptor types and subtypes. It is advised to get acquainted with their physiological and pathophysiological role in the development of opioid dependence or in the protection of gastric mucosa. The Program also covers the research into the metabolism of neurotransmitters in the CNS in order to elucidate the relationship between disturbances of neurotransmission and certain psychiatric disorders. Research in the field of presynaptic regulation of the neurochemical transmission in the peripheral and central nervous system is also included in the Program. Investigation of compounds affecting calcium and bone metabolism is also carried out. The mode of action of cardiovascular drugs and endogenous substances are planned to be tested, as well as antihypertensive agents in order to find optimal treatment of the diseases. Anticancer agents are involved in the studies to improve the effectiveness of the treatment. Structure activity relationship studies, including the chirality of drugs is also part of the Program.

### *Sub-programs*

Drug and mechanism-oriented pharmacodynamic studies  
Pre-clinical and clinical cardiovascularpharmacological studies

Separation methods and their applications in pharmacology  
Studies on compound affecting calcium and bone metabolism  
Pharmacokinetic and drug metabolism studies  
Modulation of neurochemical transmission by drugs; neurodegenerative and neuroprotective mechanisms

Clinical investigation of anticancer drugs  
The role of ion-transport mechanisms in the pre-synaptic regulation of neurochemical transmission

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a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

**AL-KHRASANI MAHMOUD (2004)****The opioid properties of endomorphins in isolated organs and rat brain slices**

*Supervisor: Dr. Zsuzsanna Fürst*

My studies were aimed at the characterization of opioid properties of recently discovered brain peptides, endomorphins (Tyr-Pro-Trp-Phe-NH<sub>2</sub>, endomorphin-1, EM-1 and Tyr-Pro-Phe-Phe-NH<sub>2</sub>, endomorphin-2, EM-2) and their synthetic analogs, using different in vitro pharmacological techniques. Since natural endomorphins have been reported to possess potent and  $\mu$ -opioid receptor type-selective agonist effect and, surprisingly, also partial agonist properties, special attention was

paid to these issues. The  $\mu$ -opioid receptor-selective agonist enkephalin analog (DAMGO), morphiceptin, morphine and normorphine were used as reference agonists. Technically two types of in vitro systems were used:  $\mu$ -opioid receptor-containing, field-stimulated i) isolated organs (mouse vas deferens, MVD hosting  $\delta$ ,  $\mu$  and  $\kappa$  opioid receptors and guinea-pig ileum, GPI,  $\mu$ ,  $\kappa$ ) and ii) rat brain slices (nucleus tractus solitarii-dorsal motor vagal nucleus complex, NTS-DVN). The endomorphin analogs were modified in position 1, 2 and 4 as compared to the parent natural peptides. 3' ring hydroxylation on Tyr1 with or without  $\alpha$ -methylation resulted in a loss in agonist potency whereas 2', 6'-dimethylation (Dmt) increased potency considerably as assayed in MVD. Substitution of Pro2 by D-Met, D-Ser or cycloSer but not by L-Ser or Hyp yielded analogs with potencies comparable to that of parent peptide. Substitution of D- or L-Ser in position 4 in the D- or L-Ser2-substituted analogs caused further loss in agonist potency. Free carboxylic terminus reduces potency whereas the change of amide function for an alcoholic one (-ol-derivatives) preserves agonist activity. In addition the agonist actions were exerted at the  $\mu$ -opioid receptor type both in MVD and GPI with the exception of the derivative with a free C-terminus. This latter tendency matches the one for morphiceptin and its free carboxylic pair. Using the partial  $\mu$ -opioid receptor pool inactivation strategy by  $\beta$ -funaltrexamine in MVD natural endomorphins, their -ol-derivatives and ?Dmt?1 -EM-1 were found partial agonists whereas ?D-Met?2-EM-2 is a possible full agonist. DAMGO, DAMGA and morphiceptin were full agonists, normorphine was a possible full agonist whereas morphine was a partial  $\mu$ -opioid receptor agonist. In adult rat NTS-DVN slices the  $\alpha$ 2-adrenoceptor agonist clonidine, DAMGO and both natural endomorphins inhibited the field stimulation-induced release of 3H-norepinephrine (3H-NE). However, DAMGO had shown dose dependent inhibitory effect but endomorphins did not even in the presence of dipeptidyl-aminopeptidase IV inhibitor, Diprotin A. One of the possible explanations of this phenomenon is that endomorphins behave as partial agonists also in the NTS-DVN complex.

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## KRISZTINA BOÓR (2005)

### Analysis of genetic polymorphisms of the dopaminergic and the serotonergic systems in drug dependent patients

Supervisor: Dr. Huba Kalász

Substance dependence is a major social and health problem worldwide. It is generally accepted that genetic and environmental risk factors contribute to the development of drug addiction, however, at present, little is known about the exact nature and effects of its genetic components. The aim of the molecular genetic researches is to identify the genetic risk factors. One of the most widely used methods is the candidate gene association study.

Neurobiological models emphasize the key role of the reward system through the dopaminergic mesocorticolimbic pathway, which is modulated by a number of other neurotransmitters, such as serotonin. Genetic polymorphisms of several components of reward system (receptors, metabolizing enzymes, transporters) have been widely studied for association with various personality traits, as well as psychiatric disorders including substance dependence.

The present theses describe the elaboration and the application of a simplified, non invasive DNA sampling method ; the genotyping of polymorphic regions including the coding region (DRD4 48 bp VNTR) and the 5' upstream region (-521 C/T SNP) and 120 bp duplication) of the DRD4 gene; the investigation of the 5' upstream region (5HTTLPR), and the intron 2 polymorphism (STin2) of the SERT gene and the analysis of DRD4 and SERT gene polymorphisms as possible risk factors for sub-

stance dependence, in a case-control association study of 73 substance dependent subjects and 362 healthy Caucasian (Hungarian) controls.

Our results indicate significant association between the -521 C/T SNP of the DRD4 promoter region and heroin dependence ( $p=0.044$ ) and an interaction between the dopaminergic and serotonergic system at molecular level. Association between the -521 CC vs. CT or TT genotypes and heroin dependence was enhanced in the presence of 14-repeat 5HTTLPR allele ( $p\leq 0.01$ ). The observed odds ratio of 2.14 for the -521 CC genotype increased to 4.82 in case of the double homozygotes (i.e. -521 CC and 5HTTLPR 14/14), emphasizing the importance of combined analysis of polymorphisms in the dopaminergic and serotonergic systems in heroin dependence.

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## EMŐKE CSUPOR (2005)

### The effect of parathyroid hormone on bone metabolism

*Supervisor: Dr. Csaba Horváth*

We investigated the skeletal effects of oversecreted PTH in autonomous parathyroid hyperfunction (primary hyperparathyroidism) and in secondary hyperparathyroidism, developed due to the regulated homeostatic functions (calcium oxalate kidney stone formers with renal hypercalciuria), furthermore in PTH resistance syndrome (pseudohypoparathyroidism type Ia). My new establishments to conclude the results are as follows: 1. In symptomatic patients with primary hyperparathyroidism age and gender influenced the effect of PTH on bone mineral density, bone architecture, bone quality and fracture risk, moreover on clinical manifestation. 2. The parathyroid hormone decrease bone mass especially in cortical bones. This skeletal effect was proven by extremely high PTH production in parathyroid carcinoma patients. 3. The investigated QUS parameters, which reflect the change of bone quality and architecture, pointed out that bone elasticity module is not considered to be responsible for increased fracture risk, even in extremely high PTH production. 4. Age is a principally responsible factor for bone fractures in pHPT patients. The frequent bone fractures in pHPT are partially explained by bone architecture, bone quality and decreased bone mineral density. 5. Bone mass similarly decreased in the investigated clinical manifestations of pHPT (patients with kidney stones or without kidney stones), therefore the clinical form did not show considerable differences. 6. The clinical manifestation of pHPT (kidney stones) may connect to the location of parathyroid adenoma. 7. In secondary hyperparathyroidism in the case of our renal hypercalciuric patients with recurrently developed calcium oxalate kidney stones cortical bone mass was pathologically decreased. 8. The difference in PTH level between patients with secondary hyperparathyroidism and pHPT was revealed only in bone mineral density and it was not considered responsible for bone fragility and architectural and quality changes. 9. Fracture risk is determined by the aetiology of increased function of parathyroid glands. Fracture risk is different between secondary hyperparathyroidism and pHPT. In secondary hyperparathyroidism with renal hypercalciuria and recurrently developed calcium oxalate kidney stones the risk factors for fracture are: increased serum sodium, decreased bone mass at femoral neck, destroyed bone architecture. 10. In PTH resistance syndrome, PHP Ia, the bone was considered to be unable to response to PTH. Therefore, bone mass measurements had not been performed before. Our results showed that the increased PTH production in PHP Ia had skeletal effects. The decrease of cortical bone mass, bone architecture and quality in PHP Ia did not differ from pHPT. 11. In P-PHP, which is a special, normocalcemic form of PHP Ia, moderately decreased cortical bone mass was accompanied by high fracture rate. The high fracture rate may be explained by decreased bone quality and hereditary bone deformities rather than decreased bone quantity. 12. Though it was beyond the scope of my investigations, my findings showed that pHPT is diagnosed very late in Hungary. The diagnosis of pHPT among asymptomatic patients lags far behind the European and American data.

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## BALÁZS DALMADI (2005)

### Predictive value of in vitro metabolism studies

Supervisor: Dr. Károly Tihanyi

The in vitro metabolism techniques play an emerging role in drug development. Here, the predictive value of the two levels of these techniques is discussed. The first level of modelling is the chemical-biomimetic level, where metalloporphyrin catalyzed biomimetic oxidation was used for the identification of nitric oxide (NO) donors with diverse chemical structure. Methodology was validated by testing known NO donors. Efficient automation of the test allowed us to investigate a subset of our corporate library. Several hits identified in this campaign were validated in both the chemical and also microsomal model that revealed all hits to be active in the biological system, as well. One of the hits showed comparable activity to V-PYRRO/NO, the prototypic liver selective NO donor.

The other level is the biochemical-biological one where human microsomal (HLM) and recombinant enzymes were used to study the in vitro metabolism of tolperisone. LC/MS measurements revealed methyl-hydroxylation (metabolite at M=261; M1) as the main metabolic route in HLM, however metabolites at 247 and M=263, were also detected. The latter was identified as carbonyl-reduced M1, the former was assumed to be the carbonyl reduced parent compound. Isoform-specific P450 inhibitors, inhibitory antibodies and experiments with recombinant CYPs pointed to CYP2D6 as the prominent enzyme in tolperisone metabolism. CYP2C19, CYP2B6 and CYP1A2 are also involved to a smaller extent. Hydroxymethyl-tolperisone formation was mediated by CYP2D6, CYP2C19, CYP1A2, but not by CYP2B6. Experiments using nonspecific P450 inhibitors - SKF-525A, 1-aminobenzotriazole, 1-benzylimidazole and anti-NADPH-P450-reductase antibodies - resulted in 61%, 47%, 49% and 43% inhibition of intrinsic clearance in HLM, respectively, whereas hydroxymethyl-metabolite formation was inhibited completely by nonspecific chemical inhibitors and by 80% with antibodies. Therefore, it was concluded that tolperisone undergoes CYP-dependent and CYP-independent microsomal biotransformations about to the same extent. On the basis of metabolites formed and indirect evidences of inhibition studies a considerable involvement of a microsomal reductase is assumed.

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- Balogh Gy, Dalmadi B, Bielik A, Keserű Gy (2005) *Identification of nitric oxide donors by biomimetic HTS application. Comb Chem High T SCR 8:347-352.*

JUDIT DONÁTH (2005)

## Clinical studies in Paget's disease with special emphasis on skeletal changes consequent to treatment with bisphosphonates

*Supervisor: Dr. Péter Lakatos*

Paget's disease is a chronic disorder of bone remodeling, characterized by an abnormal increase of osteoclast – and hence osteoblast – activity. The imbalance of bone turnover results in the formation of unhealthy and fragile bone. It leads also to the impairment of adjacent joints and establishes the risk of various complications. The objectives of my work include increasing the awareness of this bone disease, which is uncommon – and even less frequently recognized – in Hungary. As no other reviews on Paget's disease have been published yet in the Hungarian literature, my other goal is to recapitulate pertinent knowledge, to do new epidemiological, genetics and laboratorial studies and to measure the affect of bisphosphonate treatment (to illustrate these facts with experience obtained) during the follow-up of 100 patients. The first part of the dissertation presents new information on the prevalence of Paget's disease in the Hungarian population and describes currently available results of the international trials implemented by our work-group. The next part describes studies into the role of the polymorphism of BsmI (vitamin D receptor gene), of XbaI and PvuII (estrogen receptor genes), as well as of A986S (calcium sensor gene). Our work-group was the first to demonstrate that 'SS' genotype of the calcium sensor gene is more common, whereas 'pp' genotype of the PvuII estrogen receptor gene is less common in patients with Paget's disease, than in controls. Furthermore, 'xx' genotype of XbaI polymorphism has not been observed in pagetic patients. Additionally, no correlation could be ascertained between genetic polymorphism and BMD. The pedigree chart of a Hungarian family has been constructed in cooperation with international partners. Subsequent sections enlarge on essential clinical parameters, major complications, and underlying disorders – using the data of our patient population for illustration. Plasma endothelin-1 level – which can be determined by inexpensive methodology – was first studied as a reliable index of disease activity. The usefulness of conventional and novel imaging modalities was compared in the diagnostic evaluation of Paget's disease. The second part of the dissertation discusses the efficacy of bisphosphonates and changes induced by treatment with these agents.

During one-year treatment with bisphosphonates, significant reduction of tAP level (the best indicator of disease activity) occurs as early as after 6 months. This change is in agreement with the mitigation of pain, as reflected by appropriate pain intensity scales. Moderation of disease activity by early treatment is evidenced also by the improvement of the biochemical markers of bone turnover as well as of quantitative bone scintigraphy (QBS) findings. In addition to these benefits, early treatment can prevent complications. As shown by our experience obtained on a relatively small patient population, calcitonin significantly reduces disease activity and pain intensity (measured with a suitable pain scale). The results of our studies suggest that timely initiation of appropriate therapy achieves remission and moreover, it can prevent the complications and can delay the progression of Paget's disease.

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**MÓNIKA BEATRIX HANTOS (2003)**

## **The significance of the nociceptinergic system in chronic metabolic diseases and neuropsychotic syndromes**

*Supervisor: Dr. Kornélia Tekes*

The nociceptin/OP4 receptor system is present in nearly all of the nuclei of regulatory clusters in the central nervous system. Based upon the distribution of nociceptin in the organism, it could well be presumed that nociceptin influences physiological processes in a complex way, either directly or via the modulation of classical neurotransmitters.

In the present work we have studied the nociceptin and nocistatin contents of blood plasma, cerebrospinal fluid (CSF) and hepatic tissue in experimental animal models, in chronic metabolic disorders, as well as in certain neuropsychotic syndromes by means of radioimmunoassay (RIA). Based on our investigations, we were first to supply data on the nociceptin and nocistatin values of plasma and CSF of control rats, and the alteration of these values with advancing age. We have developed a method for the determination of nociceptin in various kinds of tissues (liver, spleen, testis, thymus, pancreas, brain). We were first to report elevated plasma nociceptin levels in two chronic hepatic disorders, i.e. in Wilson disease and in primary biliary cirrhosis, where decreased activity of the nociceptin metabolizing enzymes (APN, EP24.15) is suspected to be responsible for the above finding. As a result of our measurements, we were also first to report prominently high plasma nociceptin levels in hepatocellular carcinoma and in other malignant tumors. In the groups studied, no difference could be observed concerning tumor etiology and origin (primary or secondary tumor). Thus, the role of nociceptin, as a possible tumor marker in various kinds of tumor etiologies should be considered. We were first to study the role of the nociceptinergic system in primary headache syndromes (migraine, cluster). Based on our results, we assume that the nociceptinergic system exerts an effect on the trigeminovascular system, as well as on the development of neurogenic inflammation.

Our work supplies new and basic data that have not been reported hitherto in the literature, presenting novel observations and conclusions concerning the nociceptin/OP4 receptor system.

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**BORBÁLA KISS (2003)**

## **Development of bioanalytical methods for pharmacokinetic and metabolism studies**

*Supervisor: Dr. Imre Klebovich*

Pharmacokinetic investigations connect with the whole process of drug development in two main points: in the preclinical and clinical phase of original drug development and in generic drug development. During the process of drug metabolism the drug, as the substrate of metabolizing enzymes, undergoes chemical changes that help its elimination. The description of these processes is the subject of the in vitro and in vivo metabolism studies that together with pharmacokinetic studies have an important role in the preclinical and clinical phase of original drug development.

During generic drug development the therapeutic and biological equivalence of the generic and original drug is proved by bioequivalence studies. The different pharmacokinetic and bioequivalence studies require such validated bioanalytical methods, that meet the international rules of GALP and

GLP, and suitable for the quick analysis of a large number of samples as well as the selective and specific determination of the compound, the internal standard and metabolites.

This thesis – as pharmacokinetics - has multiple connections to the process of drug development. The bioanalytical methods developed for the determination of cisapride and ambroxol are the part of generic drug development. The bioanalytical method for the determination of cisapride in human plasma was developed for bioequivalence study. The analysis of ambroxol in dog plasma had the purpose of controlling the quality of the retard formulation in animal study. The metabolism of deramciclane, a novel non-benzodiazepine type anxiolytic drug, was investigated in dog and human studies as well as a part of original drug development. The isolation, purification and quantitative determination of metabolites were carried out by the recently developed liquid chromatography-radioactive detection hyphenated techniques. The applicability of the different techniques during the several stages of metabolism study was investigated. This work contributed to the discovery of the complicated and species dependent metabolism of deramciclane.

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## LÁSZLÓ KÖLES (2003)

### **Electrophysiological studies on the modulation of excitatory ionotropic receptors. Potential targets on the therapy of drug abuse.**

*Supervisor: Dr. Zsuzsanna Fürst*

Excitatory ionotropic receptors, especially ionotropic glutamate receptors in the central nervous system, are of vital importance in cognitive functions, however, their functional disturbances play an important role in the pathomechanism of neurodegenerative disorders as well as drug abuse. The influence of ATP, the possible cotransmitter of dopamine, and its breakdown products were investigated on glutamatergic transmission in prefrontal cortex and striatum, brain areas known to receive dopaminergic input. The effects of alcohols on excitatory ionotropic receptors were also investigated. Whole cell patch clamp experiments carried out in brain slices demonstrated, that the layer V pyramidal cells of rat prefrontal cortex possess P2Y purinoceptors potentiating NMDA type of glutamate receptors. It was also demonstrated, that in a subpopulation of striatal medium spiny interneurons adenosine A2A receptors are expressed, which via the phospholipase C/inositol trisphosphate/calcium/calmodulin/calmodulin kinase II pathway negatively modulate the NMDA receptor channels, but do not influence AMPA receptors. By means of whole cell patch clamp and single-cell microfluorimetry it was cleared, that ethanol as a non open channel blocker, in a voltage independent manner, inhibits NMDA receptors in primary cultures of cortical neurons. Furthermore, trichlorethanol inhibited P2X3 receptor functions much stronger than ethanol in HEK 293 cells transfected by human P2X3 receptors. The main pharmacological importance of our results is, that they might lead to new therapeutic approaches of Parkinson's disease, memory deficits, drug abuse, and schizophrenia, completing and improving the recent pharmacological treatments.

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**ILDIKÓ MIKLYA (2003)**

## **Enhancer substances: (-)-deprenyl and (-)-bpap, the specific stimulators of the brain neurons**

*Supervisor: Dr. József Knoll*

$\beta$ -phenylethylamine (PEA) and tyramine, the endogenous indirectly acting sympathomimetic amines, like their long acting synthetic analogues, the amphetamines, are generally accepted to be releasers of catecholamines from their storage sites ('releasing' effect). It was shown in the early 90s that the releasing effect obscured for decades the main effect of PEA and the amphetamines. These substances are highly potent enhancers of the impulse propagation mediated release of catecholamines and serotonin in the brain ('enhancer' effect). (-)-Deprenyl (Selegiline, Jumex) was the first, and is still the only clinically used PEA/amphetamine-derivative which lost the releasing effect but preserved its enhancer effect. (-)-Deprenyl was also the first described selective inhibitor of MAO-B and the international attention was primarily focused on this effect of the drug. The enhancer effect of (-)-deprenyl is independent from the MAO-B inhibitory effect, as proved by a (-)-deprenyl analogue, (-)-PPAP, which is an 'enhancer' substance, but not an MAO inhibitor. Furthermore, clorgyline (selective inhibitor of MAO-A) and lazabemide (selective inhibitor of MAO-B) are devoid of enhancer effect. Further studies revealed that the endogenous indol-derivative, tryptamine is a potent enhancer substance, too. This led to the development of a tryptamine-analogue, (-)-BPAP, a highly selective and much more potent enhancer substance than (-)-deprenyl. Due to its enhancer effect (-)-deprenyl was found to slow the age-related decay of behavioural performances, to prolong life and to slow the progression of Parkinson's and Alzheimer's diseases. (-)-BPAP is now a candidate to surpass the clinical efficiency of (-)-deprenyl. (Arch int Pharmacodyn Ther 328:1-15, 1994; Life Sci 56:611-20, 1995; 58:817-27, 1996; 58:2101-14, 1996; Br J Pharmacol 128:1723-32, 1999; Life Sci 67:765-73, 2000)

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**KATALIN MÜLLNER (2003)**

## **The role of the central nervous system in the regulation of gastric acid secretion and mucosal protection - involvement of central $\alpha$ 2-adrenergic and opioid-receptors**

*Supervisor: Dr. Klára Gyires*

The balance between the protective and aggressive factors is determinant in the regulation of gastric mucosal integrity. In the last decade more and more regulatory mechanisms became evident that are able to influence gastric acid secretion and mucosal protection through the central nervous system. In our experiments, we aimed to examine the possible role of the central adrenergic and opioid system, as well as their peripheral mediators in the regulation of cytoprotection and gastric acid secretion. Moreover, we studied the action of alpha2-adrenoceptor and opioid-receptor stimulants against ethanol-induced gastric mucosal damage as well as after pylorus ligation. While ethanol-induced gastric ulceration was proved to be an acid-independent ulcer model with the capability to examine the role of mucosal protective mechanisms, pylorus ligation is a widely used experimental method for the investigation of basal gastric acid secretion. The different compounds were applied either intracerebroventricularly (icv.) or intracisternally (ic.) to examine the potential involvement of cen-

tral components. Both alpha2-adrenoceptor agonist clonidine and different opioid-receptor stimulants (DAGO, beta-endorphin, DADLE, deltorphin II, DPDPE) injected either icv. or ic. inhibited the gastric mucosal lesions induced by orally administered acidified ethanol in rats.

Our results suggested that alpha2B-adrenoceptor subtype is likely to mediate the central gastroprotective action of clonidine. In contrast with cytoprotection, results from pylorus ligated rats indicated that central alpha2A-adrenoceptors may be involved in the regulation of gastric acid secretion. The possibility of an interaction between the central adrenergic and opioid-receptors is supported by the fact that opioid-receptor antagonists reversed the central gastroprotective and antisecretory effects of the alpha2-adrenoceptor agonists. The release of beta-endorphin seems to be important in the emerging interaction, since beta-endorphin antiserum inhibited the gastroprotective action of clonidine against ethanol-induced ulceration. K<sup>+</sup>ATP-channels are also likely to be involved in the mucosal protective and antisecretory actions of the examined alpha2- and opioid-receptor agonists since glibenclamide inhibited the effects of these substances by the blockade of K<sup>+</sup>ATP-channels.

As a possible link between the central and peripheral site of action, we examined the role of the vagal nerve. Vagotomy abolished the mucosal protective action of centrally administered alpha2- and opioid-receptor stimulants. In the gastric mucosa, sensory neuropeptides, prostaglandins, and nitric oxide seem to be crucial in the alpha2-adrenoceptor and opioid-receptor induced central gastroprotective action.

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## JÁNOS NEMCSIK (2005)

### **Actions of nitric oxide, estrogen, platelet activating factor and vasopressin on cardiovascular defense in rats**

*Supervisor: Dr. Ferenc László*

In the reproductive age, the incidence of ischaemic heart disease is much lower amongst woman than man. The difference disappears after menopause. In this protection the role of endogenous estrogens seems to be obvious. However, the beneficial effect of hormone replacement therapy with the combination of estrogen and progesterone could not be proven in clinical studies. The role of endogenous vasoactive agents in myocardial ischemia can lead to the discovery of new therapeutic tools. Our aim was to study the effect of endogenous estrogens and the selective estrogen receptor modulator raloxifene for the activity of constitutive nitric oxide synthase enzyme (cNOS). We also aimed to sort out the effect of hormone replacement therapy with natural estrogen or raloxifene and the administration of platelet activating factor (PAF) or vasopressin antagonists for cardiac ischemia, using new angina models. Our results showed that the decreased activity of cNOS of the rat heart and aorta and the increased susceptibility for vasoconstriction in experimental menopause could be restored with estrogen or raloxifene substitution. In our angina models lower ST-depression was observed in female rats compared to males and the difference disappeared after ovariectomy. The replacement therapy with estrogen or raloxifene abolished the worsening effect of ovariectomy, and the administration of the NOS inhibitor L-NAME aggravated the ST-depression. Administration of PAF and vasopressin antagonist decreased the ST-depression of male rats significantly. From our results we conclude that in the rat cardiovascular system estrogens and raloxifene display protective effect through the increase of the activation of cNOS enzyme. PAF and vasopressin aggravate the ischaemia, and the two endogenous agents act synergistically in the angina models. Our results support the potential cardiovascular protective role of raloxifene, PAF antagonists and vasopressin antagonists.

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**PÁL RIBA (2005)**

## The role of spinal $\mu$ and $\delta$ opioid receptors in the development of opioid tolerance

Supervisor: Dr. Zsuzsanna Fürst

It is a well-known phenomenon that in case of chronic administration of morphine tolerance develops to its most effects. Besides, its respiratory depressive effect and the risk of dependence further limit its use. Eliminating or reducing these drawbacks is one of the main purposes of the opioid research.

We studied some aspects of the opioid tolerance by means of a mouse model applying morphine pellet implantation. We found different levels of morphine tolerance in several mouse strains. The levels of tolerance did not depend on the polymorphisms of the genetic sequences of the three main opioid receptors. While significant tolerance developed to subcutaneously given morphine, we could hardly detect tolerance to it. We also investigated the analgesic effect of the highly potent and selective  $\mu$  opioid agonist peptide [Dmt<sup>1</sup>]DALDA on naive and morphine tolerant mice. [Dmt<sup>1</sup>]DALDA proved to be a much more potent and longer acting agonist than morphine given both subcutaneously and it. Cross-tolerance did not develop between morphine and [Dmt<sup>1</sup>]DALDA. Studying the interactions between the spinal  $\mu$  and  $\delta$  opioid receptors we found a potentiating synergism between the highly selective  $\delta$  antagonist TIPP $\psi$  and the  $\mu$  receptor agonist DAMGO in morphine tolerant mice when both drugs were given it.

Recent studies suggested that  $\mu$  and  $\delta$  receptors may form a  $\mu\delta$  heterodimer. During the development of morphine tolerance the  $\delta$  receptor density increases in the central nervous system and the number of the  $\mu\delta$  heterodimers may also be higher. The different behaviours of the opioid agonists and antagonists on the  $\mu\delta$  heterodimer may explain the incomplete cross-tolerance between morphine and [Dmt<sup>1</sup>]DALDA and the potentiating effect of TIPP $\psi$  on the antinociceptive activity of DAMGO at the spinal level.

- Riba P, Ben Y, Nguyen Thi MD, Furst S, Schiller PW, Lee NM (2002) [Dmt<sup>1</sup>] DALDA is highly selective and potent at  $\mu$  opioid receptors, but is not cross-tolerant with systemic morphine. *Curr Med Chem* 9:31-39.
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ANDRÁS ZOLTÁN RÓNAI (2005)

## Natural opioid peptides, synthetic analogs, peptidases: pharmacological analysis and drug design

Supervisor: Dr. Kálmán Magyar

The pharmacological properties of natural opioid peptides (endorphins, proenkephalin-derived peptides) and enkephalin analogs were analysed mostly *in vitro*, by isolated organ technique and, for correlation purposes, also *in vivo*. As a „side-line”, we detected and partially characterised a novel dipeptidyl-carboxypeptidase. As an „etude” we designed and characterised novel families of  $\delta$ - and  $\kappa$ -opioid receptor-selective peptide antagonists.

We have explored the informational value of six, opioid receptor-containing isolated organs. They were the field-stimulated longitudinal muscle strip of guinea-pig ileum, mouse, rat and rabbit vas deferens, perfused rabbit ear artery and cat nictitating membrane. We modified significantly the method for the use of mouse vas deferens and rabbit ear artery; in the latter, we have demonstrated the presence of  $\delta$ - and  $\kappa$ -opioid receptors. The pharmacological properties of  $\beta$ -endorphin differ significantly from those of (Met<sup>5</sup>)-enkephalin and shorter  $\beta$ -lipotropin fragments both *in vitro* and *in vivo*, where it was a strong analgesic in the rat tail-flick test given by intracerebroventricular route. We have developed a novel enkephalin analog, (D-Met<sup>2</sup>,Pro<sup>5</sup>)-enkephalinamide which was 80 times more potent analgesic than morphine in the same test given by the same route. We have clarified that the  $\mu$ - or  $\delta$ -receptor selection of agonism by enkephalin analogs is governed mainly by the C-terminal modifications; the analgesic analog had an increased tendency of  $\mu$ -receptor type selection as compared to (Met<sup>5</sup>)-enkephalin. The novel dipeptidyl-carboxypeptidase was detected in neural elements of rabbit ear artery and human pheochromocytoma. Its substrates should have -RF, -RY or -FR at the C-terminus. It is a metallopeptidase, and differs from angiotensin converting enzyme, neutral endopeptidase and rat brain peptidase „PDP-3”.

The pure, competitive  $\delta$ -receptor-selective antagonist peptide BOC-YPGFLT(OtBu) had a  $K_e$  of 40 nM and more than 5,000-fold selectivity over  $\mu$ - or  $\kappa$ -receptors. The  $\kappa$ -receptor agonists (e.g. BOC-YKWW-NH<sub>2</sub>) had  $K_e$  values in the micromolar range and 8-20-fold selectivity ratios. In both groups the absolute novelty was the principle of N-acylation.

- Ronai AZ, Graf L, Szekely JI, Dunai-Kovacs Zs, Bajusz S (1977) Differential behaviour of LPH-(61-91)-peptide in different model systems: comparison of the opioid activities of LPH-(61-91)-peptide and its fragments. *FEBS Lett* 74:182-184.
- Ronai AZ, Szekely JI, Berzetei I, Miglecz E, Bajusz S (1979) Tetrapeptide-amide analogues of enkephalin: the role of C-terminus in determining the character of opioid activity. *Biochem Biophys Res Commun* 91:1239-1249.
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## 4. PH.D. SCHOOL OF MENTAL HEALTH SCIENCES

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### *Head of School*

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The PhD training and research programs of the Interdisciplinary Doctoral School IV of Semmelweis University aim to offer research and methodological training for students interested in conducting research in the field of mental health. The strength of the school is the *inter-disciplinary nature of research in such areas as psychiatry, psychology and behaviour and mental health sciences*. Our research topics highlight the interactions between mental health and social sciences. We offer courses both in medical and social sciences, some of them focusing on the relationship between the interdisciplinary nature of some research areas. The school promotes initiatives related to preventative medicine. One of its goals is to facilitate the PhD candidates's skills for promoting their research topic in public.

### **4/1. PROGRAM**

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## **CLINICAL PSYCHOLOGY AND PSYCHIATRY**

### *Coordinator*

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This program offers education and research possibilities for psychologists, medical doctors and other eligible persons in behavioural sciences, clinical psychology and in the neurobiological, psychosocial, diagnostic and treatment aspects of psychiatric disorders. The Program is mainly clinical and includes applied research related to mental health, human behaviour, psychology and psychiatry. Research topics are supervised by members of four University Departments/Institutes.

#### *Sub-programs*

Psychotherapy in clinical practice

The follow-up of organically based psychopathological symptoms and their treatment with electrophysiological methods

Clinical psychopharmacology

The significance of feed back in the diagnosis and therapy in psychiatry and psychotherapy

Neuropsychology in clinical practice

Communication in family therapy

The characteristics of the trauma in the posttraumatic stress disorder, the principles of treatment

The research of anxiety and mood disorders in psychotherapy

The role of the maternal interactions aiming emotional feed back in the infants' evolvement of the emotional representation abilities

#### *Supervisors*

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Támás KURIMAY

Dóra P.-FORINTOS

Dóra P.-FORINTOS

György GERGELY

Interpersonal relationships; the interpretation of the manifest and inherent messages in family communication by various methods and psychometric coding processes	Emőke BAGDY
The research of the pharmacotherapy in neuro-psychiatric diseases	György BAGDY
Schizophrenia and emotional interactions and expectations in the family	János FÜREDI
Macro-social effects on the families and their functional disorders	János FÜREDI
Perinatal experiments of animals with hypoxia; the cellular and molecular impact mechanisms of cerebral damages in hypoxia/ischaemia	Csaba NYAKAS
The influence of interpersonal interactions to the affective and anxiety disorders	Erika SZÁDÓCZKY

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a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

KITTY ALMÁSI (2003)

## Depression and grief-reaction. Symptomatological comparison especially of suicidal symptoms

*Supervisor: Dr. János Füredi*

The aim of our study was to compare the frequency of patients with major depressive episode and bereavement in the Hungarian adult population. 4747 randomly selected subjects - aged between 18 and 64 years - were interviewed according to the Hungarian version of the Diagnostic Interview Schedule (DIS), which generated DSM-III-R diagnoses. There were only 2 persons having both lifetime grief-reaction and major depressive episode, which means that neither the risk of depression in patients who previously suffered from complicated grief nor the risk of complicated grief in patients who previously suffered from depression were higher in our sample than the general population. Thoughts of death were significantly more frequent in bereavement, while hypersomnia, feeling of guilt and worthlessness, low self-esteem, suicidal thoughts and prior attempted suicide, as well as history of comorbid panic disorder were significantly more common in major depression. In spite of the overlap in the symptomatology between bereavement and major depression, some symptoms and prior history of panic disorder and attempted suicide can help in differentiating the two conditions. Recognition of this significantly different symptomatological state can help clinicians to differentiate those patients in the early period of bereavement who are at high risk for showing (bereavement provoked) depression later.

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JUDIT BALÁZS (2002)

## The Hungarian adaptation of the M.I.N.I. and M.I.N.I. plus interviews and using them in research-psychiatric investigation of suicide attempters

*Supervisor: Dr. László Tringer*

My work is divided into two parts. In the first part, I worked on the adaptation of a short, structured psychiatric diagnostic interview, the Mini International Neuropsychiatric Interview (M.I.N.I.) and its extended variation (M.I.N.I. Plus). According to the international and my own data, the M.I.N.I. can be administered in a much shorter time than the structured interviews used before. In this way it saves much of the interviewers' and the patients' energy. For the validity study of the M.I.N.I. I chose three different criteria: the admission diagnosis of the patients, the "best estimate" diagnosis based on all documentations of the patients and another structured interview - the Diagnostic Interview Schedule (DIS). After these investigations the question arises, if there is at all a "gold standard" during the validity study of an interview. As a result, I suggested the use of the M.I.N.I. in Hungary as a diagnostic screening tool for psychiatric clinical practice and for research. In the second part of my work I examined suicide attempters using the valid M.I.N.I. interview. The aim of my study was to investigate the prevalence of mental disorders according to DSM-IV and their "subthreshold forms" among individuals who had recently attempted suicide in Hungary. I also wanted to compare first and repeated suicide attempters. The results of my study confirm those of previous findings showing high rates of DSM-IV mental disorders (the most frequent diagnosis was major depressive episode) among suicide

attempters. The results show a remarkable high prevalence of bipolar II disorders among suicide attempters. To my knowledge, this is the first study which emphasizes the importance of first major depressive episode and “subthreshold forms” of mental disorders among the risk factors of suicide attempt. The high percentage of repeaters among suicide attempters underlines the importance of careful long-term treatment of these patients. In conclusion, I would like to draw attention to the importance of early detection and treatment of psychiatric disorders, including „subthreshold forms” for the prevention of suicidal behavior.

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- Balázs J, Kiss K, Szádóczy E, Bolyós Cs, Laczkó K, Szabó J, Bitter I (2001) A M.I.N.I Plusz és a DIS kérdőív validitás vizsgálata. *Psychiat Hung* 16:5-11.
- Balázs J, Bitter I, Lecrubier Y, Csiszér N, Ostorharics G (2000) Prevalence of subthreshold forms of psychiatric disorders in persons making suicide attempts in Hungary. *Eur Psychiatry* 15:354-361.

**GÁBOR LÁSZLÓ GAZDAG (2004)**

## **Evaluation of electroconvulsive therapy use in Hungary and analysis of different drug's impact on seizure activity**

*Supervisor: Dr. László Tringer*

This work contains five investigations in three parts. All five of them deal with different questions of electroconvulsive treatment, with the goal to establish the Hungarian protocol of ECT. The first investigation focuses on the present practice of ECT use and thus explores the most important questions of the protocol. On evaluating ECT use in Hungary with a questionnaire, a very low utilization rate was found comparing with international data. ECT is mostly used in the treatment of schizophrenic patients in a lower number of sessions than the international practice and recommendations.

The goal of the two investigations in the second part of the study was to find the most suitable drug for the anesthesia of ECT concerning the impact on seizure activity and on the cardiovascular system. According to our results propofol reduces seizure duration comparing with methohexital and etomidate, but in our investigation there was no need for restimulation more frequently or with higher doses. At the same time, propofol better decreased hypertension and tachycardia, appearing during convulsion, than etomidate. These properties make propofol a good choice for the anesthesia of electroconvulsive therapy.

The impact of the concomitant psychopharmacological medication on seizure activity was investigated in the third part of the study. We could prove no influence of citalopram, but seizure duration was prolonged with fluoxetine. According to the Hungarian ECT practice the most important question is the influence of the neuroleptics on seizure activity. In our second investigation, we proved epileptogenic property of clozapine, olanzapine and zuclopenthixol. Haloperidol, fluphenazine, risperidone and sulpiride did not show any provable influence on seizure activity. There was only one substance, quetiapine, which showed antiepileptic properties, and thus might compromise the effect of the treatment.

- Gazdag G, Kocsis N, Tolna J, Lipcsey A (2004) Attitudes toward electroconvulsive therapy in Hungarian psychiatrists. *J Ect* 20:204-207.
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ÉVA JEKKEL (2004)

## Cognitive distortions in the background of suicid behaviour

*Supervisor: Dr. László Tringer*

As international statistical data reveal, the number of suicide attempts has been increasing recently all over the world - even at places, where the number of suicidal deaths has not increased. Suicidal behavior consists of a complexity of biological, psychological, and social factors. The transition of these factors to suicide attempt appears to be determined by cognitive processes. For a long time, Hungary was among the leading countries in the world regarding the number of suicides. This explains the recently intensified interest in the topic, and the process of preventing suicidal acts has been studied thoroughly. In this dissertation we present the results of our research. We tried to reveal the dysfunctional attitudes, cognitive distortions and coping strategies in the background of suicidal behaviour. The participants of our study were suicidal inpatients (N=50), and control psychiatric inpatients and outpatients (N=50). We used a matched group design: suicidal patients were compared to a non-suicidal control group of "social twins", by matching them on the following variables: 1) Age, 2) Gender, 3) Education, 4) Diagnosis according to ICD-10. The case group and the control group only differed on their history of presence and absence of suicidal behaviour. For both groups, the following test battery was used: 1) Detailed clinical interview, 2) Weissmann's Dysfunctional Attitude Scale, 3) Folkman and Lazarus's Coping Questionnaire, 4) Beck Depression Inventory, 5) Hamilton Anxiety Scale, 6) C-symptom Scale (Derogatis' Brief Symptom Checklist). The above tests were completed by all members of both groups. Suicidal patients completed the questionnaires within a week after their suicide attempt. Participants were compared based on their scores on the above 5 scales, and their subscales. The majority of our hypotheses has been proved: There was no statistically significant difference between suicidal and non-suicidal groups in their psychopathological states (BDI, HAS, BSI), but suicide patients showed specific dysfunctional attitudes, significantly higher level of irritability, an increased external control attitude, and they used asking for help, as coping strategies much less. Also they valued their situation and their future hopeless.

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DÓRA KOVÁCS (2004)

## Analysis of the symptoms of eating disorders

*Supervisor: Dr. István Bitter*

**Objective:** The purpose of this study to investigate the different symptoms of eating disorders. Tries to answer whether the excessive exercise, helps patients coping with purging symptoms. Compares purgative and restrictive anorexics, and analyses the symptom of laxative abuse. Investigates the symptom of chewing and spitting food. Examines the measure of self-esteem and the connection to the other symptoms. Examines the difference between bulimia nervosa and anorexia nervosa in the special psychopathological symptoms, and analyses the connections to the purging symptoms. **Methods:** 124 patients with anorexia nervosa and 242 patients with bulimia nervosa were studied. Demographic data and rating scales of these patients were analysed. Symptom severity were measured by the Clinical Eating Disorders Rating Instrument (CEDRI), and also the Rosenberg Self-Esteem Rating Scale (RSES), the Symptom Check List 90-R (SCL-90-R), and the Eating Disorder Inventory (EDI) were used. Comparisons were made with t test and Mann-Whitney test, predictors were defined by logistic regression, and connections were analysed by Spearman correlation. **Results and discussion:** Excessive exercise can help bulimic patients to avoid vomiting. Important differences were found between purgative and restrictive anorexics, laxative abuse played an important role in these differ-

ences, and it was responsible for the worse result on the rating scales. The prevalence of the symptom of chewing and spitting food is the same in the anorectic and bulimic group. Among anorectic patients it accompanies with more severe symptomatology, and among bulimic patients it can be an alternative of bingeing. The measure of self esteem is very low in both the anorexia nervosa and the bulimia nervosa groups. Among anorectic patients the self esteem related to binge eating and laxative abuse. Patients with bulimia nervosa tend to have higher ratings on body dissatisfaction subscale, and patients with anorexia nervosa tend to have higher ratings on maturity fear subscale.

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**MÓNIKA KOVÁCS (2003)**

## **Epidemiological and clinical implications of the comorbidity of allergic diseases and depression**

*Supervisor: Dr. János Füredi*

*Aims:* 1) To evaluate the prevalence of depressive symptomatology and disorders, and frequently related psychological problems (anxiety, sleep disturbances, suicide) in allergic patients and young women; 2) and the prevalence of allergic diseases in psychiatric patients; 3) to analyze the background factors and clinical significance of the high comorbidity; 4) and the reliability and usefulness of the questionnaires included into the analysis in this special patient group. *Methods:* Study samples: 1) Multicentric self-administered questionnaire screening (N=889) in 6 Hungarian allergy clinics with a set of questionnaires called "Allepsy" and designed for this study (allergic diseases, psychiatric scales, sociodemographic and healthcare data); in a subsample of the patients structured diagnostic interview by the MINI Plus (N=60) and follow-up with questionnaire (N=293/95/85). 2) Evaluation of a representative population sample of young women (N=3615) with the "Better Health for Women" set of questionnaires. 3) Evaluation of 323 non-selected psychiatric patients with the Allepsy questionnaire. Control sample: national representative health survey of the Hungarian population (Kopp et al., 1995) (N=12640). *Results:* 1) 50.1% of the patients had elevated scores on the depression, anxiety or neurosis scale. Depression and neurosis scores were significantly higher in the allergic than in the control sample from the general population. According to the results of the structured interview and the reliability measures of the screening questionnaires, 20.7% prevalence of depressive, and 22.4% of anxiety disorders is estimated in the overall screened sample. 2) In the young women sample allergic women scored significantly worse in all the analyzed psychological variables than non-allergic ones. 3) Prevalence of allergic diseases was significantly higher in psychiatric patients than in the general population, and in those with depressive or anxiety disorders than with other (e.g. psychotic) disorders. 4) Risk factors for comorbidity revealed to be older age, female gender, asthma, more severe or perennial allergic symptoms, multiple or unspecific triggering factors, sleep disturbances, and positive psychiatric history amongst all. *Conclusions:* Besides high comorbidity, my results show that depression has a negative impact on allergic diseases, on the other hand it is often unrecognized and not or inadequately treated. "Allepsy" questionnaire revealed a simple but reliable screening instrument in allergic patients.

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**FERENC MARTÉNYI (2005)**

## Fluoxetine in the treatment of posttraumatic stress disorder

*Supervisor: Dr. István Bitter*

Posttraumatic stress disorder (PTSD) is characterized by psychopathologic responses to severe, potentially life-threatening events. Convincing evidence has been established concerning the efficacy and safety of selective serotonin reuptake inhibitors (SSRI-s) in the pharmacologic treatment of PTSD. However there are some inconsistencies in the results of randomized, placebo controlled trials regarding gender and / or trauma related outcome differences. The efficacy and safety of an SSRI (fluoxetine) have been investigated in a trial of PTSD in the treatment of a mixed population of civilians and veterans. The study was a placebo controlled 12 week acute trial followed by a 24 week relapse prevention phase. Data about combat-related patients were subsequently analyzed. Three hundred and one patients diagnosed with PTSD met entry criteria at the baseline, with CAPS total scores of 80. Fluoxetine treatment showed statistically highly significant superiority compared to placebo in the TOP-8 and in numerous other secondary outcome measures (CAPS, DTS, MADRS, HAMA) during the 12 weeks of acute treatment, both for the mixed and for the combat-related population. Time-to-relapse was significantly superior in the fluoxetine treatment arm as compared to placebo for the mixed population, and a strong tendency of prevention relapse of PTSD was detected for veterans as well. The results of the mixed (civilian and veteran) population suggest that the required dose for the treatment of PTSD is in the upper part of the antidepressant dose-range. In contrast to other studies the patients of the present trial have been diagnosed and treated after much shorter delay. The fact, that the patients were less chronic might explain the good efficacy data regardless of the type of trauma.

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**NABIL NUMAN (2004)**

## The green leaf

*Supervisor: Dr. János Füredi*

Khat (*Catha edulis*) phenomenon in Yemeni society is considered as social custom. The general view to khat as drug addiction, like those widespread substances in the West, reflects something of exaggeration due to the ignorance in nature of the real social life in Yemen. Since the absence of knowledge of foreigners and visitors; in nature of Yemeni social and cultural life, they often have the erroneous impression about khat and Yemen; they portrayed Yemen as a country of addiction and the apathy among people due to khat addiction. Because their knowledge about khat use is not merely adequate; it is also filled with inaccuracy and misconception.

The significance of khat sittings can not be realized or understood, in terms of physical or psychological or psychopharmacological effects, unless knowing the social, cultural and ritual traditions that urge the individuals to participate in khat sittings. But, to objectively understand khat sitting, however, it is necessary to discuss khat phenomenon to give a more concrete impression of the khat sitting at the level of the place, participants and social activity. The users consume khat not only for its psychological effect (control the fear and anxiety in a hostile world as well as to give pleasure euphoria), or to relief of pain or escape from life events (reality), or to forget the harsh surroundings. But in fact, khat makes chewers contemplating in his internal world and going deeply into surrounding.

The observers, who are interested in Yemen society, will find out that the social and cultural pressures are the main factors that induce and urge the individuals to participate in khat sittings, and this phenomenon penetrates deeply into all Yemeni lives. Therefore, khat users deem that khat confirms their Yemeni identity; belongingness, social status, and source of pride, boast and sense of self-esteem. Undoubtedly, that khat sitting is considered as a field for the exchange of information, creates, and reinforces the strong and further relations between the participants. Therefore, even those who do not use khat or who rarely use it are forced or desired to participate in khat sittings in order not to be deprived of the interesting behaviour and communication. Therefore, the present study aims at assessing the psychopathological aspects of khat use in Yemeni population. I wish to characterize khat use in Yemeni population, in terms of incidence and gender distribution, to assess the frequency and severity of nine psychiatric symptoms in khat users as compared to non-users, by using SCL-90 evaluating critically the relationship between khat and psychiatric status as the present and future socioeconomic consequences of regular khat use.

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JUDIT TOLNA (2003)

## A controlled follow-up study of endogenous psychotic patients 25–30 years after the initial psychosis

*Supervisor: Dr. László Tringer*

Twenty-five to thirty years on, we conducted a multi-variable, multi-method controlled follow-up investigation across the whole spectrum of endogenous psychoses. We assessed the course and outcome of the illnesses of the patients, and the extent to which their diagnosis accorded with their prognostic indications, in other words the stability over time of the illness units described by Leonhard in the wake of Wernicke and Kleist.

In our investigation we managed to re-assess 138 of the 238 endogenous psychotic patients diagnosed and assigned to 8 middle groups in the Leonhardian system 25–30 years ago by two independent examiners. This represented a rediscovery rate of more than 96%, since in the intervening period 98 patients had died. Of the normal control group members (whom, 25 years ago, we inserted among the members of the two most different groups) we re-examined 38. In this group 6 persons had died, 6 could not be traced and 4 declined re-examination. The catamnestic examination was administered to patients while they were in the best state that could be expected of them. During the examinations we assessed background characteristics, characteristics of the course of the illness, psychopathological symptoms, personality type, quality of social adaptation, and psychological characteristics.

According to the data measured, with regard to many factors in the background and in the course of the illness the groups were clearly distinct from one another. Analysis of the psychopathological symptoms on the one hand reflected the separateness of the groups and on the other hand supported the favourable outcome of the illness expected in the cycloid, unipolar depressive and bipolar manic-depressive groups and the unfavourable outcome expected in the systematic schizophrenia groups. Using the psychopathologic symptoms measured in detail at the time of the catamnestic examination, we were able to assign patients to their original groups with a probability of between 45% and 75%. This proportion was between 60% and 95% when we did not regard as mistaken assignment to the normal control group in those groups where recovery was expected. When all aspects measured were taken into account, the assignment rate was between 73% and 100%. The stability of the different groups varied, with the affect-laden paraphrenia group proving the least stable. Our findings supported our hypotheses. The distinctness of the patient groups diagnosed in the wake of Leonhard's thinking was discernible even after 25–30 years of the illness. The outcome of the illness for the various groups corresponded to the prognostic indications of the original diagnoses. The permanence of the catatonic groups was marked, as opposed to the dispersal of the patients of these

groups that occurs in the DSM-IV system. The much-debated cycloid group emerged as a separate, stable nosological unit. On the basis of all this, the Leonhardian nosological classification, which takes into account the overall picture as well as the personality, is suitable, in the long term also, for the forming of stable, homogenous patient groups.

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## 4/2. PROGRAM

### BEHAVIOURAL SCIENCES

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The behavioural sciences constitute an integrative field which bridges the paradigms of natural and social sciences. They study human behaviour in a biological, psychological and social perspective, and provide an opportunity for establishing and analysing the components of healthy behaviour, the psychological and social risk factors of diseases, as well as investigating the background of self-destructive conduct and the development of attitudes to protect health. They examine the regularities and the possibilities of developing human behaviour from an interdisciplinary and integrative perspective relying on achievements of Medicine, Psychology, Sociology, Anthropology, Bioethics, Neuroanatomy and Neurophysiology. Nowadays, the prevention and successful treatment of diseases which impact on public health cannot rely entirely on a biomedical approach since the behavioural risk factors are highly influenced by psychological and social factors. The professional Program follows the analogue one of Johns Hopkins University (Baltimore, Maryland, USA).

#### Sub-programs

Behavioural sciences and health psychology  
Health and bioethics  
Doctors' health behaviour and morbidity  
The aspects of mental health of agony, death and mourning  
The research of correlations between personality characteristics and diseases  
The psychological research of eating disorders  
The psycho-social aspects of chronic diseases  
The effectiveness of communication in prevention and treatment  
Genetic effects in the epigenesis of the temperament and attachment behaviour  
Behaviour epidemiological research of life style and health of young people  
The correlation between sleep, cognitive activities and mood

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Mental health, disorders and quality of life- the possibilities of prevention and intervention focusing on the depressive and anxiety disorders in the elderly	Mónika KOVÁCS
Medical antropology: the cultural and inter-cultural references of illnesses and their treatment	Imre LÁZÁR
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a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

**B. TAMÁS BÁBEL (2003)**

## Neurosurgical treatment for premotor disorders. Clinical studies on functional neurosurgical treatment for Tourett Syndrome and obsessive-compulsive disorder

*Supervisor: Dr. Mária Kopp*

The increasing frequency of intractable patient suffering from mental disorders, and the advances in reversible neurosurgical technology have brought the focus on operative approaches recently. The severe symptoms in treatment refractory groups of obsessive-compulsive disorder (OCD) and Tourette syndrome (TS) lead to an incapacitating illness, with overall functional impairment resulting in suicide attempts or showing self-injuries behavior. Emerging research evidences reveal the impaired movement-, and on a most complex level-behavioral organization in underlying pathomechanismus of these disorders. These findings may delineate these diagnoses from other indications of psychiatric surgery. For highlighting the difference I introduced the alternative premotor disorders terminology. That corresponds with the shared functional neuroanatomical basis and points to the crucial importance of neurobiological substratum in stereotactic surgical method. The main objective of thesis is to assess the efficacy and safety of surgical therapy in intractable OCD and TD by clinical studies. Then I will to determine the role of surgical modality in treatment refractory groups of both disorders.

The surgical experience for Tourette syndrome limited to case reports. We conducted a retrospective follow-up study to evaluate the perioperative and late clinical data of seventeen consecutive treat-

ment refractory TD patient operated between 1970-1998 at Functional Stereotactic Department of Albert-Ludwig University (Freiburg, Germany). All cases were into subtypes according to the protocol of the Tourette's Syndrome Study Group. One patient was excluded from the study. The preoperative, postoperative and late tic severity was assessed by Tic Severity Rating Scale. The median follow-up was seven years. Six cases were lost to long term follow-up. We concluded that thalamic and infrathalamic lesioning provide a significant long-term reduction of tic severity despite of symptom severity fluctuation phenomena. Bilateral interventions have higher risk for permanent morbidity. Adequate selection of the side of first intervention might prevent the patient from increased risk of bilateral surgery. The efficiency of bilateral anterior capsulotomy in intractable OCD was examined by a prospective multidisciplinary study. We designed a patient group surgical setting, operating five extremely disabled OCD patients in a week, in order to promote the group therapy psychiatric methods in rehabilitation. The median duration of illness was 20 years (16-34 years). The median social isolation duration of illness was 9 years (5-13 years). Median symptom severity on YBOCS was found to be 38 points (36-40 points). At the one-year follow-up we observed significant reduction in all symptom dimensions. The different symptom components showed a delayed common parallel reduction dynamics during the follow-up. We were not able to detect any kind of cognitive or personality changes. Transient headache, fever, incontinence, apathy occurred. Weight gain could happen lately.

OCD and TD share a common functional neuroanatomical basis, within the impairment of premotor function might explain the clinical features. I propose a model to explain the pathomechanism as an irrelevant, extrem probability association between more and more complex motor patterns during movement- and behavioral organization. Surgical lesioning generates an irreversible re-modelling at different receptorial patterns. During this period psychiatric rehabilitation promote the re-building of pathologic behavioral patterns.

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**CSILLA CSOBOTH (2005)**

## **Psychosocial correlates of Hungarian women's mental health: focusing on the life-course perspective**

*Supervisor: Dr. Mária Kopp*

*Aims:* The epidemiologic study of depressive symptomatology, frequent anxiety, and health damaging behavior and their psychosocial determinants, namely socioeconomic status, family background, and physical and sexual abuse. Definition of the prevalence of positive mental health variables and mental health problems and the analysis of the relationship with the chosen risk factors. *Methods:* Analysis of two samples gained from representative cross-sectional studies, which gained data of 3,615 young 15-24 year old and 6,987 adult (above 18 years) women. Comprehensive and representative studies concerning the relationship of mental health and physical and sexual abuse among women have not been previously conducted in the Central-Eastern European region. *Results:* 7.6% of young women reported no goals in life, 26.9% had low self-efficacy and 48.6% had a high hostility score. Depression symptomatology was found in 26.6% of the sample. 7.9% reported frequent anxiety and 61.2% reported some type of health damaging behavior. In the adult sample 47.5% reported low well-being, 12% had no goals in life, 21.1% reported low self-efficacy and 47.1% had high hostility. 30.7% of women reported depressive symptomatology, 27.8% had frequent anxiety and 27.9% used some type of substance. Subjective financial deprivation showed the strongest association with depressive symptomatology and the second strongest association with anxiety in all age-groups. The objective socioeconomic indicators were mainly risk factors for depression and anxiety among the

middle-aged and the elderly, but were protective factors for health damaging behavior. Subjective family background variables increased the risk of mental health problems considerably. Physical and sexual abuse are highly prevalent among Hungarian women. Abuse by important person increased the risk for mental health problems in the younger age-groups and abuse by partner in the older age-groups. *Conclusions:* Women's mental health is influenced by different protective and risk factors along the life-span. Positive mental health, subjective financial deprivation, family background factors, and physical and sexual abuse all need to be taken into consideration in the prevention of mental health problems and the promotion of mental health.

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**ERZSÉBET KAPOCSI (2005)**

## **Medical profession and medical ethics in the 20th-21st centuries**

*Supervisor: Dr. József Kovács*

The role, function and social status of the medical profession has undergone fundamental changes during the last two or three decades, in nearly all the developed countries of the world. Parallel to the transformation in society and healthcare, the inner composition and structure of the profession has changed fundamentally. As a consequence, the interpretation of the profession's role, its self-identity and ethical self-assessment have suffered a disturbance. Physicians have to simultaneously face the changing relations with their patients, society and the paramedical professions, and confront the inner transformations, pushing at the limits of the profession. The aim of the dissertation was a historical, structural and functional analysis of medical profession and medical ethics. Its main principle of methodology was a model-analysis; the medical profession was analyzed as a profession on the level of abstraction, along its two main components, the healer's role and the professional status. The methods of examination employed in my dissertation were historical analysis, descriptive analysis, interpretation, comparative analysis, critical analysis and summarizing. Following a brief historical review, I mainly concentrated on the changes occurring in the second half of the 20th, and the beginning of the 21st century. I emphasized the changes within medicine, the autonomy of the medical profession, and the relationship between the profession and society. Within the field of medical ethics I examined its situation today, as well as the question of whether the traditional deontology and bioethics can be reconciled. Results and conclusions: Biological and life sciences, medical science and technology and the development of healthcare have opened almost boundless new perspectives in medicine and health preservation in the second half of the 20th century. At the same time, however, medical profession is going through one of its deepest crises in modern history at the turn of the 20th and the 21st century. Deprofessionalization, that is the erosion of the profession's classical professional and moral autonomy, is a process that is felt by doctors every day. The decreasing prestige of the profession (both material and moral), the wavering social trust, and the very little appeal the medical trade has for young people is the other aspect of this process. The way out from this crisis can be found by accommodating the classical values of the profession (professional knowledge, altruism, morality) to the demands of our modern age (partnership with patients, continuous discourse with society, and a balanced relationship with the paramedical professions). The coexistence of various professional models (contracted, service provider, entrepreneur and employee) seems very likely. Parallel to the renewal, there is a need for work out a definition for the identity of the modern physician, and a new set of roles, adequate for the new circumstances. The interpretation of the moral-ethical dimension as a constitutive element of the profession necessitates the further perseverance of medical ethics. The synthesis of bioethics and medical ethics does not seem possible due to

their difference in crucial points. However, the ethics of medical profession definitely needs to be modernized, and such a process can be augmented by bioethics. Doctors of the 21st century are going to work in a healthcare system fundamentally changed in its main attributes, and in a well-informed society, that expects quality service. In the course of preparation for the profession, and in acquirement of the attitudes and approach for the practice of modern medicine medical humanities (sociology, psychology, ethics and communication) are and will be indispensable.

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**ESZTER MAJERCSIK (2004)**

## **The elderly today; Geriatric socio-psychological study, options of improving the quality of life**

*Supervisor: Dr. Mária Kopp*

The growing ratio of the elderly is a great challenge for public health services both in field of cure and prevention, and it is the same for the whole society in respect to social welfare. In first part the study quantifies on interval scale on motivation theory approach the needs of geriatric patients. The survey of geriatric hierarchy of needs aims to analyse particular care options regarding age characteristics. The general health feeling is determined by somatic, social- and psychological factors. The well-being reflects the harmony of these factors, the socio-psychosomatic balance. The investigation (n=303) had been carried out by the method of paired comparisons of questions at the Geriatric Department of St. Margaret Hospital. Determination and quantification of the general health feeling has been performed in two ways. One of them was based on the hierarchy of needs by Maslow. Numeric values have been calculated individually for each level of the respondents' needs by the method of paired comparisons. The results have been summarized for the whole cohort by the help of "Guilford method" and general health feeling index has been quantified. The other approach was the self-rated-health method. The general health feeling of the cohort has been determined by comparison and analysis of both investigations. The research results prove that the hierarchy of needs by Maslow has been restructured with the elderly. The self-actualisation needs take the first place, while the physical ones are confined to the last grade of the hierarchy of needs of geriatric patients. The new result is in the study the numeric scaling of geriatric hierarchy of needs by psychometric assessment. In second part of the study the investigation (n=384) aimed to analyse the interactions between psychosocial factors, anxiety and anxiolytic efficacy of medication. Drug responses appear to be modulated by non-pharmacological factors and the social support among these plays one of the most important role. A low number of social contacts associated with a large number of diseases proved to be a strong risk factor for anxiety with elderly people, whereas the reverse condition (many contacts/few diseases) was associated with considerably lower HAM-A scores. Both the number of social contacts and health status were important determinants of drug response, and both showed a positive correlation with the buspirone response. It is a new finding in the study that the social support is a positive predictor of buspirone efficacy. The study aimed to contribute to facilitate the improvement of quality of life of elderly. It can be reached primarily by strengthening the protecting factors and by driving back the endangering ones. The successful aging must be actively supported by the national social- and health policy in order to improve the quality of life.

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**MIKLÓS ZSOLT MOLNÁR (2005)**

## **The effect of sleep disorders and anemia on quality of life in patients with chronic kidney failure**

*Supervisor: Dr. István Mucsi*

Several factors are known or suspected to influence the quality of life of patients with end stage renal failure. Two of these factors, anemia and sleep disorders have been analyzed in detail in this work. Sleep disorders, including obstructive sleep apnea, restless legs syndrome and insomnia, are frequent complaints in patients with end stage renal failure. Sleep disorders were assessed with standard questionnaires that have been used in several published studies. Health related quality of life was also measured with a standard, validated instrument, the KDQoL-SF questionnaire. Post transplant anemia, and factors associated with the condition was also assessed in a separate set of analyses. Insomnia was the most frequent sleep complaint in patients on maintenance dialysis. In the studies presented here the prevalence of restless legs syndrome was 11-15% in patients on maintenance dialysis and it was 4.8% in kidney transplanted patients. In multivariate analysis the modality of the renal replacement therapy was independent and significant predictor of the presence of restless legs syndrome. Comorbidity was also strongly associated with the presence of the sleep disorders in patients with end stage renal failure. The presence of sleep disorders have a negative impact on health related quality of life both in patients on maintenance dialysis and in kidney transplanted patients. In our survey on in three transplanted patient had some degree of anemia. Almost 80% of these patients does not receive any treatment for this condition. In multivariate analysis graft function and malnutrition/chronic inflammation were independent and significant predictors of the presence of anemia in kidney transplanted patients. Transplanted patients with anemia had significantly worse quality of life than patients without anemia.

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**MÁRTA NOVÁK (2005)**

## **Sleep disorders and quality of life**

*Supervisor: Dr. Mária Kopp*

Sleep medicine is a rapidly evolving field both in the area of basic research as well as in clinical research and patient care. More and more epidemiological studies confirm the high prevalence of sleep complaints in the general population, and their prevalence is even higher in patients suffering from chronic somatic or mental disorders. The goal of my work was to assess the prevalence of sleep complaints in a representative sample of the Hungarian general population as well as to analyze the potential consequences of sleep problems both on an individual and as societal level, namely, to assess the effects of sleep disorders on health-related quality of life and also their socio-economic consequences. As for this later we have found that as an indirect cost of insomnia, healthcare utilization is increased amongst those with insomnia compared to non-insomniacs. In another study, I carried out

polysomnographic assessment of sleep in cancer patients and controls and in my third study I studied sleep disorders and quality of life in patients on chronic dialysis using validated questionnaires. During my research I encountered several - both theoretical and practical - methodological challenges. During my fellowship in Canada I worked at a major university-based sleep clinic, where I have learned the basic methodology of sleep medicine, from the structured psychiatric interview, through the polysomnographic assessment to the clinical management of patients with sleep disorders. All of these areas were new to me. After returning to Budapest I have studied sleep problems in renal patients, as well as learned about theoretical and practical issues of quality of life research. To conduct our studies we had to translate and validate internationally well-known health-related quality of life scales which were not available in Hungarian. Both our study involving chronic renal patients on dialysis as well as our recently completed project involving patients after kidney transplantation showed that sleep disorders have a significant effect on quality of life of renal patients. Since there were no epidemiological data available regarding sleep complaints in the Hungarian population, I was pleased to study this question within the „Hungarostudy 2002” project. Our data confirmed that insomnia is a prevalent complaint in the Hungarian population and we have shown that the predictors of insomnia are similar to those previously described for in other studies. We also showed that insomnia might lead to increased health care costs, since increased utilization of health care services was seen amongst insomniacs. This shows that sleep problems not only have major effect on quality of life of individuals but also have significant socio-economical consequences.

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## MÁRIA RESCH (2004)

### Gynaecological psychosomatics of eating behaviour disorders

Supervisor: Dr. Mária Kopp

The first Hungarian publications on eating disorders date back to the end of the 1970s. In our study, we reviewed the gynaecological complications of psychosomatic eating disorders. Three screenings were carried out with the help of tests, complemented with an interview phase. In Hungary, the author was the first to introduce the BITE test from among the eating disorder questionnaires used (Anorexia Nervosa Inventar zur Selbstbeurteilung, Bulimia Cognitive Distortions Scale, Bulimia Investigation Test Edinburgh, Eating Attitude Test, Eating Disorders Inventory). The following of the findings must be highlighted:

1. At the National Sports Health Institute's Conditioning and Medical Division Ward the clinical and sub clinical forms of bulimia nervosa among the obese persons (n=154) were prevalent in 14% and 26%, respectively. 76% of the women surveyed experienced menstruation disorders. Severe form of depression (4%) measured according to Beck's Depression Index (BDI) could be proved only among women (n=130) (1,2).
2. Among the women turned up with gynaecological problems (n=75) at the Endocrine Polyclinic of the Clinic for Obstetrics and Gynaecology anorexia nervosa and bulimia nervosa were prevalent in 4% and 12%, respectively. Depression according to the BDI scale occurred in 64% of the cases, of which the value corresponding to clinically major depression was 10.7% (3,4,5).
3. In infertile women (n=34) prevalence of bulimia nervosa together with its sub clinical forms amounted to 48%. In the background, the abnormally low luteinizing and folliculus-stimulating as well as the abnormally high andostendione hormone levels deserve attention that correlated with PCOS (6). The abnormal eating habits associated with infertility and the gynaecological complications of eating disorders may play an important role in the birth rate.

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JÁNOS RÉTHELYI (2003)

## Epidemiological and clinical investigations on the association of chronic pain problems and depressive symptomatology

Supervisor: Dr. Mária Kopp

The epidemiology of chronic pain has attracted wide interest in the last decades because of the high prevalence rates and health care costs related to chronic pain. Similarly has abounded research on the epidemiology of depression, which is a frequent co-occurring health problem in states of chronic pain. In a sequential research project the prevalence of chronic pain problems and the prevalence of co-occurring depressive symptomatology were investigated in national representative survey data and clinical populations, as well as the role of sociodemographic background factors. Results have implications for preventive and clinical health services. In the sample of the HUNGAROSTUDY 1995 national survey (n=12,640) we found that the prevalence of chronic pain-associated disability was 32.7%. Overall pain prevalence reported by women is significantly higher than prevalence reported by men (36.8% and 27.7%, respectively), which remains statistically significant in the adjusted results (OR = 0.54; 95% CI = 0.48-0.61). Furthermore the prevalence rates were higher in older age groups, lower educational and occupational groups. The prevalence of moderate or severe depressive symptomatology was 13.4% in the whole sample. If the group reporting pain-associated disability was selected, the proportion with moderate or severe depressive symptoms was 30.2%. The co-prevalence of pain problems and depressive symptomatology was significantly higher in higher age groups, in lower educational groups, in the group of unemployed, and in the group of skilled or unskilled workers. In the representative sample of the Young women's health survey (1998), comprising 3615 female respondents aged 14-24 the overall prevalence of frequent headaches and musculoskeletal pain-problems were 46.3%, and 25.6%, respectively. Co-occurring depressive symptomatology was reported by 11.2% of the group reporting frequent headaches and 10.3% of the group reporting musculoskeletal pain problems. In the group of 91 inpatients treated for depression 43% reported pain problems during the 6 months prior to the treatment. At the time of a follow-up six months after the treatment this rate remained 40%. In the group of 57 patients with musculoskeletal problems in 58% we assessed at least moderate depressive symptoms, in the case of 10% the depressive symptomatology was severe.

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ADRIENNE STAUDER (2003)

## Psychosocial factors in allergic diseases

Supervisor: Dr. Mária Kopp

*Aims:* Epidemiological study of certain environmental, socio-economic and psychological factors related to allergy symptoms. Screening for psychiatric disorders among patients visiting the allergology-pulmonology outpatient clinic. *Methods:* Epidemiological studies based on national representative health surveys Hungarostudy1995 (12524 persons) and "Better Health for Women" (3615 young woman aged 15-25). Included psychodiagnostic questionnaires: Juhász Neurosis Inventory, Beck Depression Inventory, Folkmann-Lazarus Ways of Coping, Disfunctional Attitudes Scale, Cook-Medley Hostility questionnaire. "ALLEPSY" multi-centric self-administered questionnaire survey among outpatients (889 persons) followed by structured psychiatric interview with 60 of the respondents. Psychodiagnostic questionnaires included: Beck Depression, Spielberger Trait Anxiety and Juhász Neurosis Inventory, Marks and Matthews Fear Questionnaire. *Results:* The prevalence of current allergic symptoms was 17% in the whole population. % of young women had medical examination in their life for allergy symptoms. Allergy prevalence is higher in the Central and Southern regions and in larger cities, highest in Budapest. Higher education level of the parents, better socio-economic situation of the family indicated increased risk of allergies. Allergic persons reached higher scores on all psychological tests (neuroticism, vital exhaustion, depression and hostility scores), reported more suicide attempt, emotional coping and from dysfunctional attitudes external control, need for approval and perfectionism. The prevalence of clinically significant anxiety and/or depressive disorders was 19% among patients visiting the allergy clinic, 46% of them has never received any psychopharmacological treatment. Subclinical, or mild symptoms were also present. Risk indicators were female gender; asthma; perennial symptoms; sleep problems; a great number and non-specific allergy triggers, considerable limitation attributed to allergic symptoms, and positive family psychiatric history. Screening validity values of the questionnaires were above 80%, however they were unable to differentiate between the disorders. *Conclusions:* The prevalence of allergic symptoms is very high in young people. Modern life-style and associated environmental factors increase the risk of allergic disorders. Allergic people can be characterized by increased physical and psychological reactivity, psychical problems and inadaptive coping are more frequent among them. The rate of anxiety and/or depressive disorders is high in patients visiting the allergy clinic, however only half of the cases received any treatment. Self-administered questionnaires provide reliable help for the identification of these frequent psychiatric problems.

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IMRE SZEBIK (2005)

## Ethical questions of clinical application of gene transfer techniques

Supervisor: Dr. Mária Kopp

With the advancement of our genetic knowledge it is now possible to conduct clinical trials on Human subjects to alter the genetic substance of different cells and tissues. This paper discusses ethical, legal, psychological, theological and social questions these interventions raise. The first chapter analyses ethical issues related to germ-line genetic interventions and concludes that arguments against such interventions are not exclusive; our present medical-social praxis accepts many elements of such interventions. On the other hand, this does not mean that clinical studies to alter

germ-line cells could or should be commenced at this time. The second chapter analyses a protocol that aims to cure diseases caused by deleterious mitochondrial DNA with gene transfer methods. It concludes that at the present level of our knowledge on the function of mitochondrial DNA we cannot state that mitochondrial DNA has only basic biological role in the life of Human organisms, therefore we do not know to what extent such an intervention alters higher intellectual properties of Human beings. The next chapter analyses some lessons we learned from a gene therapy research failure by emphasizing the importance of ensuring higher level of ethical integrity of clinical research activities and by providing some practical steps. Finally, the thesis discusses some aspects of stem cell research. It raises the question whether researchers have a moral obligation to respect values and norms of people with different cultural or religious background to maintain respect and acceptability of clinical research activities.

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IRENA SZUMSKA (2005)

## Prevalence of eating disorders among young Hungarian women. Psycho-social background characteristics and comorbidity with other mental problems

Supervisor: Dr. Mária Kopp

In the past two to three decades research concerning eating disorders has been receiving escalating attention, and even though we are beginning to understand its nature, many questions are still left unanswered. Comprehensive and representative studies have not been previously conducted in the Central-Eastern European region. Therefore one of the goals of our study was the definition of the prevalence of eating disorders among 15-24 year old women, the age-group most at risk for eating disorders. Our other aim was to analyse the differences regarding psychosocial correlates and comorbidity problems between those with eating disorders and those without. We conducted a representative, questionnaire, cross-sectional survey among 3615 young women. Our results show that the point-prevalence of anorexia nervosa (AN) was 0.03%, of bulimia nervosa (BN) 0.41%, of sub-clinical AN 1.09%, and of sub-clinical BN 1.48%. Women in the eating disorders group differed significantly in many aspects from their peers. Eating disorders were more frequent among students, than non-students, and were more frequent among urban dwellers. Regarding family background factors women with eating disorders differed significantly from the rest of the sample. Women with BN reported parental psychiatric disorders more frequently. Parents with high educational level had a three-fold risk for their daughters to have eating disorders, than parents with low educational level. Of the studied mental health variables, the group with eating disorders was characterised by emotional coping strategies in stressful situations, had higher internal expectations of themselves more frequently, and had high hostility scores. From the co-morbid mental problems depressive symptomatology, suicide attempts, suicidal thoughts, and drug use were in significant association with eating disorders. Physical and sexual abuses in the past year were significantly more frequent in the eating disorder group. Results are discussed in the light of scientific literature and through the perspective of preventive interventions.

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**ERIKA TÓTH (2004)**

## **EEG-effects of gustatory and olfactory stimuli in anorexia nervosa**

*Supervisor: Dr. Péter Molnár*

*Introduction.* The pathomechanism of anorexia nervosa is complex. An important factor in the development of anorexia nervosa may be the pathological perception of gustatory stimuli. *Aims.* The effect of pleasant and unpleasant gustatory and olfactory stimuli, and neutral and provocative visual stimuli were investigated in this study on the electroencephalogram (EEG) to obtain data corresponding to the possibly altered central processing mechanisms of these stimuli. *Methods.* The pleasant and unpleasant gustatory and olfactory stimuli were exposed for two minutes after the control condition when no stimuli were presented. A neutral video film and one concerned with preparation of cake was shown, each for two minutes. Power spectrum analysis was performed on the EEG recorded according to the 10-20 system by Neuroscan. The EEG was recorded for 2 minutes during the smell exposure and visual stimuli, and right after the taste exposure. *Results.* It was found that the applied gustatory and olfactory stimuli changed the spectral characteristics of the EEG in the control and in the AN group as well and mostly in the theta and alpha1 frequency band. In the group of anorexic patients differences were found between the two frequency band: the power of the alpha1 range was lower and the power of the theta range was higher, but these were not dependent on the kind of stimuli applied. In AN patients lower dimensional complexity was observed than that seen in controls, independent of taste conditions. Higher Omega complexity was seen in control subjects in the left side irrespective of taste effects. No such hemispheric difference was observed in AN. *Conclusion.* The deviations from normal control state in AN patients as revealed by linear and nonlinear EEG analysis, may correspond to long lasting effects of brain dysfunction. The lack of a significant Omega complexity change in response to exposure of sweet taste in the left side in AN patients may correspond to a decreased sensitivity to such stimuli in these subjects. Both in the control and in the AN group the odorant and visual stimuli increased the beta1 and beta2 bands, probably corresponding to increased level of vigilance.

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**V. ANNA GYARMATHY (2004)**

## **Substance use, sexual activity and AIDS education among Hungarian secondary school students**

*Supervisor: Dr. László Molnár*

*Background:* Health related risk factors and health behaviors during adolescence are (1) very important predictors of health during adulthood and (2) are linked to morbidity and mortality among youth and young adults. *Objectives:* To examine the prevalence and correlates of legal and illegal substances (LIS: cigarettes, alcohol, and drugs); the prevalence and correlates of consistent condom use among adolescents; and to evaluate a comprehensive AIDS education curriculum in secondary schools in Budapest, Hungary. *Methods:* Between December 1996 and May 1997, 3505 students studying in Hungarian secondary schools filled out voluntary, anonymous, and self-administered questionnaires. Statistical analyses used were  $\div$  2 analyses and stratified multivariate logistic regression models. *Results:* Ever and current smoking were reported by 75.1% and 33.5%; ever and current drinking by 90% and 64.5%; and drug use by 13.3% of respondents. Students who had friends who used LIS were more likely to use LIS, and the presence developmental assets appeared to be protective. Sexual experience ever and in the past five weeks were reported by 38.0% and 20.3% of all stu-

dents. The perception that condoms were difficult to use predicted condom use when comparing non-users with those reporting any use, but did not play a significant role when comparing irregular users with consistent users. The process and outcome evaluation of instructor training and the process evaluation of educational activities for students are described. The outcome evaluation of the educational activities for students showed the importance of teacher effects and the quantity of education. *Conclusions:* Cigarette smoking, alcohol use and drug use are strongly influenced by the social and risk networks of youth. Attitudes about whether or not condoms decrease sexual pleasure may be a key turning point from not using condoms at all to using condoms at least some of the time, and direct and effective communication between teens and their sexual partners is key in negotiations about sex and using condoms. While most AIDS education curricula focus on the content of the education, other aspects – including the characteristics of those educators who appear to be most effective – also need to be considered.

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## 5. PH.D. SCHOOL OF SPORT SCIENCES

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Doctoral courses embrace the whole field of sports science. The basic subdivision that of social and natural sciences the program of which satisfy the requirements of sports sociology, sports physiology and sport policy, on the one hand and on the other, those of sports biology, sports physiology, sports medicine and sports hygiene.

Naturally, the specific topics reflect the orientation of the tutors, respectively the technical facilities of the laboratories. It is the Faculty of Physical Education and Sport Science that manages the doctoral courses, but other institutions, above all the National Institute for Sports Medicine, extend the facilities available in the institutes and chairs, both in regard of educational staff and technical equipment. The other faculties and institutes of the Semmelweis University are considered the basis of further development in this respect.

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### **5/1 PROGRAM**

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## **TRAINING AND ADAPTATION**

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The different stress factors exert considerable impact on normal functions and pathological processes during the whole life span. An adequate intensity of regular physical training positively influences the whole metabolism, thus presumably plays beneficial role in compensating the harmful effects of stress. In our earlier studies we extensively examined the effects of stress and stress hormones on the development of the brain and neuroendocrine system, as well as on adaptive behavior. Recently we aimed at examining metabolic impact of prenatal stress and its role in the development of obesity. Regular training during pregnancy might counteract the negative effects of stress by influencing the development of hypophysis-pituitary-adrenal (HPA) axis.

Regular physical training in early postnatal life also could influence brain development by acting on trophic factor production, such as NGF, BDNF. Training might enhance the resistance against harmful neonatal risk factors, such hypoxia, NMDA and ethanol toxicity. Chronic stress produces disturbances in neuroendocrine regulation and in adaptive behavior. The possible protective effects of dietary factors and physical training are also aimed at examining. Stress has a cardinal role in development and maintenance of drug addiction. The beneficial effects of regular training can be evaluated by behavioral studies (reinforcement, sensitization, anxiety) as well as by biochemical examinations (glucocorticoid hormones and receptors, neuropeptides: CRF, neurotensin). The projects claim several different scientific techniques such as hormonal, immunocytochemical and behavioral examinations. Perinatal age require special new methodology to develop considering surgical, immunocytochemical and behavioral procedures.

*Sub-programs**Supervisors***Effects of the regular training on the development of the heart, and on the prevention and rehabilitation of various diseases in childhood**

Péter APOR

*Topics*

Training effects on the morphological and functional development of the heart

Effects of the various motoric tasks, various sports on the child

The most effective rehabilitative and correction training methods in various diseases and abnormal functions

Habitual physical activity and activity patterns related to metabolic, lipid status, cardio respiratory etc parameters

Role of the individual training programs in modification of risk factors

Contact: E-mail: p.apor.md@freemail.hu

**The impact of physical training and dietary factors on neurobiology of stress response at different ages**

Klára FELSZEGHY

*Topics*

The development of neuroendocrine regulation and the effects of stress hormones on the brain development have been in the focus of our research activity

Applying different stress factors during pregnancy our aim is to extend these examinations over the foetal age. In compensation of harmful effects during pregnancy both regular training and dietary factors can be suitable natural interventions

Polyunsaturated fatty acids (PUFAs) as dietary factors elicit a considerable metabolic impact on prenatal development. The effects of PUFA diet are going to be examined on stress response following different stress procedure at postnatal age

Disturbances in neuroendocrine regulation during ageing result in anatomical brain damage and impairment indifferent brain functions. The potential neuroprotective role of dietary factors and physical training will be also investigated

The chronic stress produces variable behavioral deficits. The supposed neuroprotective influence of regular training on adaptive behavior is aimed at estimating

The development of stress response is planned to examine by assessing hormonal changes as well as determining central peptide secretion (CRF, BDNF)

The neonatal as well as old age require special techniques and methodology that provide possibility to develop new immunochemical and behavioral techniques

The role of physical training in the neurobiology of stress response

Contact: E-mail: kfelsz@helios.hiete.hu

*Sub-programs**Supervisors***Analysis of physiological and sport motion with zebris ultrasound-based motion analyzer**

Rita KISS

*Topics*

Determination of kinematics, kinetic and EMG gait parameters at healthy people

Determination of kinematics, kinetic and EMG gait parameters at professional athletes and comparison with those parameters of hobby-athletes

Effect of different injuries of lower extremities on biomechanical parameters of gait

Monitoring of effectiveness of rehabilitation after injuries and surgery

Complex motion and performance analysis of professional athletes (cyclist, fencers, light-athletes)

Contact: E-mail: kiss@vbt.bme.hu

### **Nutrition and physical activity**

#### *Topics*

The role of physical activity in the prevention of heart- and cardiocirculatory diseases

The significance of body composition in the sports specific effectiveness - the role of nutrition

Connection between nutrition and physical performance capacity of athletes

Examination of socialmetric, somatic and neuropsychological characteristics of boxers

Contact: E-mail: , martos@medisport.hu

Éva MARTOS

### **Somatic development of 7 to 18-year-old school-children**

#### *Topics*

The effects of secular growth trend on physique and body composition

Relationship between biological development and motor performance scores in prepubertal ages

Relationship between biological development and motor performance scores - international comparison

Effects of various economic conditions on kinanthropometric characteristics of Hungarian children and adolescents

Effects of overweight and obesity on somatic and motor development in children

Contact: E-mail: mohacsi@mail.hupe.hu

János MOHACSI

### **The importance of non-invasive cardiovascular examinations in the establishment of the performance ability**

#### *Topics*

Non-invasively investigated cardiovascular athletic characteristics

Performance tests examined by the cardiovascular characteristics

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Gábor PAVLIK

### **The role of the regular physical activity in the prevention of cardiovascular diseases**

#### *aspnumTopics*

The study of the relationship of sporting habits with cardiorespiratory data, lipid metabolism, sympathetic activity, platelet function as indicators of physical fitness in differently aged persons

The influence of the individual training program on cardiovascular risk factors

József PUCSOK

Performance-physiology: the effect of different sport performances on the mechanism of the formation of free-radicals and on the function of free-radical scavenging enzyme system. The study of some metabolic indices following physical exercise and sporting activity

Contact: E-mail: pucsokj@sportkorhaz.hu

### **Different exercise protocols and oxygen uptake kinetics**

Tamás SZABÓ

Relationships between the biological status (maturation status) and the functional characteristics, basic motor performances

The energy costs of the different exercise protocols using different type ergometers

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FERENC IHÁSZ (2003)

## Investigation of health promotion and health education programs in Győr

Supervisor: Dr. János Mohácsi

Body build, body composition, cardiorespiratory endurance, running speed and explosive strength were analysed in normal body composition, overweight and fat 10 to 12-year-old, non athletic boys living in city Győr. Anthropometric procedures (accepted by the international literature) and standardized motor performances were used. As a total of 616 volunteer boys took part in the investigation, beyond the agreement of children the written consents of their parents were also collected. The mean height of non selected (by the relative body fat content), non athletic 10 to 12-year-old samples were slightly taller than those of the subjects investigated in 1989. This difference can be attributed the effects of secular growth trend in part, which continuously affects nowadays in Hungary. The mean body masses as one of the consequences of dominantly sedentary life style were higher than it could be calculated by the taller stature. The high mean relative body fat contents (20-30% of the subjects were fat or obese) and lower than required relative muscle masses (consistently below 40%) are in harmony with greater body mass averages. The physique of the studied boys was picnomorphic (by using the categorisation of Conrad —1963—), their mean somatotype was meso-endomorph. The mean cardiorespiratory endurance assessed by the modified 20-m shuttle run test was statistically the same in all the three age groups, with a general qualification: very moderate. The fat and obese children represent a great ratio of the respective populations, are tall (their stature is nearer to the means of one year older subjects), but their body build is definitely picnomorph (endomorph). As one of the consequences of their high body fat content the means of relative bone and muscle masses were very small. Their motor performance were very moderate in general, neither in running tests, nor in throwing and jumping items could reach the 50% of their non fat and non obese counterparts. As one of the explanations of the taller mean stature in fat and obese boys the advanced biological maturation (according to the respective literature) can be mentioned, however, other biological factors may also have important role in the observed phenomenon. The continuous performance in simple ball games have been decreased the relative body fat content and increased the cardiorespiratory endurance described by the scores in 20-m shuttle run. The valuable changes needed more than 3 months period. Significant correlations were found between the scores in modified 20-m shuttle run test and the telemetrically measured and estimated oxygen consumption in a smaller sample of subjects. For the calculation of more accurate regressions further investigation is needed.

- *Ihász F, Király T (2003) Physical education lessons organized by basis of dominant actional series (games lessons). The Annals of the University of Oradea, the Fac. Physical Education and Sport. Oradea. (In press)*
- *Ihász F, Lee C P (2003) Anthropometric and physiological characteristics of normal, fat and obese children in primary school of Győr. RBAM Vol 19 (In press)*
- *Ihász F, Szakály Zs (2003) A kutatási eredmények szerepe a főiskolai hallgatók egészséges életmódjának tudatos alakításában. In: Katona A. (szerk.): A tanári mesterség gyakorlata. Tanárképzés és tudomány. Nemzeti Tankönyvkiadó – ELTE Tanárképző Főiskolai Kar, Budapest, 117-122.*

**ZSOLT KNOLL (2004)**

## **Biomechanics of the knee in healthy and anterior cruciate ligament injured athletes during gait**

*Supervisor: Dr. Rita M. Kiss*

The primary goal of this dissertation is to compare the gait parameters of hobby and professional athletes. Another aim of it is to determine how selected gait parameters such as kinematics and electromyography may change as a result of anterior cruciate ligament (ACL) deficiency and following ACL reconstruction in hobby athletes. Gait analysis was performed by a ZEBRIS CMS-HS ultrasound-based motion and electromyography analyser using a 19-point biomechanical model. The accuracy and correctness of the procedure was verified by comparing the results on two different treadmills, with measurements conducted by two orthopaedics. The knee is modelled by biomechanical parameters found in literature (step-length and walking-base) as well as by new biomechanical parameters such as modified knee angle, relative ligament-point-movement parameters and the neuromuscular activity of eight muscles detected by surface electrodes. On the basis of the calculated biomechanical parameters (step length, walking base, minimal and maximal knee angle, relative ligament point movement parameters and muscle activity period) of 104 healthy hobby and professional athletes, differences could be determined between the investigated groups – for the first time in Hungary – on the basis of a data bank generated by a great number of wide-ranging measurements. The results obtained from the healthy subjects were compared with those of 55 individuals with ACL damage before reconstruction and those of 33 individuals with ACL deficiency during the rehabilitation following reconstruction. The status changes of the pre-surgery and rehabilitation periods were documented by IKDC 2000 follow-up as well. The data from this study suggest that ACL injuries substantially change the kinematics and the muscle activity of the knee. The data suggest that ACL surgical repair significantly alters lower extremity gait patterns regardless of time since injury and that the re-establishment of pre-injury gait patterns takes at least 8 months to occur after surgery.

- Knoll Zs, Kocsis L, Kiss R.M.: *Gait patterns before and after anterior cruciate ligament reconstruction. Knee Surg Sports Traumatol Arthrosc* 12:7–14.
- Knoll Zs, Kiss R.M., Kocsis L: *Gait adaption in ACL patients before and after anterior cruciate ligament reconstruction surgery. Journal of Electromyography and Kinesiology* 14:287–297.
- Kocsis L, Knoll Zs, Kiss R. M. *Joint kinematics and spatial-temporal parameters of gait measured by an ultrasound – based system. J Biomech (In press).*

**PETRIDIS LEONIDAS (2004)**

## **Training induced cardiac adaptation and field-test result on young men**

*Supervisor: Dr. Gábor Pavlik*

In this present cross-sectional study I examined the echocardiography data of 457 athletes of various sport activities and of 73 non-athletes at different age groups. The study focused on two main aspects of the cardiac adaptation. As concerns the first aspect, I compared the echocardiography data of young and old athletes in order to establish the differences or the similarities that can be noticed in the cardiac adaptation at young age and also at the early stages of the athletic training compared to the adaptation at older age and to the later stages of the athletic training respectively. According to the second aspect I compared the echocardiography results of the water-polo players to the results of the other athletes, and also to the results of the swim-test, which is used for the measurement of the water-polo players' fitness level. The aim of this latter was the establishment of any possible linear relation between the indices of the two tests. Echocardiography measurements were performed with a two dimensionally guided M-mode and Doppler echocardiograph. The measured and calculated

variables were divided into morphological, functional and regulatory variables. The swim-test included the time result of a 30 m maximal intensity swim, the mean time of a 6x30 m interval swim, the number of heart beats during the four min of recovery and the difference between the first and the second half min of recovery. Results showed that at a younger age and in the early stages of the athletic training the signs of the concentric or eccentric type of hypertrophy can be better distinguished in the different athletic groups than at the later stages of the athletic training. Cardiac morphology of the water-polo players showed signs of concentric type hypertrophy accompanied by the training induced bradycardia, mostly noticed with the aerobic sport activities. Compared to the other athletes the high values in several parameters of the water-polo players are mostly an indicator of the high energy demands of the game. Between the swim-test and the echocardiography a strong relation was established. The larger cardiac morphology was associated with better swimming performance. Based on the linear and canonical correlations it seems that among the morphological variables LVWTD and LVMM showed significant correlation with all the components of the swim-test, while the weight of the LVIDD seemed not to be so determinant. Based also on the correlations it seems that both swim tasks use mostly the anaerobic energy pathways, rather than the aerobic ones.

- Petridis L, Kneffel Zs, Kispéter Zs, Horváth P, Sidó Z, Pavlik G (2003) *Echokardiográfiás jelek ifjúsági korú rendszeresen sportoló és nem sportoló fiúknál. Sportorv Szle, 44:153-167.*
- Petridis L, Kneffel Zs, Kispéter Zs, Horváth P, Sidó Z, Pavlik G (2004) *Echocardiographic characteristics in adolescent junior male athletes of different sport events. Acta Physiol Hung 91:99-109.*
- Petridis L, Kubatova J, Petridou K. *A swim- test and echocardiographic results on male junior water-polo players. FU Phys Educat Sport (In press).*

ZOLTÁN SIDÓ (2004)

## Relationships between physical performance and echocardiographic parameters in top athletes and obese persons

Supervisor: Dr. Gábor Pavlik

Physical tolerability, echocardiographic data and their changes were investigated in male obese subjects, top-athletes and leisure-time athletes in the Department of Conditioning and Internal Medicine, National Institute for Sports Medicine.

*The aims of the study were:* 1. To compare echocardiographic data of overweight, obese and obese-hypertensive patients. 2. To compare echocardiographic data of obese subjects, top athletes and leisure time athletes. 3. To examine the relationship between physical performance and echocardiographic parameters in top athletes and obese persons. 4. To investigate the long-term effects of sustained weight loss in obese patients by echocardiographic and exercise-ECG. *Conclusions and practical utilisations.* 1. In severely obese persons, a considerable pathological eccentric left ventricular hypertrophy and a relatively early diastolic dysfunction can be observed. Depending on the severity and duration of its existence, left ventricular systolic function will be deteriorated as well. 2. Association of mild hypertension to obesity brings about further changes in dimensions and functions of the heart. 3. In obese persons sustained weight loss causes a marked decrease of the left ventricular hypertrophy, a significant improvement of the systolic and diastolic left ventricular function and an increased physical performance. 4. Investigation of the diastolic function helps to separate physiologic left ventricular hypertrophy of the athletes' heart from the pathologic forms hypertrophy. 5. Regular physical activity can preserve, moreover improve systolic and diastolic function of the heart, therefore it is recommended in every age. 6. Cardiologic control of overweight and obese persons is necessary to detect the obesity induced early unfavourable alterations.

- Sidó Z, Jákó P, Pavlik G (2003) *Cardiac hypertrophy and diastolic function in well trained and in obese men. Int J Obesity 27:1347-1352.*
- Sidó Z (2003) *Az életmód változtatás szerepe a szív-érrendszeri betegségek kezelésében és megelőzésében. Sportorvosi Szemle 44:69-78.*
- Sidó Zoltán (2003) *Korunk epidémiája az elhízás. Lege Art Med 13:541-547.*

CSABA SÓS (2004)

## Effect of dietary supplement on swimmers and water-polo players power

Supervisor: Dr. József Pucsock

The aim of the study was to analyze the effect of a complex dietary supplement called Green (GDS). The questions were: What kind of anthropometrical averages characterized the examined athletes? Are there any changes in the swimmers' and water-polo players' performance after using GDS for 10 days? Does GDS have an effect on proteinuria; Does GDS have an effect on aerobic, anaerobic and cardio respiratory characteristics?

The groups compared were composed of 17, 17 year old boy and girl swimmers (n=10) and 16, 75 year old boy water-polo players (n=11) (WPP). Subjects involved in the investigation were qualified age-group athletes of the FTC. We collected data four times during the investigation both on the treadmill and in the swimming test. 1. = Characterizing the basic condition; 2.= After 10 days of GDS treatment (1 spoon of powder-15g- and 5 ml liquid per day); 3.= 14 days after the treatment was over; 4.= After 10 days of placebo treatment, on the 14th day from the last data collection.

Anthropometrical characteristics of the athletes did not change during the investigation. We had the same results as other athletes had in a similar age group. The performance did not change in either group either on the treadmill or the swimming test. The level of proteinuria was decreased by the effect of GDS in swimmers, and remained the same in WPP. We proposed that, swimmers' training volume and intensity was higher than optimal. Probably, permeability of the kidneys decreased and/or reabsorption increased after using GDS. In this way, GDS increased the optimal training volume and intensity.

Considering the most of the averages of the cardio respiratory data, there were no changes either in swimmers or WPP during treadmill tests. If there was, it was not the consequence of using GDS. Generally, there were no significant differences between the two groups either. Characteristics of aerobic and anaerobic metabolism either did not change during the investigation or they were not by the effect of GDS in swimmers. The changes in WPP showed reduced aerobic capacity and increased buffers capacity. Differences between the two groups could be explained by the differences between their preparation systems. The same swimming performance was completed with a lower blood lactate concentration and a lower heart rate in both groups. We explained these changes with the increased buffer capacity. The aerobic capacity remained the same in swimmers and decreased in water-polo players during the same time. This can happen when athletes exaggerate anaerobic work and/or over loading endurance sets while reducing the proportion of aerobic work. Dietary supplements have a beneficial effect on improving certain qualities through increasing the training volume and intensity optimum (It was the buffer capacity in our case). The improved quality appears very soon and featuring athletes. Without using GDS it would have happened later or not at all.

- Sós Csaba (2004) *Reduction of plasma lactate and proteinuria by a complex dietary supplement in over-trained athletes (swimmers). Acta Physiologica Hungarica 3-4:211-219.*
- Sós Csaba (2004) *Táplálék-kiegészítő hatása úszók fehérjeürítésére. Sportorv Szle.*
- Sós Csaba (1999) *Az úszók formaidőzítésének alapjai. Magyar Edző 6-8.*

GÉZA ZAKARIÁS (2004)

## Testing fitness of male adults in Győr

Supervisor: Dr. János Mohácsi

The purpose of present research is to describe the physique and body composition of a sample of average number of elements sorted out from male population of Győr city, aged 24-64 years. Our intention is to carry out the validity examination of the UKK walking test serving for the rating of the fitness. Relying upon the data estimated by means of walking test the cardio-respiratoric staying power

of the above mentioned sample will be described. The purpose of this research is also to examine the impact of the mode of living – especially of the physical activity – followed by the persons examined on their body composition and cardio-respiratoric fitness. The research covered 24-64 years old, living at least since 3 years in Győr city, from the point of labour physiology healthy male population (N = 903). To estimate the body composition has been adopted, the method accepted by the special literature and recommended in the „Eurofit” test-battery as proposed by Durnin-Wommersley (1974). To estimate the cardio-respiratoric capacity has been adopted the 2 km walking test, elaborated by UKK-Institute of Finland (Oja, 1991) and validated for 24-64 years old male population of Győr city (Zakariás, 2003). Elements of the physical activity have been determined on the basis of the „Baecke” questionnaire (Baecke, 1982).

It was found: That the level of physical activity – independently of age – is determinative as to the body mass index, the quantity of body fat in relation to total body mass and as to fitness of entire heart-circulation system. The physically more active manner of living has a positive impact on physiological parameters definitely constituting risk factors from the point of view of heart and blood vessel diseases. There was found a significant difference between the parameters of persons actually cultivating sports and of persons not engaged in sports. We assign this phenomenon to the fact, that positive effects of sport activity are temporary from the point of view of examined parameters, that is, only the effective sport activity implies real physiological benefits.

- Zakariás G, Petrekanits M, Laukkanen R (2003) *Validity of a 2 km walk test in predicting the maximal oxygen uptake in moderately active Hungarian men. Eur J Sport Sci 3:1-8.*
- Zakariás G, Petrekanits M, Laukkanen R (2003) *Validity of UWT in predicting of VO2max at Hungarian overweight male population. Studia Universitatis Babes-Bolyai Educatio Artis Gymnasticae 1:51-59.*
- Zakariás Géza (2003) *A telemetrikus pulzuskontroll jelentősége az optimális edzéssterhelés kialakításában. Magyar Edző 3:34-37.*

## 5/2 PROGRAM

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## PHYSICAL TRAINING, REGULATION AND METABOLIC TRANSPORT

### Coordinator

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### Sub-programs

**Investigation of biomechanical characteristics of training adaptation what are influencing the competition performance and appearing in movement realization**

#### Topics

Overuse sports injuries, knee injuries, muscle injuries

Biomechanics and histologic evaluation of Press-fit graft fixation technique for anterior cruciate ligament reconstruction

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**Latest studies in the field of muscle and joint biomechanics using Kintrex 1000 isokinetic device evaluation of these results in sports rehabilitation**

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**Hormonal and metabolic aspects of adaptation to physical training**

### Supervisors

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*Sub-programs**Supervisors**Topics*

Stress hormone responses to habitual physical exercise

Age and event dependent studies in ACTH, cortisol, STH and prolactin metabolism

Relationships between physical exercise and testosterone metabolism

Changes in carbohydrate and fat metabolism in the course of physical training in the respective events of sports

The effects of fluid and food intake during exercise on physical work capacity and metabolic balance

The microsomal and lysosomal enzyme systems of the liver

Possibility of the practical application of research results to the fields of sports activity, such as competitive and elite sports, school and leisure time sports, and to physical rehabilitation

Contact: E-mail: frenkl@mail.hupe.hu

**Mathematical modeling and computer simulation of neural control of limb movements**

József LACZKÓ

*Topics*

Natural solutions of the inverse kinematic problem: joint synergies and coordinated actions of muscles

Control of limb movements: modeling limb movements as a function of motoneuron activities and biomechanical muscle properties

Contact: E-mail: laczkoj@mfa.kfki.hu

**Somatic development of 7 to 18-year-old school-children**

János MÉSZÁROS

*Topics*

The effects of secular growth trend on physique and body composition

Relationship between biological development and motor performance scores in prepubertal ages

Relationship between biological development and motor performance scores - international comparison

pararsid11558459 Effects of various economic conditions on kinanthropometric characteristics of Hungarian children and adolescents

Effects of overweight and obesity on somatic and motor development in children

**Effects of exercise and diet on normal and pathological brain aging**

Róbert FRENKL

*Topics*

Neuroendocrinology of brain aging: effects of glucocorticoids and estrogens

Effects of exercise and diet on neurodegeneration: microanatomy of neuronal and axonal degeneration

n-3 and n-6 polyunsaturated fatty acids and brain aging

Neurological movement disorders and therapeutic movement training

Experimental models of „dementia" and their application in studying the cellular mechanism of movement therapy

Vascular dementia: prevention by exercise

Contact: E-mail: frenkl@mail.hupe.hu

**Exercise and glucose-lipid metabolism: movement therapy in diabetes and obesity**

Csaba NYAKAS

*Topics*

*Sub-programs**Supervisors*

Developmental aspects of metabolic syndrome: role of polyunsaturated fatty acids and exercise. Insulin resistance: its molecular pathobiochemistry in the muscle and other tissues.

Neuroendocrinology of diabetes: hypothalamic and neuronal regulation of hormones related to carbohydrate and lipid metabolisms

Assessment of physiological and pathological regulations after carbohydrate and lipid loading

Clinical practice of movement therapy in diabetes 1 and 2 Clinical practice of movement therapy in obesity

Contact: E-mail: nyakas@ogyik.hu

**Exercise-induced adaptation to oxidative stress and aging**

Zsolt RADÁK

During this program we deal with the source and characteristics of free radical species. The mechanisms and the consequences of oxidative damage and repair (lipid, protein and DNA) are deeply discussed. The effects of regular exercise and aging on the oxidative damage and repair on the nervous and muscle system are in the focus of the program.

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**In vivo mechanical behavior of the musculo-skeletal system and mechanical, hormonal and genetic respond to strenuous exercises**

József TIHANYI

*Topics*

Specific tension of the skeletal muscle in vivo under different muscle contractions

Alteration of mechanical characteristics of the patellar tendons induced by stretch

Genetic basis of sarcomerogenesis due to frequent and strenuous muscle stretch

Effect of short and long term stretch-shortening type training on neural and tissue adaptation

Effect of vibration on bone mineral density in different ages

Neural regulation and control of movement velocity

Contact: E-mail: tihanyi@mail.hupe.hu

**The effect of lower education in the somatic development**

Márta WILHELM

*Topics*

Somatic measurement of preschool, kindergarten and children in the lower grades of primary school (3-10 years). Using validated tests of motor skills for this age group, and also many studies in the Hungarian population. Scanning international data, and analyze the differences, and their reasons also

Creating new motor tests and validating them for the age group of 3-8 years. Analyzing motivation, the reasons for the lack of motivation in the early years of development

Creating a new database for the 5/1, 5/2, and 5/3 programs. Following the development (in its complexity) of children from 3 to 18 years of age

Analyzing different curricula, with special attention to physical education. Comparing the effects of these curricula in different school systems with motor development and performance in the Hungarian and European population

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József Tihanyi
János Mészáros
Róbert Frenkl

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Péter Hidas	pt
Mónika Horváth	na
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Miklós Zsidegh	na

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Carmelo Bosco
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Róbert Frenkl
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Róbert Frenkl
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Anikó Barabás
János Mészáros
János Mészáros
János Mészáros

a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

BEN-ZION ADAR (2005)

## Risk factors of prolonged sitting and lack of physical activity in relate to postural deformities, muscles tension and backache among israeli children. A clinical cross sectional research

*Supervisor: Dr. Róbert Frenkl*

Modern society is regarded as belonging to a 'sitting culture' with continuous reduction in physical activity. Already with a child's entry into primary school he spends hours sitting on a chair, and after school hours he continues with similar pas-sive activities mostly watching television, playing video games or sitting at a computer, etc. The continuous sitting culture and lack of physical activity seems to effect the postural development and the muscular-skeletal of the back and the lower limbs. *Objectives:* To examine the effect of the prolonged sitting parameters (duration, type) and physical activity parameters, and their influence on the tightness of the hamstrings and iliopsoas muscles, the range of pelvic rotation, the spinal curvatures, postural de-formities and prevalence of complaining back pain. *Methods and Measures:* 303 pupils (154 boys and 149 girls) were randomly selected from four primary schools in three different Israeli cities. All the research population was divided into three age groups according to age and sex. Straight leg Raising (SLR), Pelvic Rotation (PR), Hip Extension (HE), Lumbar Lordosis (LL), Thoracic Kyphosis (TK) angles and postural deformities were measured, and past complains of Back pain, 'Physical activity parameters' and 'Sitting parameters' were recorded. *Results:* A significant negative correlation was found between the SLR, and LL angle ( $r = -0.14$ ,  $P = 0.01$ ). A greater range of motion of both SLR and PR was found in those in the "Good category" posture, "Poor" and "Fair" posture score showed higher levels of hamstring tension. 136 children (45.4%) reported back pain. Among the girls, significant differences were found in hamstring tension, both in SLR and PR, between the groups with and without back pain ( $p < 0.05$ ). The mean lumbar curve angle was greater in the symptomatic group ( $F = 5.152$ ,  $p = 0.02$ ). The average sitting was 3 hours/day. The commonest leisure activity was watching television. Television watching of 15.4 hrs/week in boys was double that of using a computer (7.3 hrs/week). Among the girls watching television occupied 13.7 hrs/week and using a computer only 5.6 hrs/week. There was a reduction in the range of all motion parameters with increase in the number sitting hours involved ( $p < 0.05$ ). Significant differences were found in the frequency of use of computers and back pain ( $p = 0.007$ ). Among all the children who complained of back pain 73.9% defined them-selves as computer users. 98% of computer users reported 'Sitting type1 (Sitting with hips and knees at 90 degrees or less). Sitting with legs extended forward (Type 2) showed the highest incidence of back pain in relation to all sitting postures among TV watchers ( $p = 0.06$ ). 37% (112 of the whole population) were in the non-active group. The percentage of children involved in physical activity levels increases with age. There was a tendency towards better ranges of motion, both with regard to SLR and PR, in those more involved in physical activity ( $P = 0.06$ ). 55% of the "non-active" group were in the "Poor" postural category. The "very-active" group showed the low-est level of postural deformity ( $r = -0.156$ ,  $p = 0.06$ ). Dorsal kyphosis was greater in the "non-active" group and there was tendency towards increased kyphosis with decreased physical activity ( $F = 2.694$ ,  $p = 0.006$ ). *Conclusions:* Prolonged sitting and reduced activity are noted to involve a greater in-cidence of postural deformities and a higher frequency of complaining back pain, espe-cially in young children and increased muscle tension. The most harmful sitting posture was found to be while sitting at a computer. This position seems to generate the great-est amount of pressure on the lumbar intervertebral discs. The sitting position most commonly noted when watching television was with both the back and legs extended and this found to be the highest incidence of backache among the sitting types of watching television. *Recommendations:* Great importance must be attached to encouraging physical acti-vity and sport exercises as an inseparable part of our cultural life. This is a relatively inexpensive method of improving the overall health of the population, both physically and mentally, especially if it is commenced at a young age. This situation, which is con-sidered to be the most dangerous, chronic epidemic of the modern era, will, probably, become worse in the future because the amount of sitting hours and inactivity will in-crease as modern high technology continues to develop.

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SÁNDOR BÉRES (2005)

## The biomechanics of last strides and take-off of the long jump

Supervisor: Dr. József Tihanyi

*Introduction:* The research introduces the biomechanics of the last three strides and the take-off phase of the running long jump in three studies. In the literature there was not enough attention paid to investigate how the slower approach speed effects the kinetics and kinematics of take-off at shorter run-ups. Therefore, the aim of the study was to analyse the effect of the short run-up on the take-off time characteristics, on the ground reaction forces, on the CG path before the jump, and on the distance of the jump. Although, there are several studies on female long jumping little attention has been paid on the comparison of the jumping technique of male and female jumpers. According to those studies significant differences were found between the horizontal take-off velocity, between the proportion of horizontal and vertical velocities, between angle of projections and among other parameters. The main reasons of the differences between the sexes were the differences in take-off velocities, and that the take-off CG heights of women was lower than those of men. We have not found a study that presents the technique of the two genders at similar resultant velocity. Beside the physical and technical preparation, it is essential to strengthen the mental qualities of the athletes. The bases of the psychic preparation of the competitor are to know his/her own abilities and to know the expectable performance realistically. It is not favourable for the performance if the competitor undervalues or overestimates his/her abilities. Because of these factors it is necessary to find a method to determine the expectable result with an acceptable tolerance from the data measured in the course of preparation. It is easy to get the velocity results of the short run-ups applied in technical training with the widely used photocells. The personal prediction could help the work of long jump trainers and athletes. *Hypothesis.* Research of long jumps from different run-up distances. We hypothesized that applying 6, 8, 10 and 12 strides approach runs the run-up speed will gradually increase and as a consequence the CG velocity parameters measured at the moment of the take-off will change. Presumably the proportion of the horizontal and vertical take-off velocity components will also change, which will result in the changing of the angle of projection. We hypothesized that the vertical velocity component will change less than the horizontal velocity component, and as a consequence the angle of projection will also decrease. As the horizontal velocity increases gradually with the increasing number of strides, it is probable that the biomechanical parameters of the last three strides will be different too. We suppose that the extent of lowering the CG is proportional to the extent of run-up speed increase. Based on literature data it can be stated that the result of long jump is influenced first of all by the final run-up speed. It seems logical to suppose that at run-up distances with various numbers of strides also the final run-up speed will be the main determinant of the distance jumped, even though the biomechanical parameters are different. It is the more so, as the path, time and velocity characteristics of the CG during take-off are influenced by the final run-up speed. Biomechanical comparison of female and male long jump techniques at similar run up speeds. Theoretically, it can be assumed that men whose height is considerably greater than those of women have higher CG at the take-off and so may have higher angle of projection that may result in longer jump. However, we do not suppose that there may be significant differences in the preparation of the jump. Prediction of jumping distance with full-length run-up. We suppose that the increase in velocity with run-ups with increasing number of strides can be described with a non-linear equation, as the number of strides cannot be increased to the infinity and the increase of speed is also limited considering the run-up distance. If there is significant relationship between the number of strides in run-up and the final run-up speed and between the final speed and the jumping distance and those data can

be fitted to some kind of function, then the length of the jump with full-length run-up can be predicted. If the data can be fitted to a polynomial equation, the optimum number of run-up strides can also be determined. *Methods, Subjects.* Eight international and national calibre male long jumpers volunteered as subjects for the short run-up and the prediction of jumping distance study. Their age, body weight and body height were 22.1  $\pm$  2.4 years, 75.2  $\pm$  2.2 kg and 188.0  $\pm$  4.2 cm, respectively. The biomechanical parameters of the best jumps of the finalists of the women's long jump at the European Youth Athletic Championship in Nyíregyháza, 1995 and the 12-stride run-up jumps of the six best jumpers from the eight subjects in the short run-up study were compared.

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**MARCO CARDINALE (2003)**

## The effects of vibration on human performance and hormonal profile

*Supervisor: Dr. Carmelo Bosco*

This thesis examined the effects of vibration on human performance and hormonal profile. Following an extensive review of the scientific literature, it was determined that vibration affects neuromuscular behaviour and produce specific adaptive responses both with acute and chronic exposure. There was lack of information referred to the effects of vibration exercise. Further, the effectiveness of vibration as an exercise intervention was not clearly identified. The experiments were conducted to investigate the effects of various vibration exercise protocols on neuromuscular performance and hormonal profile. The first experiment was aimed to analyse the effects of a chronic exposure to vibration exercise for 10 days on vertical jumping ability of physically active subjects. Average jumping height during 5s continuous jumping was shown to be significantly improved by 11.9% in the experimental group. The height of rise of centre of gravity and average power of the best jump recorded during 5s continuous jumping was also shown to improve. No changes were observed in counter movement jump. It was suggested that the adaptive response to vibration exercise was connected to neural factors since no increase in muscle size could be detected in less than 2 weeks of exercise. Also, vibrations were shown to affect stiffness modulation suggesting strong influence of vibration exposure on the Ia loop.

The second experiment was aimed to investigate the acute effects of vibration exercise on the force/velocity relationship of lower limbs. Five minutes exposure to vibration exercise in static position was shown to shift the force/velocity and the power/velocity curve to the right in professional volleyball players. It was suggested that the improvements in F/V and P/V relationship were due to neural factors connected to force generating capacity in human skeletal muscle. Neural adaptations have been quoted to be the main adaptive response leading to an increased force-generating capacity without a concomitant increase in cross-sectional area (i.e. Sale, 1988). Due to the short application of vibration treatment (five minutes) and the early gains observed, the neuromuscular aspects were underlined as predominant in determining the observed response.

The third experiment tested the efficacy of vibration treatment on the upper limbs of well-trained international level boxers. It was found that five minutes vibrations with a protocol similar to the one used in experiment two were capable of enhancing arm flexors mechanical power by 13 %. EMG analyses showed a reduction in EMGrms activity following vibration exercise concomitant to an increase in average power. This finding suggested an increased neuromuscular efficiency following vibration exposure. EMGrms measured during vibration exposure was found to reach levels higher than 200% of the EMGrms measured in normal conditions, supporting the occurrence of the tonic vi-

bration reflex with vibration exposure. The results supported the idea that acute adaptive responses to vibration exposure were connected to neural factors. In particular, the reduced EMG and the parallel increase in mechanical power suggested an increased efficiency of the?? and ? loop leading to an improved joint stiffness modulation.

The fourth experiment was conducted in order to verify acute hormonal responses to vibration exposure in well-trained subjects. For this aim, a total of 7 minutes vibrations were administered through a vibrating plate to well-trained handball players. Vertical jumping ability was shown to decrease together with serum testosterone and serum cortisol concentrations. The results suggested that 7 minutes vibration represented a stressful treatment protocol leading to an impaired neuromuscular performance. The parallel decrease in Testosterone and cortisol levels also showed an impaired activity of pituitary-adrenocortical and pituitary-testicular axes with a 7 minute protocol. The fifth experiment was conducted to analyse the effectiveness of a vibration exercise protocol different from the one used in experiment four on hormonal profile and vertical jumping ability. For this scope, a total of 10 minutes vibration treatment were administered divided in two sets of five sub-sets lasting one minute each, with 6 minutes rest in between sets. Testosterone levels were shown to improve by 7% following the vibration treatment. Growth hormone levels increased by 460% and cortisol levels decreased by 32%. A parallel enhancement of vertical jump was observed (+ 4%), together with an increased mechanical power of lower limbs during leg press exercise (+7%) and a reduced EMG activity of leg extensors muscles (-10%). The results suggested that the physiological responses to vibration exercise can vary depending on the protocol utilised. Moreover, considering the observed hormonal responses and the adaptations in neuromuscular performance, vibration exercise can represent an effective exercise intervention for increasing force-generating capacity and affecting hormonal profile even in well-trained populations. The final experiment compared the effects of fatiguing exercise protocols with and without superimposed vibrations. From this study it was concluded that dynamic muscle activation with superimposed vibration produced an average power 8% higher than in normal conditions. EMG activity recorded during arm flexion with superimposed vibration was shown to be 14% higher than during the same task performed without superimposed vibrations. Peripheral factors were mainly involved in determining fatigue during the superimposed vibration task as shown by the negative relationship found between power and LA ( $r = -.83$ ;  $p < 0.05$ ). This phenomenon suggested that during superimposed vibrations larger motor units were most probably recruited leading to an increased power performance and to lactate accumulation as compared to the normal condition.

**TAMÁS HALASI (2004)**

## **Biomechanical aspects for treatment of upper ankle instability**

*Supervisor: Dr. Anikó Barabás*

My study was the first in Hungary to compare results of three basically different kinds of surgical techniques of ankle ligament reconstruction. Tenodesis, anatomical reconstruction and periosteal flap plasty were never before compared to each other in one of any of the previously published foreign papers. Based on the results of a complex follow-up anatomical reconstruction was selected as the most suitable method for the surgical treatment of athletes. I proved, that with equal mechanical effects anatomical reconstructions provide better functional and subjective results than the other two techniques. I could also prove, that anatomical reconstruction is the method for ankle stabilization, which enables the highest rate of returning to sport at preinjury level. Internationally I was the first to examine the effects of surgical intervention on the proprioceptive function of the ankle. Furthermore, I was the first to prove the beneficial effects of anatomical reconstruction (Karlsson procedure) on joint position sense. The normalization of impaired pre-injury joint position sense of the operated patients provided evidence on the role of proprioceptive deficit in the development of functional instability. The proprioceptive investigation led to another practically important conclusion concerning the surgical method-of-choice, namely that restoring the tension of the anterolateral structures are vital regarding the success of surgery. Thus, performing this as a single or complemen-

tary method is beneficial for broad use. An internationally new and void-filling ankle-specific activity score was developed. I used the new score in the practice on patients operated for chronic anterolateral ankle instability and proved its reliability and higher validity and sensitivity compared to the Tegner score. Although the system was tested on patients treated surgically for ankle instability, it can be used to evaluate other ankle problems or treatment modalities, too. As an important complement of complex clinical evaluation, the new score will enable a more precise comparison of different authors' results on an international level. As first author domestically and internationally, I proved the efficacy of proprioceptive training on joint position sense of the ankle. With evidences on the improvement of joint position sense, I provided further support to the theory of parallel working and co-existing proprioceptive receptor systems. I gained useful information for the practice by determining the effective period of the training at six weeks. I proved the importance of the broad use of proprioceptive training in the prevention, conservative treatment and rehabilitation of injuries or operations.

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**PÉTER HIDAS (2005)**

## **Incorporation and fixation strength of the press-fit femoral fixation after ACL reconstruction; A biomechanical and histological study in porcine models**

*Supervisor: Dr. Róbert Frenkl*

Novel rehabilitation protocols after ACL reconstruction suggest early mobilization and loading as soon as possible. So as to realize these scopes the graft and its fixation has to be strong enough right after surgery. In this study the biomechanical and histological properties of the femoral press-fit fixation were examined. In an excessive study, also in domestic and international respect, the biomechanics and tissue incorporation were examined in a porcine model. The biomechanical and histological examinations proving graft-incorporation on cadavers and animal models known in the international literature were synthesized in accordance with the aims of this work. It was proved that the tensile strength and stiffness of the press-fit fixation did not differ significantly from those of the nowadays widely accepted and used interference screw fixation. The results of the biomechanical experiments were confirmed by the histological examination of the bone block incorporation in the early postoperative period in the animal model. In a short-term follow-up study it was proved that the press-fit fixation did not differ from the interference screw fixation either in the clinical results. On the basis of this knowledge it became possible to safely apply the accelerated and effective rehabilitation protocol also in the case of the advantageous press-fit fixation. This way the rehabilitation after ACL reconstruction and requirements of rapid return to sport or work can be realized in accordance with modern, state of the art guidelines. All of these experiments served the aim of widespreading, acceptance and safe usage of the advantageous and economically friendly femoral press-fit fixation in Hungary and abroad.

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MÓNIKA HORVÁTH (2005)

## Biomechanical characteristics of gait and postural stability of patients with hemiparesis

Supervisor: Dr. József Tihanyi

The study investigated the stroke induced impaired motor function, the hemiparesis and hemiplegia and the related functional disorders. Walking is often the prime target of rehabilitation because of its importance to functional independence and a key ingredient in functional competency. The degeneration of the balance control can be caused by several functional disorders, such as the mechanical disorders (decreased muscle strength, reduced range of joint movements), muscle-nerves components (decreased or increased muscle tone), disorders in motor control and sensor coordination (dysfunctions in the visual, the sensorimotor, and the vestibular system). The aim of this study was to further investigate the deficiencies of hemiparetic gait pattern and more closely examine and quantify the asymmetry, which occur in hemiparetic gait. Our aim was to define the main kinematic, kinetic, electromyographic characteristics of hemiplegic gait and the balance disorders. 1. It was proved that the instrumented gait and balance assessment methods detected the functional state with higher sensitivity and with more accuracy than the global motor function assessment scales. 2. We experienced that the asymmetry of the hemiparetic gait appeared in the temporal-distance parameters. The stance phase of both the affected and unaffected sides is longer in duration and occupies a greater proportion of the full gait cycle in subjects with stroke than in the able-bodied during walking. The stance phase is both longer and occupies a greater proportion of the gait cycle on the unaffected side than on the affected side. Greater proportion of the gait cycle is spent in double support than that of able-bodied walking at a given speeds. The stride length and cadence of the hemiplegics were lower than values for healthy subjects. 3. We experienced that if we artificially increase the speed of the gait by the means of a velocity adjustable treadmill, the change in the gait velocity concomitantly changed the other gait parameters like stride length, cadence, stride duration and double support duration. It was experienced, that the increase of the velocity of the hemiparetic patients caused decrease of the stance phase and the double support. Although the cadence increased by the increased velocity, the step length did not show significant increase. It was proved, if we "force" the hemiparetic subjects to higher velocity by means of the treadmill, the asymmetry of their gait pattern gradually smoothed to the pattern of the healthy subjects. 4. We can declare that the disorders of gait as a result of congenital or acquired childhood hemiplegia and the hemiplegia acquired at adult age are significantly different. The results of the present study indicate that patients with chronic hemiplegia (being hemiparetic from early childhood and more than ten years) learned an individual gait pattern adapting themselves to specific circumstances. Therefore is very difficult to recruit them into homogeneous groups. It is proved, in a good agreement of the previous studies that joint movement coordination strategy on paretic side differs from the normal gait. The disturbed joint movement in the paretic limb modifies the joint movement pattern and the force development on the ground on the non paretic side. 5. The paretic patients displayed unequal limb loading during standing and significantly greater body sway in eyes closed than in eyes open condition. It was proved, that that crossed stand means higher instability for hemiparetic subjects especially when the affected limb is positioned ahead. 6. Characterising the postural stability by head movement we found similar result as in COP movement test. There was a significant relationship found between the area of head excursion and the area of centre of pressure in both left and right sided paretic groups during both eyes open or closed conditions. In the left sided paretic group the length of the COP excursion showed a significant correlation with the area of head movement. 7. We declare that in case of the hemiparetic people the side of the lesion and the side dominance determine commonly the amount rotation and the direction of the lateral deviation. 8. We found significant differences between the left and right sided paretic groups considering the total length of body sway during eyes open condition. The right hemisphere lesion resulted in greater body sway and larger excursion of COP than left hemisphere lesion. They also showed shorter step length, longer double support and higher cadence during gait.

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**HELGA OGOVOSZKY (2005)**

## Overtraining and oxidative stress

*Supervisor: Dr. Zsolt Radák*

Overtraining is per definition a condition wherein an athlete is training excessively yet performance deteriorates. This is a process included the whole organism that can be associated with vegetative processes, neuroendocrine, somatic and psychological functions. Exercise increases the formation of free radical species enhanced the oxidative stress. It was hypothesised that during overtraining there is a marked increase in the generation of free radical species, due to physical activity and inflammation. The aim of present study was to measure the effect of moderate, hard and strenuous exercise on certain brain functions, antioxidant enzyme activities of liver and muscle, and stress markers of lipids (muscle, brain) and proteins (liver, brain). Rats were divided (Wistar, aged 5 months) into four groups and exposed to swimming: control, constant duration (1h/day, 5/wk, for 8 wk), abruptly increased duration (1h/day, 5/wk, for 6 wk, then 3.5h/day, 7/wk for 2 wk), and continuously increased duration (same as trained group, but the duration increased by 30 min in each wk). In the last week open-field test was used to measure emotional stress. Memory was assessed by the passive avoidance test and the acrobatic-locomotor (balance) ability was evaluated by the rotarod test. We used body weight (measured each week), ACTH and corticosterone levels as a marker of overtraining. Eight week of swimming resulted significant increased emotional and behavioral stress. The size of thymus decreased and the adrenal increased with increased duration of exercise. The changes in body weight, ACTH and corticosterone levels of each group demonstrated an increased stress condition. The changes in antioxidant enzyme activities and levels of RCD and TBARS proved the positive effects of moderate training, but did not support our hypothesis of overtraining. We did not found any relation between the activity of antioxidant enzymes and the rate of oxidative stress markers of lipids and proteins. Our hypothesis that overtraining causes massive oxidative stress is not supported. Further investigations are necessary to study the possible relationships between trainingload and antioxidant enzymes, and oxidative repair systems.

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**ATTILA PAVLIK (2005)**

## Femoral press-fit fixation for anterior cruciate ligament reconstruction

*Supervisor: Dr. Róbert Frenkl*

The rupture of the anterior cruciate ligament is a serious knee injury, which frequently refers to young patients capable of working. The surgical reconstruction has rapidly developed for the last decades due to the developments in the technical possibilities and the thousands of scientific studies on this topic. Because of the improving results more and more ACL reconstructions have been per-

formed all over the world, however, there are a lot of debates about how to treat ACL injured patients. The main goal of our study is to prove that the femoral press-fit technique is an appropriate method for bone-patellar tendon graft fixation. Based on the biomechanical measurements and the clinical results the most important conclusion of the summary is that the femoral press-fit fixation technique is a proper, alternative method for graft fixation in ACL plasty.

The study entirely fulfilled its purposes, namely produced a biomechanical basis for the femoral press-fit fixation by summarizing the results of cadaver and animal measurements. The good biomechanical properties were supported by the histological issues of our study. The results of our midterm, prospective follow-up study on 285 patients corresponds with the published data of the international literature. After the operation our patients were able to return to sports activity in high rate. With the knowledge of these facts the application of the accelerated rehabilitation protocol - used successfully at the interference fixation technique - became possible also at the press-fit fixation group.

Many disadvantages with the use of metal or absorbable material can be avoided with the application of the press-fit fixation technique, while it is not necessary to modify the postoperative rehabilitation protocol. The significant reduction of expenses with the use of this method is also not negligible. Thus, an accelerated rehabilitation of the patients can be performed after ACL reconstruction accordingly the modern professional principles; and there is a good chance to return to work and sport as early as possible according to the demands of present times.

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JÓZSEF PUCSOK (2005)

## The effect of training on steroidhormone profile and metabolic parameters in combat sports

*Supervisor: Dr. Róbert Frenkl*

The purpose of our study was to analyze and compare the effect of acute, all out type physical exercise on the level of serum and urine anabolic-catabolic hormones and the cyclooxygenase system mediators in combat sports. The concentration of anabolic hormones in the serum, such as testosterone, DHEA and androstendione have significantly changed. The concentration of the main catabolic hormone, cortisol increased in judo players, but not in the karate group. The results of our study supports, that elite judoists were in excellent physical condition. The hormonal response due to the catabolic/anabolic effects is balanced. The karate players demonstrated no significant increase in serum cortisol level, the intensity of the treadmill test primarily strengthened the anabolic effects. We experienced no changes in catabolic effects. The changes of serum DHEA concentration is worth to note. We suggest, that the changes in the level of DHEA and the hormonal adaptation are important demonstrators of the improved physical capacity. The change in concentration of adrenal glands originated DHEA is less known, compared to cortisol and testosterone on the effect of exercise. We found significant increase in both groups (judo, karate), which supports that DHEA has an important effect on the adaptation of physical exercise.

The urine steroid profile showed a decrease in the level of inactive metabolites, androsterone and etiocholanolone in elite competitors. Among the analyzed hormones, we measured increase, although not significant, only in the concentration of DHEA and 11-beta-OH androsterone. This increase was negligible, so the level of 11-beta-OH androsterone eventually remained stabile throughout the exercise test. We suggest, that the elevated serumconcentration of DHEA and the metabolism in the liver explains the higher concentration. The changes on the level of cyclooxygenase enzyme mediators are inversely related to exercise intensity. The changes in TXB2 are related to exercise in-

tensity. We found, that physical activity has an effect on the level of PGE2 and TXB2 in the airways. We conclude, that these mediators have an important effect on the adaptation of the airways. The examination of biologically active substance - PGE2 and TXB2 - is a useful indicator of exercise-induced airway adaptation.

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## KATALIN ROSTÁS (2005)

### Investigation of respiratory functions in smoking and non smoking boys

Supervisor: Dr. János Mészáros

The aim of the study was to compare the resting and exercised respiratory functions of 16 to 17-year-old regularly smoking (n=44) and non-smoking boys (n=41). The habitual physical activity was close to the Hungarian average in both groups. The smoking boys can be characterised by 0.5-5.5 year smoking history. The smoke 1 to 40 cigarettes every day. As one of additional control group the peak exercise physiological respiratory characteristics of qualified athletes of the same age were also compared. Physique was described by using standard anthropometric techniques and also their weight-related body fat content was estimated. The resting and post-exercise respiratory parameters were collected by Micro Medical Printer Spirometer. The all-out physical exercise was performed on treadmill. The exercise induced respiratory changes were measured by Jaeger-DATASPIR, and Sensormedics Vmax29 analyser. The very moderate anthropometric differences among the three compared groups, and the slightly higher relative body fat content of the non-athletic subjects cannot be related to the resting, exercised, and post-exercised respiratory functions. No significant differences were found between the means of resting vital capacity, Tiffeneau index, peak expiratory flow, and mean expiratory flow of the regularly smoking and non-smoking subjects. Neither the peak minute ventilation, the respiratory rate, nor the peak exercised tidal volume were different in the smoking and non-smoking groups, however, these functional characteristics of the qualified athletes were remarkably higher. The means of vital capacity, Tiffeneau index, and the mean expiratory flow were also statistically the same measured 5 times during the 3 minutes recovery period. The relative expiratory flow (relative to the respective resting value) did not change during the 3 minutes recovery in the group of smokers, nevertheless it was higher at the beginning of recovery and decreased almost linearly during the observation period in the group of non-smokers. Although statistical differences were found between the respiratory characteristics of smoking and non-smoking groups neither at rest, peak intensity exercise nor during the 3 minutes recovery, nevertheless the means of non-smoking subjects were consistently more high (more favourable), or they were closer to the average of respective physiological range. In their opinion consequently the result of statistical analyses are less reliable, and because of the consistent, but small differences the evaluation based on physiological standpoints are the determinative. In conclusion the negative consequences of some year regular but moderate smoking can already be assessed by the differences between the resting respiratory parameters, further, the decreased respiratory functions can also be described by the various recovery patterns, the qualitative and quantitative values of the compared two groups.

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- Rostás K, Mészáros J, Mohácsi J (2000) Pulzusplató és aerob teljesítmény gyermekeknél. *Kalokagathia* 38:51-56.
- Rostás K (2005) Exercised respiratory characteristics in postpubertal boys. *Hung Rev Sports Med* 46:1. 17-26.

**ANDRÁS TÁLLAY (2004)**

## **Evaluating the roles of sporting activity and biomechanical risk factors of the lower extremity in the etiology of the patellofemoral pain syndrome**

*Supervisor: Dr. Anikó Barabás*

Our cross-sectional epidemiological study performed on 586 secondary school students was aimed to explore the prevalence of the patellofemoral pain syndrome (PFPS). In order to improve the primary prevention of PFPS, its major intrinsic and extrinsic risk factors were disclosed. In a pilot study completed on top athletes, the impact of proprioceptive prevention training was analysed. In the course of the epidemiological study completed on 586 students selected randomly – a study without any precedent in international terms and representative from the statistical point of view, – students were surveyed by various anthropometric, knee stability, and leg structure measurements besides taking physical tests and recording patient history. Data were analysed by parametric statistical methods. PFPS prevalence was (N=60) 20.41% for males and (N=61) 20.89% for females, representing 20.65% for the entire age group. Our study confirms the role of overload: significant correlations between PFPS-prevalence and various levels of sports activity were proven by evidence. As regards intrinsic risk factors, evidence could be provided on increased Q angles for males; and higher age, increased varus alignment position, muscle balance disorders, and higher levels of sports activity coupled with larger weight for females. Extrinsic risk factors included hard sports track surface in each case. Furthermore, perhaps the most important perception of the study is that the majority of these risk factors are gender-specific. Besides the treatment of various biomechanical abnormalities, our attempts were made to correct proprioceptive disorders as described in the literature, the intrinsic risk factors explored by us as well, and the lack of flexibility. A stretching proprioceptive prevention training method was introduced for a first-class women's handball team 1.5 years ago. Our initial short-term experiences are highly favourable: the prevention training method proved to be extremely effective in the prevention of LCA injuries, lateral ankle ligament injuries, and PFPS. Based on our survey experience and a review of literature, it is proposed in the case of PFPS that students should not overload their problematic joints and should avoid sitting for long periods of time triggering pain – e.g. with the knee flexed, – walking on stairs, and squatting. In agreement with Thomeé's opinion, we see no reason for totally prohibiting PFPS patients from practising sports. Sports loads on the students with the risk factors may require training modifications. A well-designed training programme lead by a physiotherapist may form an important part of treatment besides modified sports activities.

- Tállay A, Kynsburg Á, Pavlik A, Halasi T, Berkes I *Patellofemoral Pain Syndrome in Adolescents - Evaluation of the biomechanical risk factors of the lower extremity. Flam J Sports Med (In press)*
- Halasi T, Kynsburg Á, Tállay A, Berkes I (2004) *Development of a new activity score for the evaluation of ankle instability. Am J Sports Med 32:899-908.*
- Halasi T, Tállay A, Berkes I (2003) *Percutaneous Achilles tendon repair with and without endoscopic control. Knee Surg Sports Traumatol Arthrosc 11:409-414.*

**ANDRÁS TATÁR (2004)**

## **Body build, body composition and motor performance in 9-14-year-old schoolboys living in various living standards**

*Supervisor: Dr. János Mészáros*

The aim of the study was to compare the morphological characteristics, body composition and physical performances of 9 to 14-year-old boys with different levels of habitual physical activity (non-athletes n=2593– and athletes n=365–) and different socio-economic conditions (as well as Hungarian Euroid n=2958–, Roma n=618–, Hungarians living out of border n=628–). Body build and composition were assessed by anthropometric techniques (metric and plastic indices, somatotype com-

ponents, and relative body fat content) accepted by the international literature, and the physical performance was estimated by the results of 30 m dash, standing long jump, fist-ball throw and 1200m run tests. No significant differences were observed between the body dimensions of non-athletes and athletes, nevertheless the growth type of the athletes was significantly more leptomorphic, their somatotype was more ectomorphic than those of the non-athletes in all the 6 age groups compared. A remarkably lower relative body fat content was characteristic in the groups of athletes. Although the effects of spontaneous selection cannot be excluded in the observed differences, the anthropometric differences between the two samples can be attributed to the markedly higher relative body fat content in the non-athletic samples. The mean motor performances were obviously higher and more homogeneous in the group of athletes, however, the mean scores of the athletic children and adolescents in 2003 have differed moderately from the means of non-athletes investigated in the mid 1970's. The anthropologic variability between the Hungarian Europid and Roma subjects have manifested firstly in smaller body dimensions and significantly more picnomorphic constitution (as one of the inherited characters) of Roma subjects, although the distribution of relative body fat content in the two samples was also different. The ratios of extremely lean or obese individuals were remarkably higher among the Roma boys. The physical performance capacity (estimated by the short burst activities) of Roma subjects differed just moderately from those of Europid boys, and their performance in 1200m run was characteristically lower. The differences have been source from the more hypoactive lifestyle of the Roma boys, and the different levels of motivation may also have importance in this respect. The boys living in Hungary were significantly taller and heavier than the Nagyvárad subjects, but the relative body fat contents were lower in the boys living out of border. The body dimensions and physique characteristics of Nagyvárad children was almost the same to those that characterised the Hungarian boys in 1983. The physical performance differences between the samples were consistent and very high. The scores of Nagyvárad boys were significantly higher, and the individual performances were more homogeneous, in spite that they are all in one curricular PE class per week.

- Tatár A, Zsidegh M, Mészáros Zs, Ihász F, Prókai A, Vajda I, Mészáros J (2003) *Physique body composition and motor performance in Hungarian and Roma boys. Rev Portug Cienc Desp 3:122-124.*
- Mészáros J, Zsidegh M, Ihász F, Mészáros Zs, Tatár A, Prókai A, Vajda I, Mohácsi J (2003) *Physique body fat content and motor performance in twins. Rev Port Cienc Desp 3:124-127.*
- Zsidegh M, Mészáros J, Mohácsi J, Uvacsek M, Tatár A, Mészáros Zs, Prókai A, Vajda I (2003) *Growth type and motor performance in obese children. Rev Port Cienc Desp 3:139-141.*

**MARTINA UVACSEK (2005)**

## **Secular growth changes in body dimensions and the body composition in 10-18-year-old girls**

*Supervisor: Dr. János Mészáros*

The aim of the study was to analyse the effects of secular growth trend on body dimensions and physique characteristics of 10-18-year-old Budapest girls between 1983 and 2003. The somatotype, relative body fat content, and some lifestyle attributes were also estimated in the high-school girls of the second investigation.

Standard anthropometric field procedures were used, accepted by the international literature. A total of 2708 volunteer girls took part in the second investigation. Significant differences were found in the growth trend of body height, and body mass, the girls of the second investigation were generally higher and heavier. The height-related means and standard deviations of body mass, and especially the BMI indicated that close to 30% of the subjects were overweight or obese at the time of second data collection. For the significantly taller stature did not refer significantly wider bone diameters in this comparison.

The body build of the Budapest girls were more leptomorphic and their mean bone-muscle development (described by the plastic index) were consistently lower than those of the girls 20 years ago. As one of the consequences of higher skinfold thicknesses the meso-endomorph mean somatotype was characteristic already at 10 years of age in the second investigation.

The physique and body composition differences can be attributed to the environmental effects especially to the generally characteristic hypoactivity.

The results of questionnaire indicated that the investigated high-school girls were well-informed about healthy lifestyle and diet. The physical activity over the curricular PE lessons was usually popular among them but it did not reach the required weekly frequency and duration. The study time out of school was many times longer than the spent time in front of the television or computer screen. The demand of proper alimentary habit was proved by the correct fruit and vegetable consumption and by the refuse of the variety of snack bars.

- Uvacsek M, Mészáros J, Mohácsi J (2002) *Secular growth trend in Hungarian girls. Stud Kínanthr 3:107-112.*
- Uvacsek M (2003) *A kövérség és az életmód összefüggése. Magy Sporttud Szemle 1:22-25.*
- Uvacsek M, Szmodis M (2003) *A táplálkozás és a kövérség kapcsolata középiskolás leányoknál. Sportorvosi Szemle 44:79-84.*

MIKLÓS ZSIDE GH (2005)

## Anthropometric characteristics and motor performance scores in fat and obese boys. International comparison

Supervisor: Dr. János Mészáros

The increasing ratio of fat and obese individuals became characteristic in the economically developed countries during the past decades. The marked changes can be observed both in growing and adult populations (142). The aim of the study was to compare the human biological characteristics and motor performance scores of fat and obese 10-13-year-old boys, belonging to definitely different anthropological races, living in various geographic regions but very similar socio-economic conditions. Volunteer Hungarian, Cypriot, and Malaysian children (N= 1195) took part in the investigation, and following the suggestions of Lohman (73) they were grouped into two fat categories (F% = 25.00-29.99 fat and F% ? 30.00 obese).

The anthropometric techniques used for the description of physique and body composition are accepted by the international literature. For the estimation of growth type the Conrad (21) method was used. Somatotype components were determined by the suggestions of Carter and Heath (19), and the relative body fat content was assessed by the calliper-metric technique, described by Parízková (104). Physical performance capacity was estimated by the scores of 30 m dash, standing long jump, fist ball throw and 1200 m run. The fat and obese children were significantly taller than their non-fat counter-parts in all three nations and four age groups. Nevertheless their taller stature can be attributed to the more or less advanced biological development, which has ranged within the physiological range. The difference of means in the case of fat boys is not consistent but at least reveals the anthropometrical differences among the different populations. The morphological and constitutional differences can not be proven between the different human-races among obese age groups. The extremely picnomorphic but on height and plastic index based robust constitution is a general characteristic of the Hungarian, Cypriot and Malay measurement. The motor performance of fat and obese sample is very weak. There is a real difference among the identical age and anthropometrically similar origin based classification between the means of fat and obese sample. Among the same categories the performance of the Hungarian sample is the best followed by the Cypriot and by the weakest Malay sample.

Based on the results of the Cluster analysis of fat and obese 10-13 year old boys the national distribution of the cases more and less was different, but the distributional differences were not consistent. The experienced frequency in Cluster 2, that represented the given age groups the best, was smaller than in Cluster 1 and Cluster 3. The multivariate judgment that differs from the original classification allows the creation of a general classificatory and orientational methodology that is missing from known and existing daily practices.

- Zsidegh M, Mészáros J, Mohácsi J, Uvacsek M, Tatár A, Mészáros Zs, Prókai A, Vajda I (2003) *Growth type and motor performance in obese children. Rev Port Cienc Desp 3:139-141.*
- Zsidegh M (2003) *Growth type and motor performance in obese children. In: Bánhidi, M. (Ed.): Apáczai-Napok 2002 Tanulmánykötet. PALATI, Győr 444-450.*
- Tatár A, Zsidegh M, Mészáros Zs, Ihász F, Prókai A, Vajda I, Mészáros J (2003) *Physique, body composition and motor performance in Hungarian and Roma boys. Rev Port Cienc Desp 3:122-124.*

## 5/3. PROGRAM

### SPORT AND SOCIAL SCIENCES

#### *Coordinator*

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#### *Sub-programs*

#### **Sporttourism**

##### *Topics*

Validity study of the scientific measuring methods for sports tourism.  
 Influencing factors of sports infrastructure concerning geological location and research of intervention possibilities.  
 Contact: E-mail: banhidi@atif.hu

#### *Supervisors*

Miklós BÁNHIDI

#### **The role of sport in the socialization process, pedagogical aspects of sport**

##### *Topics*

Characteristics of the physical activity of youth, influence of the living style by the conscious increasing of the physical activity  
 Pedagogical investigation of the effect of the school physical education and sport, some didactical problems and specific methodic of their solutions in the subjects of physical education and in school sports  
 The value proceeds of the sport movement on the totality function of pedagogy: analysis of the effectiveness of sport pedagogy  
 The pedagogical activity of the coach, his didactical and educational praxis in Hungary the turn of the century  
 The characters of the education work of the sport clubs in Hungary in the years of the change of political system  
 The development and preserving of the psychosomatic abilities with the means of the different sports  
 Studying the human behavior in extreme situations  
 Investigation of the student-teacher, respectively athlete-coach relation by pedagogical aspect, interactive actions analysis  
 Comprehensive, theoretic analysis on Sportpedagogy  
 Contact: E-mail: peter.biro@freemail.hu

Edit NAGY BÍRÓ

#### **Examination of pedagogical and didactical situations in the physical education class, sport activities and teacher training institutions**

##### *Topics*

Learner and content centered education-instruction  
 Purposefulness, teaching for understanding, critical thinking, and problem solving skills in education  
 Lifelong learning and sport  
 Health, education, physical education, sport

József BOGNÁR

*Sub-programs**Supervisors*

Pedagogical questions of talent selection, identification, and talent management

Physical education teacher education programs at the local and international level

Teacher's role, career selection and development in our field

Pedagogical research relating the physical education teacher's role.

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**The methods of pedagogical research, didactic, analysis of the activity of the teacher, teacher training**

Iván FALUS

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**Organic background of Learning-, and behavior problems (hyperactivity) - a neuropsychological approach**

Rita FÖLDI FODOR

*Topics*

The aim of the research is the investigation of the background and characteristics of the functional disorders caused by the early injuries of the nervous system. The understanding of the organization of the psychological function systems, and the characteristics of the information processing helped by understanding of the structure of the nervous system, the maturation of the nervous system's processes, and the properties of the brain integration

The development of the regulation of motor activity, the role of stimuli (proprioceptive-, and nociceptive) during motor activity in the process of the maturation reveal the importance of the motor development in the maturation of the nervous system, and forming of the cognitive functions

The differential diagnostic problems in the partial dysfunctions and hyperactivity are studied both from neuropsychological (cognitive neurosciences) perspective, and from the point of view of dynamic theories

Contact: E-mail: foldi.rita@kre.hu

**Sport in modern society**

Gyöngyi SZABÓ  
FÖLDESI

*Topics*

Transformation of sport in post-communist societies

The constitutionalization of sport in Western- and Eastern European sports models (relationship of elite and recreational sports)

Equality of social chances

Social behaviour in sports

Deviant behavior in sports

Opportunities for decreasing racism, xenophobia and prejudices through sports

The social, political and economic significance in sport

The contribution of public, civil and business sectors of sport

Sports as means for publicity

Various models for sports financing sports marketing

Sport marketing

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*Sub-programs**Supervisors***The role of sport in the socialization process, pedagogical aspects of sport**

János GOMBOCZ

*Topics*

Characteristics of the physical activity of youth, influence of the living style by the conscious increasing of the physicals activity

Pedagogical investigation of the effect of the school physical education and sport

The value proceeds of the sport movement on the totality function of pedagogy: analysis of the effectiveness of sport pedagogy

The educational practice of the coaches in Hungary at the turn of the century

The characters of the education work of the sport clubs in Hungary in the years of the change of political system

The development and preserving of the psychosomatic abilities with the means of the different sports

Studying the human behavior in extreme situations

Investigation of the student-teacher, respectively athlete-coach relation by pedagogical aspect

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**Theoretical and methodological fundamentals of physical education curriculum's aims, content and requirements of the course**

Pál HAMAR

The course realizes a scientific program based on curriculum theory - built upon didactical, sport pedagogical and theoretical knowledge of physical education towards PhD graduation - which contains: the public education politics, educational control and school P.E. connection, theoretical and practical questions of general school P.E. and sport science, potential ways of scientific researches of P.E. curriculum from the point of methodology and its perspectives

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**Scientific basics of living standard (recreation)**

László JAKABHÁZY

*Topics*

Training specialists who promote a sport-orientated lifestyle

Analyzing universal culture from recreational point of view

Training of specialists who transfer the values of recreation for all age-groups on a very-high level, based on scientific knowledge and practice

Training of specialists who - in possession of psychomotor abilities and movement culture - can put his/her knowledge into practice, and is able to teach motor activities on high level

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**Application possibilities of new didactics methods in various sports movements' education**

Katalin KERESZTESI

*Topics*

Development and result inspection of new didactics methods

Movement therapy development of students suffering from dyslexic and psychiatric patients

*Sub-programs**Supervisors*

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### **Historical role and social background of the ancient and modern Olympic**

István KERTÉSZ

*Topics*

The role of Olympism in the development of Pan-Hellenic idea in the period of the Greek poleis

The development of the social equal chausses in the ancient Greek democracy and the Olympism

The role of Olympism in the Hellenization of the ancient eastern world

Sport and military training in the ancient Greek and Roman world

Olympians and bourgeois development in Hungary in the 19th century

The connection between the international peace movement and the modern Olympism at the end of the 19th century

The role of Olympism in the coming abreast of the developing world

The communal organizing power of Olympism in the development of Hungarian society in the 20th century

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### **Youth and socialization**

Péter MOLNÁR

*Topics*

International comparative youth research, enhancing the youth's future orientation and socialization

Free time and sport

The notion of modern youth culture and demography

Motor control, motor learning

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### **Relationship between society and local state institutions, task orientations in sport**

Csaba NAGYKÁLDI

*Topics*

Personality characteristics of the athletes, personality development effects of sport and its pedagogical applications. Social psychological characteristics of sport teams, dynamics of the groups and building of the teams

Psychological possibilities of enhancement of sport performances and the effects of psychoregulation. Analyses of the combat and action-efficacy and its pedagogical and psychological regulation

Contact: E-mail: nagykal@mail.hupe.hu

Theory and methodology of training; scientific information on sport training, measurement, and evaluation; improvement and achievement concerning personality and health issues; talent selection, talent issues, and talent management

László NÁDORI

Contextual and organisational modernisations of graduate and post-graduate education of Sport experts in considering European integration and legal harmonization.

András NEMES

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*Sub-programs**Supervisors***Sport event management (conceptual framework of managing sports events)**

Mihály NYERGES

Basic methodological elements of major international sports events, the planning process-conceptual framework-effective management process, the event marketing process-project management techniques-case studies

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**Motor performance diagnostic and methodological basis of it**

Károly OZSVÁTH

Motor performance is fundament of the evaluation. Generally we can state that the main influencing factors are ability of physique (conditional and coordinational), the technique of movement (forms of execution, connections among parts of movement) tactical setting (application) and other factors from surroundings (weather, adverse part, equipment etc.) To find the acceptable diagnostical and prognostical methods it has to make measurable the subject of investigation. The conditions of measurability is concerning to the certain motor quality or parameters of the complex motorium. It has very high importance to look together the measured parameters and to know the feature of the differentiation and groupings. It means the investigation of the structure. At least there has to give the referential values.

The evaluation of test units, formation of norm-system (standards) raises several questions concerning the methods and measurement. Instead of independent evaluation of certain test (items) it has to analyze the test-results all together, this is the "sum score" analysis. The independence of item will loose.

In the specialized literature they call the "dimensionalism" in that relation the composition of test unit as to appear earlier than workout of norms. The dimensionalism is the question of validity. The basis is "what measure" the questions: how, to which relation, how precisely are on the second row. The technical background is the multivariation and multidimensional statistical methods.

Contact: E-mail: ozsvathk@mars.tofk.elte.hu

Movement and performance constancies in result-orientated movements. Researches in theory of (sport) games in the background of movement games. Techniques of observing movements during matches and applying of gained experiences in sport games. Theoretical and methodological problems of increasing human performance in elite sport.

Endre RIGLER

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**The application of the autogenic training in sports and in school**

Kornél SIPOS

*Topics*

Personality examination by self-evaluational questionnaires (anxiety, self-efficacy, coping strategies, etc.)

Public health strategy: a common perspective of Health psychology - Sport psychology (The effects of regular physical activity on physical and psychological health. Sports of handicapped and quality of life)

*Sub-programs**Supervisors*

Dropprevention in school (epidemiological examinations)  
 Cross-cultural comparison of psychometric scales (STAI, STPI-Y, CSAI-2, ACSI-28, LDM, SASC, etc.) equivalence examinations  
 Examination of the affectivity with the erotrop-trophotrop index of the Liischet-test  
 Psychological self-regulation techniques for school children and elite athletes  
 Health psychological examination with complex Cerberus® method  
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**Sport history and sport politics**

Katalin SZIKORA

Interest has increased towards the scientific research of these topics among the students graduating both from the University of Physical Education and Sport Sciences, and from other universities, during the last decade. The theses written within this sphere of interest analyze the economic, socio-historic, political background of history of sport and mainly Olympics, the Olympic idea, the Olympic Games with the method of history and political sciences. Besides investigating the so called performance sports", other domains of body culture, e.g. recreation, leisure sport also form part of these researches  
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**Philosophical problems in physical culture**

Ferenc TAKÁCS

*Topics*

Historical antecedents of sport philosophy  
 Concept of culture and physical culture  
 Sport and its art representation  
 Value preferences in sport education  
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**Role of mentalhygiene in retaining physical/mental health and in the healing of illnesses**

Teodóra TOMCSÁNYI

*Topics*

Role of mentalhygiene in retaining physical/mental health and in the healing of illnesses  
 Mental health and pastoral care  
 Types of relationships and helping professions (equal, ambivalent, and asymmetric relations)  
 Value seeking and retaining, networking building role of communities  
 autoAdult trainings and their quality assurance in mentalhygiene, sport pedagogy and pastoral care  
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**Problems of sport and social integration, relationship between sport and deviant behaviour This issue touches the characteristics of social integrative role of sport with due regard on these disorders.**

István VINGENDER

*Topics*

*Sub-programs**Supervisors*

Functions and disfunctions of the sport as a social integrative institution

Deviancies, maladaptive behavioural forms, and its' disorders in the social milieu of sport

Protective and predictive factors of sport, problems of the sportsmen's social integration, questions of their sociability

Process and stages of deviant carrier in the sportsmen's walk of life

The social context of deviancies in the social millieu of sport

The social representations of deviancies in the social milieu of sport

The social context of deviancies in the social millieu of sport

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Katalin Keresztes
Gyöngyi Szabó Földesi
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Endre Rigler
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Katalin Keresztes
Péter Molnár
László Jakabházy
Pál Hamar
János Gombocz
Katalin Szikora
Kornél Sipos
Mihály Nyerges

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**TIBOR BAKONYI (2004)**

## **Civil dream and political state Contributions to the contemporary Hungarian political history**

*Supervisor: Dr. Gyöngyi Szabó (Földesiné)*

Although there is a lot of truth in the view which has often been mentioned in the field of sport, according to which sport was the losing party of the 1989-1990 political transformation, it is a fact that with the political transition the opportunity for professional political debates has been given. Consequently, the institutions for public debates have been created. No professional analysis has been carried out in the field of Hungarian sport science. So far the inevitability of the resocialization and 'denationalisation' of sport has primarily been described with the help of the related branches of knowledge. As a consequence of all these facts the studies concerning the transition of Hungarian sport have a reason for their existence. This thesis paper deals with the political and the concepts concerning the social status of sport and the rule of state intervention and responsibility. Moreover, it also analyses the paraconcepts and reconstructs the debates. The author concentrates on the great promises of the political transformation which even today often seem illusory and he emphasizes the fact of their transition. The author's main purpose is to analyse the sets of relations between the civil sports organizations and the examination of their main purpose. Basically the thesis paper is based on document analysis. During the work the following types of documents are in the focus of attention: party decisions in connection with sport, directives, submitted laws and bills, the minutes of parliamentary debates about sport and the sport law. Another important method of the research was the depth interview. The author talked to six important sports managers and politicians who had key positions in the particular period.; consequently they played a crucial role in the field of sports policies. The results of the research show that the general opinion was held immediately after the political transformation that the market, the democratization, and the strengthening of the civil organizations created the contents and the forms of the welfare state. This view was shared by many of the shapers of sports policies as well. It is also for this reason why wide set of the laws concerning the transformation of the sports system could not be passed during the first parliamentary and governmental term after the political transformation. If we look at the minutes of the first-term parliamentary debates, it turns out that the judgement concerning the lack of sports political concept is too brief. There were categorical sports political paraconcepts in this era. It is especially interesting to examine the author's view, according to which it was impossible to define the liberal, the conservative, the socialist and the Christian democratic, etc. sports policies. These were such trends and ideologies which often appeared parallel in the sports policies of executive power, and in the sports political arguments and discourses. The self-organizing professional sport turned up late, and even after the period following 1994 the directions in the field of seeking new ways and means were dictated by the wider policies. If we analyse the parliamentary debates of the sport law, it turns out that in 1996 the politicians created a frame law as a result of an actual political decision, but it did not provide an appropriate background to the inevitable reforms of the sports sphere. The declaredly centralised, paternalis-

tic sports policies of the state created a moral crisis in Hungarian sport. Moreover, this weakened the previously-born civil control and it made its survival impossible. The sports policies at the turn of the millennium and its supportive legal background encoded the excessive influence of governmental power, paternalism, subjective decision-making, the defenceless nature of sports organizations and a lucrative statutory interpretation. All in all, the author claims that in operating welfare societies the responsibility of the state is much smaller than in Hungary and it is much less spectacular in the field of political interventions in sports policies. If we look at all these facts, we should not only be aware of the benevolent self-controlling attitude of the welfare state or the positive effect of normativity, but we must also be aware of the fact that in welfare states sports managers participate in genuine debates about the preparation of decisions. An effective national sports concept and sports strategy can only appear in Hungary if decision-making will not be dominated by political arguments instead of a professional approach.

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**ATTILA BORBÉLY (2005)**

## **Eastern martial arts in the mirror of the change of sport politics and sport economy**

*Supervisor: Dr. Gyöngyi Szabó (Földesiné)*

This dissertation is a historical overview on the judgement of the Eastern Martial Arts and on the philosophy of the Eastern sports in connection with the change of regime in Hungary. This paper is based on a document analysis, a survey and a deep-interview method, which has been made among Eastern Martial Arts sportsmen in Hungary. The paper aims to analyze how the Eastern Martial Arts have become independent, civil and operating sport organizations during the last sixty years in Hungary, in spite of being politically controlled and partially prohibited during the socialism. In the first part of the paper the author gives a brief overview on the socialist sport model and sport politics behind that. He tries to reveal the factors which had made an influence on the judgement of the Eastern Martial Arts from the period of time after the Second World War till the end of the Socialism during the late eighties. In the second part the author deals with the effect of the economic changes on the Hungarian sport model.

He discusses how the functions, the structure, the finance and the organization of sport have been changed after the state had partly withdrawn from the immediate political direction of sport. The author examines how the Eastern Martial Arts were able to find their positions within the new sport system. Parallel with this how the reason for the existence of Eastern philosophy and ideology has been accepted by the above mentioned society. The survey and the deep-interview analysis and conclusions could show the habits, behaviors and aims of the Eastern Martial Arts sportsmen in connection with their sport activity. In conclusion, the author makes proposals how the values of the Eastern Martial Arts could be more and more spread and approved.

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**TAMÁS FREYER (2005)**

## **Fighting of the security forces against the football hooliganism in Hungary**

*Supervisor: Dr. Gyöngyi Szabó (Földesiné)*

The aim of the research was to examine what are the possibilities of security forces (policemen, organizers) to stop football hooliganism. Three distinguished areas were analyzed; the legal and infrastructural background of securing football matches and the training of security force specialists. Research was carried out in the period between 1999 and 2003 among the security force specialists at the matches of four football clubs in Budapest. Participant observation, press and document analysis, questionnaires and interviews were used as the major methods. The following answers were received to the hypothesis: The hypothesis that football hooliganism is not mere a question of security, but a social problem as well, was justified. First of all political will and decision is required to fight against hooliganism. Secondly the deviant fans should be removed from the stadiums. Thirdly, stress should be put on prevention, and accepting of the rules of fair play and supporting should be preferred and taught already in the childhood. The legal background should be created to reach all these aims. In contrast to the opinion the laws are available, according to the security force specialists, to stop football hooliganism. The enforcement mechanism of these rules are weak in their opinion. The courts are indulgent, the security systems in the stadiums are very poor and not adequate. That is why the rules cannot be effective either. According to the hypothesis the Hungarian football stadiums are unsuitable to organize matches there safely. Reconstruction of these stadiums started during the research. 13 stadiums have been already finished, and the future of the others depend on the economic situation of Hungary and on the actual sport political decisions. The reconstruction works were carried out in the spirit of the new international and national security regulations, and practice will give new information about their suitability. Several critics were formulated on the systems built into the stadiums. Even the most modern ones would not be effective enough; without security force specialists able for a well-prepared cooperation. The training of the specialists are different everywhere, so it weakens the cooperation. The researchers supposed that the policemen and the local organizers were not satisfied with the effectivity of their work. The research findings did not prove this assumption. The presence of hooliganism and its spreading to other areas are explained by outer factors by them. The activity of the security force specialists would be aided with a comprehensive cross-sectional survey, which aimed at the demographic and sociological characteristics of the fans and paid attention to the changes in the rule, to the training of policemen and local organizers and to the developing of stadiums. The researchers intend to continue their research in this direction.

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**SHAUN GALLOWAY (2004)**

## **An in-depth analysis on elite karate athletes concerning motivational theory: focus on borg RPE as an achievement process**

*Supervisor: Dr. Csaba Nagykáldi*

The purpose of this study was to determine over the course of two three week long training blocks (six weeks in total), whether the training intensity will increase self-efficacy levels investigating two experimental and a control group. The first experimental group (training) received high intensity

training loads. The second experimental group (Borg) received the same training loads and was also asked to keep a log book of their exertion levels compared to the levels which were set for each training session. This was done to see if cognition of the training intensity would better increase self-efficacy levels. The data was collected from these three groups of elite karate athletes. The psychological measurements included: State sport confidence inventory (SSCI), Trait sport confidence inventory (TSCI), Borg rating of perceived exertion (Borg RPE) and a questionnaire to evaluate the athletes' motivational background. Repeated measures ANOVA showed that the training group's SSCI had no significant differences between any of the treatments while the TSCI scores were significant between the first and second treatment,  $p \leq 0.004$ . The Borg group TSCI scores had significant findings between the first and second treatments  $p \leq 0.05$  and the second and third treatments,  $p \leq 0.01$ . There were also significant differences found between the first and second treatment for the SSCI scores,  $p \leq 0.05$ . The results for the MANOVA (using all three groups as dependant variables) were mixed: the TSCI scores were not significant while the SSCI scores were significant,  $p \leq 0.002$ . Using discriminant analysis the separation was found between the Borg group and the control group. There was also borderline significance found between the training group and the control group,  $p \leq 0.08$ . Regression analysis showed that prior TSCI and SSCI scores were the best predictor of self-confidence scores where high intensity training loads are used. Total results indicate that the TSCI scores are unstable in the face of intense training load. However, the SSCI scores were not affected significantly, except for the Borg group, between the first treatment and the second treatment. Differences in SSCI scores between groups were found in favor of both the Borg and training group in comparison to the control group at  $p \leq 0.002$  and  $p \leq 0.08$ .

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**GÁBOR GÁLDI (2004)**

## **The structure of leisure time and physical recreation in Hungary between 1963 and 2000, in the light of the use of time studies**

*Supervisor: Dr. Gyöngyi Szabó (Földesiné)*

On the basis of our top athletes' achievements, Hungary is considered worldwide, to be a real sports empire. However, as for leisure sports and physical recreation, which improve the quality of life and condition of health, there is not so much to be proud of. The aim of my dissertation was to highlight the rules and tendencies that are characteristic of the leisure time structure and sporting habits of the Hungarian population. I have endeavoured to describe the changes that occurred in the leisure time structure of the population, in sports and I have tried to show what difficulties people faced during their slow adaptation to the changed functions. At the same time, I have attempted to explore what impact the political, economic and social changes of the examined time period had on sports. Furthermore, I have examined how the population's leisure time structure – with special emphasis on physical recreation and sports – changed in the year 2000. It was interesting to analyse the differences between the lifestyle of people who pursue sports activities and of those who do not; it was equally interesting to see what characterised the days when the respondents did some kind of physical recreation activity. I used various research methods in my paper, primary and secondary data collection as well as the analysis of qualitative and quantitative data. I conducted structured interviews with leading sports politicians of the past number of years, and about the most important legal and political issues as well as about the relevant institutional matters. In addition, other information sources also helped me with my work, for instance, previous lifestyle studies and use of time analyses performed by the Hungarian Statistical Office (KSH), which enabled me to have an insight into

former eras, too. The results showed that the worktime spent in full-time employment in the case of men continuously decreased between 1963 and 1993, but the work necessary to supplement the full-time income doubled. Looking at the differences in leisure time in the various dwelling places, we can see that those in the capital have the most leisure time while village dwellers have the least. Only in 1993 did this latter group reach the 1977 amount of leisure time of those living in the capital. The time spent in front of the television screen continuously grew to the detriment of other leisure time activities, and by 1986, in the case of women, it neared the percentage of all the other activities together. In the case of men, this tendency reached this level only in 1993. Despite the fact that the leisure time grew gradually until 1993, the proportion of active leisure time activities declined, which was mainly influenced by the settlement's level of urbanisation. According to the 1999-2000 use of time analysis, 70.3% of the Hungarian population between 15 and 84 do not perform any sports activities whatsoever. 24% of those with an elementary school qualification and 54% of those with a university qualification do sports activities with some kind of regularity. 67% of those under 20, while 6% of those over 70 perform some kind of sports activity. The regional differences are also noticeable, since 40% of those living in the capital do sports, as opposed to only 22% of those living in small community settlements. Only one-tenth of the population pursue more than one sport. Those men who do sports, in all age groups use less time for satisfying their physiological needs. The amount of social time as well as the working capacity of those who do sports is higher than that of their inactive peers. The socially bound time of those women who do not do sports is significantly higher than that of their sporting peers, therefore we can presume that their greater workload prohibits sporting activities to be incorporated into their everyday life. Most men and women who do sports have more free time than their inactive peers. In the active age groups' of men who do and who do not do sports, the strong dominance of income earning work can be observed, this dominance is even stronger in the case of sporty men between 40 and 59 than in the case of their inactive peers of a similar age. The fact that in all age groups of both sexes who do sports spend less time on watching television, sleeping and passive relaxation, demonstrates the more active use of their leisure time. In all age groups of men and women who do sports, the percentage of the time spent on watching television within the leisure time is significantly lower even on an average day of the year.

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**CSABA HÉDI (2005)**

## **Contribution of the state, civil and business sector in university sport in the 1990's**

*Supervisor: Dr. Gyöngyi Szabó (Földesiné)*

Birth of modern sport at the end of the XIXth century can be considered as a product of civil society. The years following the World War II. brought on radical changes when political regimes called first popular democracies and later socialists were established in countries of Eastern Europe including Hungary and when in the bipolar world both sides blew up political function of sport, using it different ways and intensity, with different purposes. Sport was transformed into a state's activity, while active civil society ended by being suppressed at the cost of some difficulties. The politically bipolar world came to an end with the changes in Eastern Europe in 1989-1990, and the system of one party was suppressed. In the middle of the 1980'ies a relatively autonomous students' sport federation was founded. The change of the political regime made it possible for institutions of university sport to become independent from state and autonomous. The realisation of this process passed off in a contradictory way which is examined by our research through the analysis of the participation of state, civil and business sphere. The purpose of the present essay is to reveal: How the change of the political regime happened in the institutions of university sport?

What was the development of the structure and functioning of university sport in the decade following the change of the political regime, particularly concerning the participation of state, civil and business sector in university sport? We suppose that the laws and decrees having entered into force in this field since 1989 had a contradictory impact on the institutions of university sport. We suppose that, in the period of erosion of the so-called socialist regime, university sport began to become civil at an earlier stage than other sectors. It could profit from this advantage only for a short period mainly because of the contradictions of its financing. The essay is based essentially on analysis of documents, while the research is based on surveys. The scope of our empirical study was the presentation of changes in the structure and the contents of the high education at the end of the 90'ies and beginning of 2000.

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LAPPAS KLEOMENIS (2005)

## Deviant behaviour of greek football spectators

*Supervisor: Dr. Gyöngyi Szabó (Földesiné)*

In Greece deviant behaviour within different spectating groups has been witnessed to a greater extent than in many European countries. While in many European countries, football deviance has been treated in a preventive phase and sanctioned more or less efficiently if occurred, in Greece the frequency and intension of deviant episodes is steadily increasing. There have been few researches in Greece dealing with football deviance but this is the first comprehensive study focusing on this problem. The major purpose of this study was to discover how the demographic characteristics, the socio-economic composition, the customs and motivations of Greek football spectators have changed in the last years and what are the main manifestations of deviance in Greek football stadia as well as the spectator's views on football fans' behaviour in and out of the stadia. The deepest causes of football deviance related to the changes in wider society and in football spectating subculture have been also studied. In our research the following methods were used: survey method, in-depth interviews, press analysis and analysis of documents. The sample of our survey consisted of football spectators attending first league football matches and it was selected randomly. The questionnaires consisted of opened and closed questions. In-depth interviews were made with spectators, coaches, managers of first league teams, and media experts. Participant observation consisted of structured and non-structured observations. The task of participant observers was to register any kind of disorders, misconduct, and aggressive and violent episodes. The related documents, which were analysed, were issued by the National Statistical Service of Greece and the Ministry of Cultural Affairs. Football hooliganism, and the reaction of media to football deviance has been studied from the daily newspapers and sport magazines. The data from the questionnaires were analysed and evaluated through SPSS Statistical Program while the data from in-depth interviews and press were analysed through qualitative analysis. The results focus on the behavioural patterns of Greek football spectators, which have radically changed mainly because of the changes, which have occurred in the demographic characteristics, the socio-economic composition in the last years. The results show that the number of young people attending football matches have remarkably increased. Still the males dominate the grandstands at the stadia although the amount of females has relatively increased. The greatest part of spectators comes from the big economical and administrative centres, that is from Athens and Thessalonica. The majority of football fans belong to the middle socio-economic classes. Notable changes occurred also in the customs and motivations of spectators towards football matches. Hooliganism appears to be one of the major problems. It is surprising that manifestation of racism and xenophobia have been very rarely noticed within football spectating subculture. The reasons of the

above mentioned changes are deeply rooted in the transformation of Greek society over the last years, in the impact of global and local factors, in the changes in football spectating sub-culture and in the impact of mass media on sports and particularly in football. Finally recommendations are made in order to counteract football deviance and particularly hooliganism in Greek football stadia.

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**MÁRTA LÓCZI (SEBŐKNÉ) (2003)**

## **The teaching and learning process of the special sports programme in teacher**

*Supervisor: Dr. János Gombocz*

Lack of exercise as a way of life is in close connection with the gradual decay of national health indexes. The habit of doing exercises in one's free time has to be formed at an early age. Lower primary teachers responsible for the personality development of the 6 to 10 age group have a key role in children's habit formation. Do we prepare our trainee teachers for this important task? To find the right answer we have examined teacher training in special respect of the cognitive, social and emotional aspects of physical education, and the teaching and training process of sports. The findings of the survey: - The exploration of the cognitive sphere was carried out with the help of syllabus analyses of Physical Education and by tracing the changes in the methodology of Physical Education. Based on the survey we can state that the practise of forming cultural instrumental knowledge isn't in accordance with the expectations defined by the teacher trainers' professional committee. The integration of higher education does not favour the methodology of action and skill based subjects. With the help of questionnaires we revealed the socio-economic background of our trainees. Based on the findings of the survey we can state that the majority of the students come from lower middle class families living in little provincial settlements, and they will be first generation intellectuals. The emotional aspect of Physical Education (value judgments, attitudes and psychical qualities) and the process of studying and teaching sports were examined by questionnaires. Based on the survey we can state that: their value judgments concerning exercises are positive but their attitudes are unstable. The recorded data among the first year beginners and the fourth year seniors show positive tendency in favour of the fourth year, but the difference does not reach the desirable level. The results of the examinations of psychical qualities, which can be formed, with the help of motional exercises, show the same tendency. If we sum up the opinion of our colleagues teaching Physical Education at our Teacher Training College we can state that the expectations of the training requirements are not fulfilled during the training. The reasons may lie in the insufficiency of the students' preliminary training and also in the poor facility supply of the educational and training institutions. Suggestion: The efficiency of the training should be raised by creating favourable conditions, by applying modern teaching material and by effective organization of the learning process.

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IMRE MAKSZIN (2005)

## Teleological relationships in the curriculums, in the planning of the educational -instructional work and in the instructional process

Supervisor: Dr. János Gombocz

We are demonstrated the determinant role of teleology in the everyday pedagogical praxis. The philosophical side of the pedagogical activity first of all the issues of teleology, are connecting to the praxis organically, and these two sides are inseparable from each other. The issue of the teleology appears at the curriculum theory in general and concrete form, on the all levels of the planning of teaching-learning and in the instructional process.

Step by step we demonstrated the different goal levels as the result of the demolishing of the goals, in every chapters of the thesis. These goal levels helped to understand the contact between the theoretical and practical side of the pedagogy. We demonstrated that the planning work, to make syllabus, instructional process, goal realization, can be plan only understandable form to the students. The reason of this is that to the students only in this form are revealing the goals, what means measurable forms. It is important to clear, on philosophical base, the relation of the need-interest-value, which determines our every day life, and without educational relations of that we can't be successful.

The base of the educational work of the teacher should ensure by the suitable planning, with the exactly prepared task hierarchy, and with the measurable form identified performance objectives. With the evaluation forms, which are going through the whole educational process, are ensured the feedback to the students and to the teachers as well. We ensure the process character and this bring nearer the goals to the students, i.e. the requirement became values.

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EMŐKE MARTOS (BUCSYNÉ) (2003)

## Research on sociopedagogy student's health-cultural behaviour, with special respect to physical activity

Supervisor: Dr. János Gombocz

The dissertation summarizes the results of our pedagogical research programme, which has been going on for five years. The aim of our research was the examination of sociopedagogy students' health - cultural behaviour, respectively the change of this behaviour affected by college education. We put an emphasis on the effect of college education on the subjects' physical activity, respectively the change of physical activity. Institutions of higher education with identical educational profile, but working with different syllabi in the field of health - and body - culture took part in the research. The staff members at the Department of Physical Education, Sopron College Faculty had worked out a special syllabus in the mentioned educational domain with the purpose that by means of the knowledge, skills, behaviour patterns learned and the abilities developed the students - as graduated educators - convey proper behaviour patterns to their environment. Forming a physically active lifestyle gained a central part in the educational conception. The main course of our research was the comparison of the data of female students in Sopron (110 people) and in the associate institutions (199 people). We approached the problem of research from several angles. The examination of health-cultural behaviour was completed by the examination of sport motivation, furthermore by the examination of the students' fitness and personality. Assessing the data of the questionnaire compiled for the investigation of health - cultural behaviour we found that students in Sopron have a physically more ac-

tive lifestyle in spite of the fact that there are more sick students and students suffering from lesion. Our presupposition that the physically more active lifestyle influenced Sopron students' dietary and health-affecting habits (smoking, alcohol consumption) was not proved. During the analysis of the questionnaire about sport – motivation we found that at the examined age cognitive and moral motives gain a great role in the formation of the individual's motivational structure. The examination results of the strength of sport motivation verified that sport is important in our students' lives; they are planning a more intensive practice of sport activity in the future. The cross-sectional and longitudinal examination of Sopron students' fitness was tightly connected to our pedagogical research topic, since the change of fitness indicates the change in physical activity in an indirect way. On the basis of the performance shown in motoric tests we can say that the students reacted to the training stimuli affecting their organism with an intensive development. The data confirmed the results we received via written investigation that the physical activity of the students in Sopron had changed positively as a result of college education. The hypothesis of the cross-sectional and longitudinal personality research that during the education certain characteristics of sociopedagogy students alter - features necessary in the helping profession improve - proved true. The results of our research confirmed that behaviour - even that of young adults - can be formed; moreover, sport plays a significant role in the process of becoming an educator.

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**KATALIN MIZERÁK (2005)**

## **Sports history of the Central-Asian Mongol people from the cultic roots to the 20th Century Olympic Games movement**

*Supervisor: Dr. István Kertész*

Explanation of the topic, objective of the research: The dissertation dealing with the sports history of the Central-Asian Mongols is to be regarded as a gap-filling research. The novelty of the dissertation is that not only does it publish the most recent data but it also drafts the analogies regarding the Hungarian and the global sports history. It also details war history, historic and literary sources not analyzed in Hungarian so far. The I. part deals with the cultic times of the Mongol sports history and on the other hand it focuses on the transformation of the Mongol sports history and sports philosophy during the Manchurian empire. The II. part shows the transformation of the Mongol sports history of the 20-21th century from the aspect of ideological changes. Methods of the research: the history of the traditional folk games and their cultic background has been described mainly based on Mongolian historical and literary sources. The published historical and sports historic data were obtained partially by personal collection and also by using the works of domestic and foreign orientalists, famous Mongolian, Russian, Polish, German, English, French and Hungarian historians, ethnographers, anthropologists, archeologists, relying on the information of Mongolian data providers. Information about the age of tribe alliances has been significantly widened by the descriptions provided by ancient Greek, Roman, Chinese historians, geographers and travelers. The material mostly supported by sources and data could be collected from the heyday of the Mongolian regime. The works of travelers and linguists of the 19-20th century as well as the imperial regulations effected at that time helped a lot in analyzing the Manchurian empire. As far as information collection was concerned, the biggest challenge was the compilation of the results of the 20th century, because the articles, studies and interviews have not been systemized in the Mongolian sport science yet. Scientific results of the dissertation: After reviewing the notions in connection with the traditional competitive and folk sports in Central-Asia it became apparent that to the Mongols, physical exercises had been essential part of the daily life beginning from the age of tribe alliances. To the nomad Mongols the

regular physical exercises had been a condition to survival for thousands of years. The dissertation includes the description of traditional folk games and reveals sports history in connection with the dual approach of the body and its ritual role, which contributed to the Mongolians' world conception. It can be stated that the maintenance of physical fitness containing traditional cultic elements as well, was not simply the preference of some, but it had become a vital necessity strengthening and keeping together the entire Mongolian society. The conclusions of the dissertation elaborating the Mongolian sports history can be summarized in four points: 1.) The sources of history, literature, the religious texts, legends and tales and the information given by data providers proved the conclusion saying that the Mongol sports had started from religious practices 2.) The sources also proved that the changes in the Mongolian sports and in the society were realized in close connection to each other. 3.) The up going stages of the Mongolian history (age of tribe alliances, the reign of the Genghis) always were of high importance in the Mongolian sports history. 4.) Following the system change a positive procedure started in the Mongolian sports history. An unexpected possibility was offered to Mongolia by the co-existence of the traditional and modern competitive sports. In the knowledge of the data available, we can state that the country with rich body cultural habits might cause as unexpected surprises when joining the international competitive and recreation movement as it did with its speed it had caught up with infrastructure and culture.

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ÉVA ANETTA MÜLLER (2004)

## Movement examinations on the example of movement consistency and performance constancy

Supervisor: Dr. Endre Rigler

In the world of sport definitely but also in course of physical education in schools we will have to try to answer the following questions: When can we consider the process of movement successful and completed? What are the criteria for the acquired movement? Evident as it may seem, further questions arise during analysis. These are: Is it enough to show the movement? How do we know that the observed movement meets the previously set requirements? Is one presentation of the movement enough or should there be more to signal the acquisition of the movement. How much are we able to carry out movement reproduction? Which of the reproductions –with possible differences- can we consider good? Is the reproduction of the movement enough in itself or do we have a further purpose with it. Which aspects of movement should be considered when analysing? (It is especially interesting when we do not have special measuring and testing devices at our disposal to get information quickly. In a P.E. lesson for instance.) Even today we often have to rely on our own personal observations. In our view, in the classical process of movement the level of automation and the dynamic stereotype could be the central elements. We think that the consistency of success of performance in movement reproduction is a sound basis to draw conclusions about the level of acquisitions. *Objectives:* The specific purpose of my research is to examine the development of movement and learning of movement. *Methodology:* My general questions are the followings: How the accuracy of performance influenced by different ages, sexes, experience in sports, part of the day. How faults of movements are influenced by complex and complicated movements. Local and general tiredness how influences the level of movement reproduction and constancy. In case of several movement attempt (serial of throw and serial of jump) how accuracy and constancy of movement change in respect of result. My methods are the following: Statical balance. Dynamical balance. Accuracy of throwing and kicking (to aiming at a target, the target means the concentric circles on the floor and on the wall). Serial throws and jumps (constancy of movement). Time assessment. *Significance:* The significance of

the project is the fact that one of the central questions of sport and physical education is movement precision as this is the basic of successful performance. The significance of reproduction of movement and movement precision at school is also evident as they serve as a basic for grading. The research is relevant as we observe the process of movement acquisition and perfection because it helps answer question like: When can we consider the process of movement acquisition complete? What are the criteria for acquired movement?

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LEE CHEE PHENG (2003)

## Comparison of anthropometric characteristics and motor performance scores in Malaysian boys. An inter-disciplinary approach

*Supervisor: Dr. János Gombocz*

The general pattern of healthy growth is quite similar from one individual to another, but there is considerable individual variability in size attained and rate of growth at different ages, with respect both to the body as a whole and to its specific parts. Both the whole body and its parts, therefore, must be measured, and the study of growth is synonymous to a large extent with measurements. The aims of this comparative study was to characterise the physique, body composition and motor performance of Ipoh children and adolescent aged between 10 and 13 years. The investigated 1.922 boys mean 8-9% of the respective Malaysian, Indian and Chinese population. Standard anthropometric procedures were used for the estimation of growth type (Conrad 1963), somatotype (Heath and Carter 1967) and relative body fat content (Parížková 1961). The actual level of physical performance capacity was assessed by the result of 1200 m run, 30 m dash, standing long jump and fist ball throw. The significant differences between the height, body mass and growth type characteristics can be attributed to the racial variability, however, the very great ratio of fat and obese children and adolescent were independent of the anthropological differences, Nevertheless, significantly more picnomorphic growth type was found in the fat and obese subjects irrespective of racial differences. The physical performance capacity of the investigated Malaysian children is in a definitely lower level, than their European and North American counterparts. The absence of practice in some of the test exercises means just one side of the problem. The learning effect can be excluded from the evaluated results consequently. The 80 minutes regular physical activity alone cannot compensate the negative consequences of their sedentary life style.

Bearing in mind the complexity of these relationships, and recent evidence that children are not as active as they should be and are becoming fat, such problems in childhood and adolescence may lead to serve adult health consequences, it seems prudent to stress primary prevention strategies in childhood aimed at controlling obesity and promoting aerobic fitness, such strategies should include regular testing of fatness and fitness as well as the promotion of habitual, vigorous physical activity.

A potentially important mechanism linking exercise to long-term weight loss is a collection of psychological factors. These factors are important to explore for several reasons. Exercise prescription might be affected, and there is a possibility, judging from clinical experience and recent research (Bouchard 2000), that improved well-being and enhanced self-esteem produced by physical activity generalise to other areas and lead to improved dietary adherence. A review of literature on exercise and psychology suggest several possible effects of physical activity on psychological factors that may be related to weight control. These factors are mood, body image, self-esteem, self efficacy, and coping. The general aims and goals of schools physical education must be separated in and must be fill up with special contents in case of fat and obese children and adolescents. The most important

goals are: the goals of physical education and sports, the nature of physical education and competitive sports with respect of educational goals, achievement of the physical goal in leisure-time physical activity and in school, achievement of the goals and motor skills, achievement of socioethical and personality goals.

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**MARINA SALVARA (2003)**

## **Elementary school physical education teachers' representations on instruction: a cross-national comparative perspective between greece and hungary**

*Supervisor: Dr. Edit Nagy (Bíróné)*

This research was conducted to describe and compare the Greek and Hungarian elementary school PE teachers' representations on instruction and the teaching styles they employed through a triangulation multidimensional instrument method. Some of the purposes of this research were to demonstrate the teaching styles employed by the teachers in Athens and Budapest and to identify which of the teaching styles are in the spotlight, involved in the personal teaching theory, and the point of the spectrum at which the teachers in the countries have arrived in conjunction with the National Curricula. Teachers consented to participate were initially examined with the Physical Education Teachers' Representation Questionnaire (PE-TRIQ I & II), responding for their represented importance (I) and frequency of employment (II) on the items, which were distributed with a time-lag of 1 month. Exploratory factor analyses with PCA, using varimax rotations, conducted separate for the teacher samples, resulted in the development of two 28-item, 4-factor, PE teachers' perceptual models. The reproductive factor indicated knowledge declared and imposed by the PE teacher. The assimilative factor reflected knowledge achieved with a teacher-pupil agreement and distribution of roles. The discovery factor was composed of items that represented knowledge achieved through logic reasoning. Finally, the productive factor was indicative of knowledge reconstructed by means of transformation leading to the construction of new knowledge. By means of Pearson product-moment correlations, a high convergence was revealed between I & II measures. Lessons taught by teachers to pupils in 4th, 5th and 6th grades of elementary schools were videotaped and coded with the system designed for Identifying Teaching and Learning Behaviours (I ITLB), with which the Greek and Hungarian teachers' employed spectrum of teaching styles was constructed and compared. Thereafter, the Cheffer's Adaptation of Flander's Interaction Analysis System (CAFIAS), was used for the depiction of teachers-learners interactions along the spectrum and their everyday practices. A structured informal interview followed each session with each of the PE teachers to further add clarity to the observed data. Descriptive statistics were computed for all 84 observed lessons and measures. MANOVA techniques, follow-up ANOVAs, and post-hoc Games-Howell procedures, were conducted, separately for each instrument, to examine whether any of the Greek and the Hungarian teacher differences or indifferences could be attributed to the characteristics of teachers' gender, years experience, postgraduate studies, school grade and pupils' socioeconomic status at which they taught. In short, the results indicated that experienced and well-educated teachers had richer, better instantiated cognitive representations of the subject matter, instructional strategies, classrooms, and the nature of children than do inexperienced teachers. Interestingly, it was found that both teacher samples teaching pupils belonging to low class tended to apply more direct teaching styles, while for high class pupils indirect styles were their preferred practices, embodied to the reproduction and production clusters respectively. An oversimplification of this finding should be avoided. Greek teachers tended to spend most of their time using direct teaching styles, compared to Hungarian

teachers that tended to use indirect along with teacher-centred approaches. From the analyses of the data, the differences that were revealed reflect the differences in the National Curricula.

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## LÁSZLÓ SZEPESI (2004)

### Competition training and competitive preparation of national team level fencers

*Supervisor: Dr. Endre Rigler*

Preparation of the best French sabre fencers for the world championships and Olympic Games was found unsatisfactory with respect to both competitive practice and training work, and therefore needed fundamental reshaping. To gauge the effect of the changes introduced the number of the lessons, bouts, touches, and intraseason victories were carefully observed and recorded through ten years. This paper deals with some particular aspects in the analysis of these data. The marked differences in almost all the studied quantitative measures of competitive preparation and training work disallowed a common treatment of these elite sabreurs, the 8 who performed well enough to become members of the national team had to be discriminated from the second ranks of swordsmen. In order to extract the greatest possible amount of information latent in the data iterative techniques of regression were applied in addition to the conventional direct approach. In search of the best predictor for the end-of-season success rate, specific techniques were required to handle the problems associated with the complex relationships between the observed data. Multicollinearity was strongest between bouts and touches, but even the number of lessons happened to relate too closely to the success rates of certain periods. The really efficient technique to deal with the multicollinearity problem was the use of residuals of which the collinear contaminant variance was partialled out. The question of whether autocorrelation did or did not bias the estimated regression could not be settled to satisfaction: first-order differences were associated with information loss, and the results were largely similar but not directly comparable to those with the original data. By applying iterative regression techniques several models were developed to account for various aspects of the complex relationship between the studied quantitative measures of preparatory work and the success rate at the main contests of the season. These models drew attention to unexpected, sometime surprising effects: reciprocal influences, effects developing after considerable time lag (e.g. suggesting that certain lesson effects may become manifest in the next season only), and – last but not least – the variable importance of the respective World Cup competitions for the eventual success rate. The best fitting models for the team members were:

$$S8 = 0.027 + 0.237 S1 + 0.365 S2 + 0.217 S4 + 0.001 B5 \pm 0.061; \text{adj.R2} = 0.666;$$

$$S8 = 0.541 - 0.002 L1-7 + 0.0039 B1-7 + 0.0005 T1-7_{\text{res}} + 0.906 S1-7 \pm 0.065; \text{adj.R2} = 0.630;$$

$$S8 = 0.184 - 0.002 B4 + 0.001 T4 + 0.257 S3 + 0.393 S7 \pm 0.074, \text{adj.R2} = 0.515.$$

For the non-team members they were:

$$S8 = 0.188 + 0.488 S1 + 0.325 S7 - 0.014 L6 \pm 0.084; \text{adj.R2} = 0.432;$$

$$S8 = 0.152 + 0.534 S1 + 0.352 S7 - 0.001 L1-7 \pm 0.087; \text{adj.R2} = 0.384;$$

$$S8 = 0.546 - 0.002 L1-7 + 0.0037 B1-7 + 0.00041 T1-7_{\text{res}} + 0.857 S1-7 \pm 0.097; \text{adj.R2} = 0.324.$$

where B: number of bouts, L: number of lessons taken during both the training sessions and competitions, S: success rate = the number of victories divided by the number of bouts, T: number of touches given, res: residual of which the effect of the collinear variables had been partialled out, while the numbers following the variables denote the periods of the season of which the eighth referred to the period of the world championships and Olympic Games.

The standard error and adjusted R-square of the model is shown on the right of the equations. The

quantitative analysis of the competitive and training stress contributed a lot to the understanding of the improving international performance of the French sabreurs in the studied period and, to some extent, in the times following it. Jean-François Lamour became Olympic champion twice: in Los Angeles in 1984 and in Seoul in 1988, and became individual champion of the Lausanne world championship in 1987. The team of the Raçing Club (Lamour, Guichot, Delrieu, Ducheix and Bolle) won the European Cup of the Champion Teams in 1990. Every member of the French national team (Lamour, Guichot, Delrieu, Ducheix, Daurelle and Granger-Veyron) was either a medalist or a participant of the individual finals at the recent world championships or Olympic Games. These are unique and unparalleled events in the one hundred year history of French sabre fencing.

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LÁSZLÓ TÓTH (2005)

## Complex study of physical development, motor performance, academic achievement, and emotional characteristics for 10-15-year-old school children

*Supervisor: Dr. Kornél Sipos*

The aim of the study was the complex examination of emotional characteristics of 10-15-year-old school children (N=299). The state-trait anxiety, arousability, physical self-concept, [general-, and situation specific] self-efficacy, were those variables according which the gender differences and age-related changes were analyzed. Body height, body weight, Body Mass Index, and plastic index were recorded for characterization of the physical development. Standing long jump, 20m-, 60m dash, 10m slalom with ball, Burpee test, 6min-, and 12min run (Cooper test), hip joint mobility test, static balance were the physical performance variables. The cognitive-emotional functions were measured by the Hungarian forms of anxiety/arousability scales (STAIC-H, and AAI-H), Tennessee Self-concept Scale's physical self-concept scale, and Schwarzer's generalized-, and situation specific self-efficacy scales [self-efficacy towards physical exercise]. In the academic achievement we distinguished the grade point average (GPA) for mathematics + Hungarian language + foreign language (GPA1), and GPA2 (physical education + practical work + drawing). The gender differences and the age-related differences were explained according to the results of the international literature. The conditional characteristics (strength, speed, endurance) were not separated in this age from each other by factorial analysis. In the differentiation of the skills, according to our opinion, the morphological background factors and the learning processes play equally important role. The results of the multivariate regression analysis revealed that the higher boys of 10-11 years, and the low-built 12-13-year-old girls had significantly higher anxiety. With other words, the somatic changes of the puberty (changing of the body shape) mirror in the affectivity (provoke the anxiety). The motor, cognitive, and affective functions (components of the personality) in childhood have temporarily changing connections which have age-group related shaping characteristics in case of optimal conditions. According to the cluster analysis, the motor performance differences in puberty can't be explained by the level of the bodily development. Boys perform better than girls in all age groups in general. At the same time, the essential differences in these age groups in the complexity of the motor performance and anxiety are determined by the functioning of an organ or the appropriate organ system.

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## 6. JÁNOS SZENTÁGOTHAÏ PH.D. SCHOOL OF NEUROSCIENCES

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### *Head of School*

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The Neuroscience Graduate School blends the theoretical and clinical neuroscience research topics, treats the basic questions of the structure and function of the nervous system in a synthetic view as well as the normal and pathological functioning of the human brain as observed by the clinicians. The research topics as listed below and arranged in three basic science and three clinical research Programs witness the large array and variation of supply.

### **6/1. PROGRAM**

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#### **NEUROMORPHOLOGY AND CELLULAR BIOLOGY**

### *Coordinator*

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### *Sub-programs*

Structure and function of higher order thalamic nuclei	László ACSÁDY
Striatal circuits in relation to learning and motivation	András CSILLAG
Endocannabinoids in the brain: a novel type of communication among neurons	Tamás FREUND
Subcellular localization of GABA and glutamate receptors in the hippocampus	Tamás FREUND
Synaptic plasticity in the spinal cord	Gábor GERBER
Histological and immunohistochemical investigations on the brain of <i>Mobula japonica</i> (Myliobatiformes)	Mihály KÁLMÁN
The glial reactivity and neural regeneration in relations of the glial histogenesis and evolution	Mihály KÁLMÁN
Junctions between glia and connective tissue: functional morphology	Mihály KÁLMÁN
Neurogenesis in vitro: cell biological and molecular biological studies on neural progenitor cell lines	Emília MADARÁSZ
Fate of neural progenitor cells implanted into various regions of the brains at different ages	Emília MADARÁSZ
Epileptic reorganisation in surgically removed hippocampi of TLE patients	Zsófia MAGLOCZKY
Synaptic information processing in the olfactory bulb	Zoltán NUSSER

Structure and function of higher order thalamic nuclei	László ACSÁDY
Subcellular distribution of ligand- and voltage-gated ion channels	Zoltán NUSSER
Topographical localization and chemical characterization of thermo-sensitive neurons in the central nervous system	Miklós PALKOVITS
Neuronal pathways of the vestibular stress (vestibular-hypothalamic connections)	Miklós PALKOVITS
Sensory processing in the spinal cord with emphasis on the nociception	Miklós RÉTHELYI
Sensory and motor projection of the viscera into the spinal cord	Miklós RÉTHELYI
The role of cannabinoids in CNS regulatory mechanisms	Tibor WENGER

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Miklós Antal	ft
Csilla Ari	ft
Péter Barthó	ft (a)
Ágota Bíró	pt (a)
Csaba Boros	pt (a)
Gábor Zoltán Csáki	pt
Nóra Hádinger	ft
Davis Jonathan	it (a)
Márk Kozsurek	ft (a)
Anikó Ludányi	ft
Anikó Reichart (Guttmané)	pt (a)
Zsolt István Kovács	pt (a)
Balázs Markia	pt
Károly Markó	ft (a)
Ferenc Mátyás	ft (a)
Kinga Meltzer	pt
Tünde Molnár	pt
Rege Papp	pt
Adrienn Regina Szabó	pt
Virág Takács	ft
Anita Zádori	ft

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Márta Jelitai	ft
Éva Mikics	ft
Viktória Vereczki	pt
Natalya Zayats	pt

**Ph.D. graduates**

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Frigyes Helfferich	pt
Attila Kófalvi	pt
Attila Losonczy	pt
Szilvia Mezey	ft
Gábor Nyíri	ft
Tamás Sebestény	pt
Patrícia Varjú	ft
Lucia Wittner	ft

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István Katona
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András Csillag
Tamás Freund
Teréz Tömböl
Emília Madarász
Tamás Freund

a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

BÉLA AJTAI (2003)

## Comparative morphology of reactive gliosis

Supervisor: Dr. Mihály Kálmán

The reactive gliosis is a reaction to injuries to the central nervous system, where the main role is played by the astroglia. The reactive gliosis is thought to be responsible for the lack of regeneration after injury. In early stages of development regeneration is still possible, but no reactive gliosis is formed, in fact, the astroglia is essential to support and guide nerve fiber growth in the developing brain. In my work I investigated the reactive gliosis, in the following comparative morphological studies. 1. in the developing rat brain: i. in a given region (e.g. cerebral cortex), at different stages of development (pre- and postnatally). ii. in different brain regions (cortex, corpus callosum, diencephalon) in the same age. iii. following mechanical lesions of different severity, to find the earliest age when a glial reaction similar to that of the mature animal may be formed. 2. In the developing rat brain, in different brain regions (corpus callosum, diencephalons) and in different ages to analyze the relationship of reactive gliosis and nerve fiber growth. 3. In brain regions with special astroglial structure: i, rat and chicken Bergmann glia; ii, ependimoglia of the goldfish brain, following stab wound lesions. The analysis of the experimental material was based on immunohistochemical stains. According to our results, in the rat diencephalon, even embryonic (E) lesions were followed by reactive gliosis (E18 and later). In the cerebral cortex, if severe enough, even injuries to the newborn (P0) may provoke reactive gliosis, therefore the elicibility of the reactive gliosis appears earlier than generally thought and its time of its earliest onset is different in different brain region. The reactivity of the astroglia in the rat cerebral cortex becomes similar to that of the adult after the 5th postnatal day (P5). Between the newborn age and P5, the appearance of the gliosis depends on the severity of the lesion, but it always manifests on P7-8. It is therefore the appearance of the reactive gliosis that can be linked to a certain developmental age, not its elicibility. The appearance of the glial reactivity occurs by the end of neuronal migration and the transformation of the radial glia into astrocytes.

Following transections we observed that those performed on E17 never, those performed on E20 always disrupt the rat corpus callosum. Since reactive gliosis appears after lesions performed on P2 or later only, in the meantime factors other than the gliosis might be responsible for the inhibition. After lesions performed between E18 and the newborn, the disrupted fibers grow well in the developing cortex. Along their course GFAP-immunopositive astrocytes appear, which may support their growth. In the diencephalons, lesions performed on P5 or earlier result in reactive gliosis that is morphologically similar to the one seen after P2 or later corpus callosum transections. Contrary to that, however, an aberrant nerve fiber bundle appears in the territory of the diencephalic reactive gliosis. We think that the growth of these fibers was stimulated by the reactive gliosis. No such phenomenon has been proven until now in vivo. Following P5 or later lesions, the diencephalic gliosis will be inhibitory to fiber growth, too. These results indicate that the relationship of reactive gliosis and fiber growth may be different, depending on region and stage of development. Depending on region and age, morphologically similar reactive gliosis may have different effect on axonal growth. In our experimental model the transition from growth-promoting to a growth-inhibiting environment has a well known temporal profile, which might prove to be very useful for studying the lack of regeneration on a molecular level. Our lesion resulted in the activation of the cerebellar Bergmann glia and the ependimoglia of the goldfish tectum, but this reactive gliosis has a special morphology: their glial processes do not rearrange themselves. Another new observation is that even the chicken Bergmann glia is able to express GFAP, when activated. In the GFAP-immunonegative ependimoglia of the goldfish brain, however, even the lesion failed to provoke GFAP expression as opposed to the GFAP-immunonegative areas of the rat and chicken brain, where the lesion results in the appearance of GFAP-expression.

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**FRIGYES HELFFERICH (2004)**

## Hearing and stress: investigation of the central pathway of the audiogenic stress in rat

*Supervisor: Dr. Miklós Palkovits*

The aim of our study was to describe the ascending pathway of the audiogenic stress, using modern neuromorphological techniques, in rat. In the preface the definition of the stress and some stress-theories are delineated. The classification of the stressors is followed by the description of the audiogenic stress. The brain regions, which belong to the ascending and the descending auditory pathways, are reviewed, shortly mentioning their function and their interconnections. Some known stress-pathways are presented and the ideas about the audiogenic stress are delineated. Two basic experimental techniques were used. In the first step those brain regions were identified, which became activated by noise stimulation. The expression of the proto-oncogene, c-fos was used as a marker of the neuronal activation in the experimental model of the intensity-dependent audiogenic stress. The immunohistochemical identification of the activated neurons was followed by the definition of their corticotropin-releasing hormone (CRH), catecholamine and TIP39 content. Beside the activation of the auditory pathway, some stress-related regions showed an elevated c-fos induction, as well. CRH/c-fos double labeling was found in some hypophysiotropic neurons of the hypothalamic paraventricular nucleus (PAmp). It has been established, that the extrahypothalamic, CRH-containing neurons did not show activation following noise stimulation. Activated neurons were present in the A1, A2, A6, A10, A11 and A15 catecholaminergic regions. In the second step of the experiment, a neuronal connection needed to be found between the audiogenic pathway and PAmp. A modern tract-tracing technique was used: pseudorabies virus was injected in the parvicellular part of the hypothalamic paraventricular nucleus. The virus spread from the injection site, and following an appropriate survival time, the infected neurons were visualized by immunohistochemistry. The specific, retrograde, transynaptical spread of the virus delineated the primary, secondary and tertiary afferentation of the PAmp. Infected neurons were found in all known regions, which directly innervate the PAmp, among these, in the auditory-responsive posterior thalamic nuclei: the parvicellular part of the subparafascicular nucleus and the posterior intralaminar nucleus. Our finding confirms the assumption, that these multisensory nuclei are obligatory relays in the ascending pathway of the audiogenic stress. Some more infected neurons were found in the auditory pathway, previously not described as afferents of the PAmp: in the medial paralemniscal nucleus, in the dorsal cortex of the inferior colliculus, among the dorsal periolivary neurons and in the dorsal cochlear nucleus. These regions innervate the PAmp indirectly; thus they represent secondary or tertiary afferentation. Based on the results of the two experimental techniques, the ascending pathway is figured as follows: the noise-generated action potential reaches the dorsal cochlear nucleus via the auditory nerve, makes a short cut from here to the medial paralemniscal area, which projects to the posterior thalamic nuclei. From here, directly (or indirectly via the forebrain) it reaches the parvicellular hypophysiotropic part of the hypothalamic paraventricular nucleus, which in turn controls the endocrine stress response. The bidirectional connection between the PAmp and the DCN was proven by pseudorabies injection into the DCN, which lead to neural infection in the PAmp. Based on the activation of the TIP39 neuropeptide-containing neurons, and the infection of these areas following PAmp virus injection, it is assumed that TIP39 serves as a neuromodulator in the transmission of noise to the hypothalamus.

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ATTILA KÓFALVI (2004)

## Role of nicotinic acetylcholine and cannabinoid receptors in the regulation of neurotransmitter release in the central nervous system

*Supervisor: Dr. Beáta Sperlágh*

Last decade the lipid messengers got in the lime-light, which is partly due to the research of the cannabinergic system. The nicotinic acetylcholine receptors have drawn increased attention because of smoking and the Alzheimer's disease as well. The most conspicuous effects of cannabinoid intake both in animals and humans are the hampered cognitive and motor functions. Thus the objects of our investigations were the rodent and human hippocampus, and the rat caudate-putamen. We have shown that the expression and function of the CB1 cannabinoid receptor is highly conservative at the cellular and subcellular level in the rodent and human hippocampus: the receptor is expressed on the axon terminals of CCK-positive interneurons. We demonstrated with direct neurochemical methods that activation of the receptor by exogenous agonists results in the same extent of inhibition of GABA release both in rat and human as well.

Cannabinoids inhibit the release of glutamate also from rodent hippocampal synaptosomes. Our results, which are based on neurochemical approaches, involving a CB1 knockout mouse strain, and immunocytochemistry, however, clearly authenticated that it is not CB1 receptor that mediates presynaptic inhibition onto glutamatergic terminals. This subserves the theory of the existence of a new central G-protein coupled cannabinoid receptor. In the rat caudate-putamen, our goal was to explore how cannabinoids affect the release of several neurotransmitters. We found that CB1 receptor activation decreases the release of GABA according to that CB1 receptor is located to medium spiny interneurons. Cannabinoids decreased the release of glutamate as in the hippocampus, whereas they didn't influence the release of dopamine.

In the hippocampal slice, nicotinic agonists didn't evoke GABA release per se. However, after their washout, a dramatic increase of the release was observed during a subsequent electrical field stimulation. The agonists enhanced the transporter-dependent release of GABA via activation of the  $\alpha 7$  nACh receptor. The effect of nicotine depended on external  $Ca^{2+}$ , of voltage-dependent  $Na^{+}$  channels and the frequency of electrical stimulation. The underlying mechanism needs further investigation. The evolutionary conservative expression and role of CB1 receptors assume important regulatory function. The effect cannabinoids and nicotine on cognitive function, and the cannabinoid effect on motor function is clearer in the light of our results.

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ATTILA LOSONCZY (2005)

## Short-term plasticity in the synapses of hippocampal interneurons

Supervisor: Dr. Zoltán Nusser

Information processing in the hippocampal neuronal network relies on precise, spatio-temporal interactions between apparently homogeneous pyramidal cells (PCs) and highly diverse GABAergic interneurons (INs). A powerful way of scaling the impact of the INs on their postsynaptic target cells, thus interfering with hippocampal network behaviours, is modulating the short-term plasticity of synapses established and received by hippocampal INs. Our goal was to reveal the cell type dependence and the underlying mechanisms of short-term plasticity in the synapses of hippocampal INs. Using a combination of electrophysiological and anatomical approaches, we tested how the short-term plasticity of excitatory postsynaptic currents (EPSCs) depends on the postsynaptic IN type in the hippocampal CA1 area. Three distinct types of short-term synaptic behaviour (facilitating, depressing, and combined facilitating-depressing) were defined by fitting a dynamic neurotransmission model to the data. The short-term plasticity of EPSCs was cell type dependent, but with significant heterogeneity within all three studied IN populations (O-LM, O-Bi, and basket cells). We tested the contribution of the activation of presynaptic metabotropic glutamate receptors (mGluR) to the short-term plasticity of glutamatergic inputs to hippocampal GABAergic INs. Our results demonstrate that persistently active mGluRs reduce the EPSCs in vitro recorded from some hippocampal INs. The magnitude of the reduction of EPSCs was cell-type specific; excitatory synaptic inputs to O-LM cells were ~7 times more affected than those to basket cells. Glutamate released by 10 stimuli at 33 Hz did not appear to increase mGluR activation further. The results obtained using mGluR agonists and antagonist point to mGluR2/3/8 being responsible for the cell-type selective, persistent regulation of glutamatergic postsynaptic responses.

We identified a novel form of GABAergic input onto hippocampal PCs. In paired recordings between cholecystinin-immunopositive, mossy fibre-associated INs and their target CA3 PCs, no postsynaptic currents could be evoked with single presynaptic action potentials or with repetitive stimulations at frequencies <25 Hz. Even at presynaptic firing rates of 100 Hz, at least twenty action potentials were needed to evoke postsynaptic currents in PCs. Cannabinoid receptor antagonists drastically increased synaptic transmission through this connection, converting the 'mute' synapses into high-fidelity ones. Our results reveal that the synapses of some hippocampal interneurons are specialised to function as use-dependent high-pass filters, allowing only high presynaptic firing frequencies to be transmitted to the postsynaptic cells. This special synaptic operation is achieved by tonic cannabinoid receptor activation in a presynaptic input specific manner.

Our results show that the short-term plasticity is highly regulated by tonically active presynaptic metabotropic receptors in the synapses of hippocampal INs in a cell type specific manner. Consequently, the cell type specificity of short-term plasticity may contribute to the differential functional role of distinct interneurons in the hippocampus.

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SZILVIA MEZEY (2004)

## Brain regions and pathways involved in learning in the domestic chick (*Gallus domesticus*)

Supervisor: Dr. András Csillag

Changes in nicotinic and muscarinic cholinergic receptors in 15 forebrain regions 30 minutes after one-trial passive avoidance training were studied in day-old chicks, by quantitative receptor autoradiography. Nicotinic receptors bound significantly more [<sup>3</sup>H]- $\alpha$ -bungarotoxin bilaterally in the lobus parolfactorius (LPO), while muscarinic receptors bound significantly less [<sup>3</sup>H]-quinuclidinyl benzilate bilaterally in the hippocampus, hyperstriatum ventrale, LPO and posterolateral telencephalon, pars dorsalis. The data support an involvement of cholinergic receptor types in the neural mechanisms underlying passive avoidance learning.

The tracer fast blue (FB) was injected into the ventral tegmental area (AVT) or substantia nigra (SN) of week-old domestic chicks and the position of retrogradely labelled neurons was mapped in striatal subregions. In a double retrograde labelling experiment using FB and red microspheres we established whether striatal neurons projecting to the AVT and/or SN are the same cells. The anterograde tracer biotinylated dextran amine was used to confirm the results of retrograde tracing. The neurons projecting to the SN or AVT considerably overlap in the viscerolimbic parts of the striatum and in the ventral paleostriatum. Exclusive striatonigral afferents arise from the paleostriatum augmentatum and paleostriatum primitivum. Of all labelled striatal neurons, 0.22% were double labelled from both the AVT and SN. Thus, the AVT and SN are innervated from distinct and partially overlapping subregions of the striatum. At the cellular level, however, striatonigral and striato-ventrosegmental neurons represent separate neuronal populations, even in overlapping regions. Given the arrangement of striato-ventrosegmental neurons, the nucl. accumbens probably does not have a distinct boundary with the LPO but extends into the anatomically defined LPO, colocalizing with medial striatal neurons.

We also investigated the morphology and connectivity of striato-ventrosegmental neurons in the medial LPO. Neurons in the medial LPO labelled by FB from the AVT were targeted and injected with lucifer yellow (LY) in fixed slices. The fluorescent label in the filled neurons was then photoconverted, and the ultrastructure of cells was investigated. The soma of striato-ventrosegmental neurons is rich in organelles and they possess a large and slightly eccentric nucleus. The LY-labelled cells possess relatively few sparsely spiny dendrites. Axospinous synapses on the labelled cells are asymmetric and correspond morphologically to glutamatergic excitatory type of terminals. Both symmetric and asymmetric axodendritic and axosomatic synapses were detected. Some symmetric synapses were GABA immunolabelled, whereas some asymmetric synapses were immunopositive to glutamate. Axon collaterals of labelled cells formed symmetric or asymmetric axodendritic synapses. Direct contact without interposing glial processes was observed between one of the FB-labelled neurons and an adjacent neuronal soma. There was also a microneuron attached to one of the labelled cells, which we identified as a neurogliaform 'dwarf' cell.

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**GÁBOR NYÍRI (2004)**

## **Quantitative analysis of GABA and glutamate receptors in the synapses of hippocampal interneurons**

*Supervisor: Dr. Tamás Freund*

Hippocampal glutamatergic pyramidal cells receive input from several types of GABA (gamma-amino-butyric acid) -releasing interneurons (IN-s) and innervate them reciprocally. Our goal was to characterise the receptor content that plays a role in the synaptic interactions of these neurons.

Pyramidal cells expressing different GABAA receptors receive input on their axon initial segment from axo-axonic cells and on their soma from two kinds of basket cells, containing either parvalbumin (PV), or cholecystokinin (CCK). Using a quantitative electron microscopic immunogold technique, we proved that the synapses formed by the two types of basket cell show a difference in the subunit composition of GABAA receptors. We found that synapses made by PV positive basket cells showed five times less immunoreactivity for the  $\alpha 2$ -subunit than synapses made by PV negative (CCK positive) cells. This difference is due to specific GABAA receptor  $\alpha$ -subunit composition, because neither synaptic size, nor total receptor content was different in these two synapse populations. Synapses established by axo-axonic cells showed an intermediate immunoreactivity but the highest density of the  $\alpha 2$ -subunit in their efferent synapses.

Glutamatergic activation of IN-s involve N-methyl-D-aspartate (NMDA) type glutamate receptors expressed in type I synapses, mostly on their dendritic shafts. To compare the NMDA receptor content of synapses, we analysed four populations of synapse. Synapses i) on spines of pyramidal cells; ii) on PV positive IN dendritic shafts in str. radiatum; iii) on randomly found IN dendritic shafts in str. oriens and iv) on somatostatin-positive IN-al dendritic shafts and somata in str. oriens. The four populations of synapses significantly differed in labelling for the NR1 subunit; pyramidal cell spines having the highest and PV positive dendrites in str. radiatum the lowest immunoreactivity. In str. oriens, IN segments had a high variability of synaptic receptor content.

These results show that synaptic GABA and glutamate receptor content is highly regulated in synapses established and received by hippocampal interneurons. Consequently, the synapse specific receptor expression contributes to the differential action of distinct interneurons and to the precise timing of network operations in the hippocampus.

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**TAMÁS SEBESTÉNY (2004)**

## **The terminals of optic fibres in the primary centres of avian visual system**

*Supervisor: Dr. Teréz Tömböl*

Birds process visual information by means of two visual pathways, the tectofugal and thalamofugal pathways. The primary centres of the thalamofugal and tectofugal pathways are the nucleus geniculatus lateralis dorsalis thalami (GLd) and the tectum opticum (TO). Behavioural and physiological studies point to a dominant role for the GLd and the tectum opticum in visual discrimination. We attempted to map the arborisation of the optic fibres of the primary centres, which have not been

completely elucidated to date. Golgi impregnation, GABA-immunogold methods and anterograde: phaseolus vulgaris leucoagglutinin (PhL-A), dextran-amin (BDA) and retrograde tracing horseradish-peroxidase (HRP), were applied, and the results analysed under the light- and electron microscope (EM). In Golgi preparations of the chicken GLd, various types of cells and fibres were studied. Two groups of neurons were found: projection neurons with long axons and interneurons with locally branching axons. Medium-sized cells with curving spiny dendrites (prn1) of projection neurons in anterolaterally area of the nucleus were investigated. The HRP-filled projection neurons were investigated using EM. The interneurons were GABA-positive. In the GLd, Golgi-impregnated thick, afferent optic fibres developed their terminal branchings among the neurons. The terminal pattern of these fibres resembled the optic terminals in the LGB of the mammalian brain. Layer 7 is one of the avian TO layers that receives retinal input. Golgi impregnation was used to investigate the afferent and connections of layer 7 in both the light- and EM, together with GABA-immunogold. The radial neurons studied emit dendritic side-branches within layer 7, which interdigitate with the arborisations of the optic fibres and have contact with them. In the EM preparations, asymmetrical synapses of large, PhL-A and BDA- labelled optic terminals located on dendritic profiles were observed that we think are the dendritic profiles side-branches of radialneurons. The retinal input to layer 7 may be able to modulate the transmission of information to the visual thalamus by way of a feedback loop to layers 4-5 of the TO involving the nucleus isthmi pars magnocellularis.

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**PATRÍCIA VARJÚ (2003)**

## **Glutamic acid and GABA in the early stages of neurogenesis: studies in the course of in vitro neural cell fate decision**

*Supervisor: Dr. Emília Madarász*

In the adult nervous system glutamic acid and GABA are mediators of synaptic communication. During embryonic development these neurotransmitters regulate mitotic division of neural progenitors and influence migration, neurite elongation and synapse formation. Continuously proliferating cell lines capable to differentiate into neural cells could serve as in vitro models for studies on neurogenesis by early embryonic neuroectoderm. Previous works from our laboratory demonstrated that immortalized neuroepithelial cell lines could be established from the anterior vesicles of 9-day-old p53<sup>-/-</sup> mouse embryos. The NE-7C2 cell line was one of the isolated p53<sup>-/-</sup> neuroectodermal clones. NE-7C2 cells represent neuroectodermal progenitors in an early developmental stage. All-trans retinoic acid treatment of NE-7C2 cells elicited the establishment first the neuronal and then the astroglial features during well-characterized developmental phases. The aim of my work was to determine the time-schedule of the appearance of ionotropic glutamate receptor subunit proteins and the fully active forms of these receptors in the course of in vitro neuron formation and to study the expression pattern of embryonic and adult forms of glutamic acid decarboxylases (GADs) during the establishment of neuronal phenotype. Molecular biological and biochemical methods were used to describe the expression pattern of the AMPA and NMDA type glutamate receptors. The possible roles of these receptors were investigated by Ca<sup>2+</sup>-imaging techniques during the well-characterized stages of in vitro neurogenesis. The time-schedule of the expression of embryonic and adult forms of GADs were also analyzed and some possible functions of the protein isoforms have been suggested. The distribution of cells containing glutamate receptor subunit proteins or GABA was analyzed by immunocytochemical methods. The results demonstrate that the activation of ionotropic glutamate receptors can influence the neuronal cell fate determina-

tion. AMPA-gated channel activation may alter the mitotic activity of progenitors and the network formation of young neurons, while NMDA receptor functioning gets role in later phases of neuronal cell differentiation. The increased responsiveness to glutamate indicates that both AMPA and NMDA receptor mediated activities can influence the process elongation and network formation by maturing neurons. The embryonic forms of GAD67 enzyme could play a role in early steps of neuron formation while the full-length forms were detected at the period of neuronal maturation.

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LUCIA WITTNER (2004)

## Hippocampal interneurons in human temporal lobe epilepsy: differential changes of perisomatic and dendritic inhibition

Supervisor: Dr. Tamás Freund

The morphological changes of hippocampal interneurons in human temporal lobe epilepsy have been investigated. Epilepsy is thought to be related to a changed balance between excitation and inhibition. The sprouting of mossy fibers and the supramammillary afferent pathway provide an excess excitation to the dentate gyrus, whereas the CA1 region receives an enhanced excitatory input from the CA3 region and from the sprouting of CA1 pyramidal cell axons. It is still not clear whether inhibition is decreased or preserved in the human epileptic hippocampus. There is evidence for interneuronal cell death, as well as for the preservation of GAD-positive cells. It has been reported in a rat model of epilepsy that dendritic, but not perisomatic inhibition is decreased in epilepsy. To examine the fate of perisomatic and dendritic inhibitory cells in human temporal lobe epilepsy functionally and neurochemically different interneuron types were studied in control and epileptic hippocampi. Dendritic inhibitory cells are thought to control the efficacy and plasticity of input of principal cells, whereas perisomatic inhibitory neurons control the output of principal cells. Our results suggest that an intense synaptic reorganisation takes place in the human epileptic dentate gyrus and CA1 region, even in the non-sclerotic tissue, before the death of considerable numbers of pyramidal cells. The examined calbindin-immunoreactive dendritic inhibitory interneuron type survives in large number, and participates in this reorganisation, it shows plastic changes in response to epilepsy, whereas other dendritic inhibitory cell types are vulnerable to epileptic injury. In the dentate gyrus, parvalbumin-immunostained perisomatic inhibitory interneurons hyperinnervate granule cells in epileptic patients, and may participate in the induction or maintenance of hypersynchronous population events. The lack of profound changes in perisomatic inhibitory input in the CA1 region suggests that other factors are likely to account for the selective vulnerability of pyramidal cells to epileptic injury. Future strategies considering the possibility of both hyperexcitation and hyperinhibition might result in the development of new therapeutical approaches to prevent epileptic seizures.

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*Sub-programs*

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Asymmetry of brain structures involved in the control of gonadal, functions	Ida GERENDAI
Neural and local control of testicular functions.	Ida GERENDAI
Long-term effects traumatic stress on behaviour and endocrine responses	József HALLER
Fast, non-genomic effects of glucocorticoids on aggressive behaviour	József HALLER
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The neural background of glucocorticoid-related abnormal forms of aggressive behavior	József HALLER
Neuroendocrine and autonomic responses to stress	Krisztina KOVÁCS
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Role of PACAP in the regulation of the gonadotropic hormone secretion	Katalin KÖVES
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cAMP and regulation of prolactin secretion	György M. NAGY
The role of new hypothalamic signal transduction mechanisms in the function of the pituitary gland	György M. NAGY
The role of prolactin in the regulation of endocrine-immune interactions	György M. NAGY
Effect of prenatal stress on the postnatal development of the neuroendocrine system	Ágnes NEMESKÉRI

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**PÉTER BANCZEROWSKI (2003)****Effect of extrahypothalamic structures on testicular functions, with special emphasis on asymmetry**

*Supervisor: Dr. Ida Gerendai*

The aim of our studies was to investigate the involvement of extrahypothalamic structures in the control of testicular functions, with special emphasis on the effect of right- and left-sided structures. We performed lesion of the insular cortex, and the amygdala, interrupted part of nerve fibers to and from the insular cortex, and cut the major commissural pathway of the brain the corpus callosum in adult male rats and studied the effect of the interventions on testicular steroidogenesis, serum testosterone and gonadotrop hormone concentrations. Following lesion of the insular cortex on the right side serum testosterone level and steroidogenesis of the testes decreased (in the case of the left testis the difference was significant). Similar lesion on the left side did not change the parameters studied. Both right- and left-sided lesion induced a significant increase in serum LH concentration. The effect was more pronounced after right-sided lesion. Interruption of nerve fibers above the amygdala by a paramedian sagittal knife cut on the right or on the left side resulted in opposite effect on testicular steroidogenesis: right-sided intervention increased while left-sided one reduced testosterone secre-

tion. Only left-sided cut influenced (decreased) serum testosterone level. Both right- and left-sided lesion of the amygdala induced a significant decrease in basal testosterone secretion in vitro of both testes and in serum testosterone level. However, serum LH concentration decreased only after left-sided surgery. Interruption of the corpus callosum in animals with left-sided orchidectomy induced a significant rise in steroidogenesis of the remaining (right) testis. Both sham surgery and callosotomy combined with left orchidectomy resulted in a significant increase in serum FSH level. Results of our studies suggest that extrahypothalamic structures and interventions influence endocrine functions of the testis through the hypothalamo-hypophyseal-testicular axis and by a direct neural route. Certain components of the regulatory system exhibit functional asymmetry.

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## IBOLYA BODNÁR (2003)

# Role of hypothalamic structures and factors in the regulation of prolactin secretion

*Supervisor: Dr. György Nagy*

Prolactin (PRL) is a polypeptide hormone that is synthesised in and secreted from the mammatropes of the anterior pituitary gland. In mammals PRL plays an essential role in the initiation and maintenance of lactation. The mediobasal hypothalamic dopaminergic system is the main physiological regulator of PRL secretion, however in the last few years several prolactin releasing factors have been postulated. Significant part of these factors is synthesised in the hypothalamic paraventricular nucleus. Suckling stimulus of pups is the most effective physiological stimulus of PRL secretion. The information on the brain structures involved in this reflex is fairly limited. The aims of the studies presented in this dissertation were to reveal 1. which brain structures are involved in the mediation of the suckling stimulus-induced release of PRL, 2. the site and mechanism of the PRL releasing effect of pituitary adenylat cyclase-activating polypeptide (PACAP), 3. the role of the hypothalamic dopaminergic and L-DOPAergic neurons in the control of PRL and alpha-MSH. In summary, we have demonstrated that; (a) the connections of the brain stem with the hypothalamus are required for the expression of the suckling-induced release of PRL; (b) the medial parvocellular subdivision of the hypothalamic paraventricular nucleus plays an important role in the mediation of PRL response to the suckling stimulus and is essential for the transfer of the neural signal of this stimulus, (c) serotonergic innervation of the paraventricular nucleus is involved in the mediation of the neural impulses of the neuroendocrine reflex from the brainstem to the hypothalamus; (d) neonatal treatment with monosodium glutamate primarily affects LDOPAergic neurons located in the ventrolateral part of the tuberoinfundibular dopaminergic system, and these L-DOPAergic nerve cells are not involved in the regulation of basal PRL secretion; (e) the site of action of PACAP inducing PRL release is not the anterior lobe of the pituitary gland, and alpha-MSH, secreted in the intermediate lobe is probable involved in the mediation of the PRL releasing effect of PACAP.

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**Hajnalka Bokor (2003)**

## Connection of the nucleus reuniens thalami and the supramammillary nuclei to the septo-hippocampal system

*Supervisor: Dr. József Kiss*

The septo-hippocampal system is one of the most important player- in addition to several other cortical and subcortical nuclei, which takes part in memory formation. Our goal was to identify and characterize its connections with two diencephalic nuclei, the nucleus reuniens thalami (RE) and the supramammillary nucleus of the hypothalamus (SUM). By using the transmitter-selective retrograde tracer [<sup>3</sup>H]D-aspartate, combined with immunocytochemistry we show that the RE innervates both the hippocampus and medial septum via an aspartate/glutamatergic pathway. Moreover, the two projections derive from two spatially separated populations of projection neurons. Similarly, the SUM also innervates both elements of the septo-hippocampal system with aspartate/glutamate fibers. While the hippocampus receives afferent input exclusively from the lateral part of the SUM, the septum is innervated by fibers originating in the medial part of SUM and in other neighboring hypothalamic areas. In parallel, most septal nuclei, the lateral part of the habenula, several hypothalamic nuclei and the raphe nuclei also project to the SUM via an aspartate/glutamatergic pathway. Thus, the SUM is located in a central position to integrate inputs from endocrine, extrapyramidal and lower brainstem centers. Finally, we uncovered that the SUM and the RE are also connected by calretinin-immunoreactive aspartate/glutamatergic axons. Taken together, we suggest that the RE and the SUM may play an important role in the modulation of the septo-hippocampal system via their extensive connections.

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**CSABA FEKETE (2003)**

## Central regulation of hypothalamic-pituitary-thyroid axis in fasting animals

*Supervisor: Dr. Zsolt Liposits*

The hypothalamic-pituitary-thyroid (HPT) axis regulated by the hypophysiotropic thyrotropin-releasing hormone-synthesizing neurons plays important role in the control of energy homeostasis. During fasting, the falling levels of leptin centrally inhibit the HPT axis via the hypothalamic arcuate nucleus. We studied the role of the anorexigenic  $\alpha$ -melanocyte-stimulating hormone- ( $\alpha$ -MSH) / cocaine-and amphetamine-regulated transcript- (CART) containing neurons and the orexigenic, neuropeptide Y- (NPY) / agouti-related protein- (AGRP) containing neurons of the arcuate nucleus in the regulation of HPT axis in fasting animals.

Our light- and electron microscopical studies provided evidences that  $\alpha$ -MSH- and CART-containing neurons of the arcuate nucleus innervate the hypophysiotropic TRH neurons. Administration of these peptides into the lateral cerebral ventricle prevented the fasting induced inhibition of the TRH-synthesis in the hypophysiotropic neurons. Furthermore, we have demonstrated that a population of TRH-producing neurons in the PVN receives dual innervation by fibers containing  $\alpha$ -MSH and AGRP, the endogen antagonist of  $\alpha$ -MSH. We have also revealed that central administration of NPY results in inhibition of HPT axis reminiscent of the effect of fasting on the thyroid axis.

In summary, our results provide evidence that the  $\alpha$ -MSH/CART- and NPY/AGRP-containing neurons of the arcuate nucleus play a key role in the regulation of HPT axis in fasting animals.

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## JÓZSEF HALÁSZ (2004)

# The effect of glucocorticoid hypofunction on aggression and related disorders - behavioural, neuronal and autonomic changes

Supervisor: Dr. József Haller

Pathological aggressive behaviour is associated with consistently low basal glucocorticoid levels and lower reactivity during environmental challenges (glucocorticoid hypofunction) in different human populations. Male Wistar rats were used to examine the possible casual relationship between aggression and low glucocorticoid levels. In addition, the underlying neuronal network was investigated. Experimentally induced glucocorticoid hypofunction changed aggressive behaviour dramatically as animals oriented their biting attacks toward vulnerable body parts of their opponents without proper signalling. This behavioural pattern is extremely rare in controls. c-Fos protein immunocytochemistry was used to clarify neuronal activation patterns during this behaviour. In controls, aggressive behaviour activated the medial amygdala, the hypothalamic attack area and the periaqueductal grey matter. Similar activation was noticed in models of pathological aggression (both in glucocorticoid hypofunction and in aggression evoked by electrical stimulation of the attack area). However, nuclei connected with the stress response (paraventricular nucleus) and anxiety (central amygdala) showed a glucocorticoid dependent activation. Glucocorticoid hypofunction induced anxiety in social context only, and this effect was accompanied by a change in the anxiolytic effect of the 5-HT<sub>1A</sub> receptor partial agonist compound buspirone. Heart rate reactivity was also monitored by a radiotelemetric system during glucocorticoid hypofunction-induced pathological behaviour, and similar to human pathological aggression, a significant decrease occurred during social challenges compared with controls.

As a conclusion, it can be stated that consistently low and non-reactive glucocorticoid levels constitute a link between pathological aggression, anxiety and change in autonomic reactivity. The strong activation in the areas involved in the control of anxiety may induce pathological behaviour, and this abnormal activation can influence the function of areas directly connected with the central regulation in aggressive behaviour.

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ILDIKÓ MIKLÓS (2004)

## Gabaergic and histaminergic regulation of the corticotropin-releasing hormone secreting neurons of the hypothalamic paraventricular nucleus

Supervisor: Dr. Krisztina Kovács

Stress-related afferent inputs converge on the corticotropin-releasing hormone (CRH)- synthesizing neurons in the medial parvocellular subdivision of the hypothalamic paraventricular nucleus (PVH) to initiate the neuroendocrine stress cascade by releasing CRH into the hypophyseal portal vasculature that stimulates adrenocorticotropin-mediated release of corticosteroids from the adrenal cortex. Numerous pharmacological studies confirm the regulatory role of GABAergic and histaminergic systems, however the synaptic interaction between these two system and the CRH-positive hypophyseotropic neurons has not been exactly demonstrated yet. Our aims were to characterize this GABAergic and histaminergic inputs to the CRH neurons and study their functional impact in the regulation of acute and chronic challenges.

Using combination of pre-embedding immunostaining and post embedding immunogold methods direct synaptic contacts were identified between GABA-containing terminals and CRH neurons. The majority of axo-dendritic synapses were symmetric inhibitory type. Using the stereologic disector method it was revealed that 78.8% of the GABAergic synapses terminated in CRH-positive neurons in control, colchicine-treated rats. Following adrenalectomy and chronic stress (both increase the synthetic and secretory activity of CRH neurons) the total number of synapses, total GABAergic synapses and those GABAergic synapses that terminated in CRH-positive profiles was significantly increased.

In the second part of the study histaminergic axons were revealed by pre-embedding immunocytochemistry, that rarely formed asymmetric axo-dendritic and axo-axonic type synapses in the dorso-medial parvocellular region of the PVH. Synaptic contacts between histaminergic fibers and CRH secretory neurons were not detected. To assess the functional impact of histaminergic influences on the stress-related neurosecretory neurons we combined the immediate early gene (IEG) member c-Fos in histamine synthesizing neurons. Only restraint, footshock and hypoglycemic stress induced significant c-Fos immunoreactivity in HDC mRNA expressing histaminergic neurons.

In summary, our data provided the first direct evidence for synaptic contact between GABA and CRH neurons in the PVH. We also revealed the functional impact of the GABAergic and histaminergic system in the regulation of chronic and acute stress responses. Our ultrastructural data support the direct synaptic control of CRH neurons by GABAergic inputs and favors the neuromodulatory function of histaminergic system on the regulation of hypothalamic stress-related neuron population.

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E. BÉLA TÓTH (2003)

## Effect of neuroendocrine regulatory mechanisms and endocrine factors on the cells of the immune system

Supervisor: Dr. György Nagy

The natural compound salsolinol (SAL) was detected within the *hypothalamo-hypophyseal system*. Its direct effect is the elevation of prolactin (PRL) levels and the inhibition of the synthesis of dopamine (DA) that causes the release of pituitary PRL. Specific receptor binding sites differs from the known D2 receptors are hypothesised in the pituitary and in the hypothalamus. Prolactin releasing effect of SAL is demonstrated in pituitary cells as well. It is proposed that SAL acts through the phosphorylation of AADC, changing the decarboxylation process of L-DOPA, in concert with the effects on DA-transporters and the vesicular monoamine transporters. This description of the hypothalamic „*prolactin releasing factor*” may lead us to an original and new approach in aspects of the physiology and pharmacological regulation of other dopaminergic systems within the central nervous system. The importance of our finding is a better understanding of the regulation of dopaminergic system, and the mechanism of tonic dopamine release, as well as the characterisation of changes occurs in dopaminergic terminals during the rapid and transient release of PRL.

The immunological aspects of our results concerned of SAL, are: (i) the changes in DA signalling acting through PRL release may modulate the response of the immune system; (ii) the theory of the “pre- and postsynaptic” DA/SAL effect may provide additional details in understanding or in treatment of pathologic immune responses. The receptors of DA coupled with the Gi and Gs proteins, resulting in changes in levels of cAMP and Ca<sup>2+</sup> can modulate the mitogen/PRL induced multiple intracellular signalling pathways. It is also hypothesised, that the autocrine /paracrine balance between DA/SAL/PRL is also important in development of the proper immune response.

The role of PRL is considered as a permissive/co-mitogen factor within the immune system, however more attention should be given also in some of the pathological conditions. The immune-stimulatory effect of milk is parallel with the amount of milk-PRL. That effects the overall responsiveness of immune system and the development of the hypothalamo-hypophyseal system in neonate. Milk also contains an anti-proliferative factor that increases in concentration by the end of lactation. Its role as immune-modulator for the neonate may influence the mother as well. The amount of the milk-derived inhibitors modulates the effect of mitogens, stopping the cell cycle in S-phase, but without any sign of direct toxicity.

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BALÁZS RADNAI (2005)

## Salsolinol: A new regulator of prolactin secretion and the possible mechanism of action

Supervisor: Dr. György Nagy

Prolactin is a polypeptide hormone that is synthesized in and secreted from mammatropes of the anterior lobe of the pituitary gland. This hormone not only sub serves multiple roles during reproduction but it also plays an essential role in the general homeostasis of the organism. The secretion of prolactin from mammatropes is under a dominant inhibitory control that originates in the me-

dial-basal hypothalamus. The bulk of previous and recent evidence support the view that dopamine is the neurohormone which is the physiological prolactin-inhibiting hormone. Dopamine is delivered to the anterior lobe through the vascular connection between the hypothalamus and the pituitary gland and maintains mammatropes in their tonically suppressed secretory state. In mammals, however, several exteroceptive stimuli such as suckling of the nipples of lactating mothers by their litters sharply elevate plasma prolactin. These stimuli may act by decreasing the inhibitory influence of hypothalamic dopamine and/or enhancing the activity of unknown hypothalamic neurons that secrete a prolactin-releasing hormone (PRF). Surgical removal or denervation of the neuro-intermediate lobe of the pituitary gland blocks or attenuates the secretory bursts of prolactin induced by different physiological stimuli including suckling. These observations have suggested strongly that a PRF may exist in the neuro-intermediate lobe. Indeed, it has been also shown that perchloric acid extracts of the neuro-intermediate lobe can stimulate the release of prolactin both in vitro and in vivo. In spite of relentless efforts to isolate and identify this PRF from neurointermediate lobe, the identity of this substance has remained elusive.

Analyzed the perchloric acid extract of the neuro-intermediate lobe and of the median eminence, a dopamine-derived compound, R-salsolinol (SAL), has been detected in high concentration that has a selective and dose-dependent prolactin-releasing activity both in vivo and in vitro. Moreover, the concentration of SAL in neuro-intermediate lobe extracts varies in parallel with the suckling-induced prolactin secretion and is markedly reduced following disruption of dopaminergic innervation of the neuro-intermediate lobe. Lack of interference of SAL with 3H-spiperone binding to AP homogenates indicates that SAL does not act at the dopamine D2 receptor. Moreover, 3H-SAL binds specifically to homogenate of AL as well as neuro-intermediate lobe obtained from lactating rats. These data clearly indicate that SAL does not act at the dopamine (DA) D2 receptors, and suggest that SAL supposedly has a binding site through which the secretion of PRL may be affected. Therefore, binding of 3H-SAL to different regions of the central nervous system (CNS) has been investigated.

Specific and saturable binding have been detected in the striatum, cortex, median eminence and in the hypothalamus as well as in the AL and the neuro-intermediate lobe (NIL) of the pituitary gland. KD values of the bindings were in the nanomolar range in all tissue tested. 3H-SAL displacing activity of several agonists and antagonists of known DA receptors have also been tested. Salsolinol may regulate DAergic neurotransmission of hypothalamic neuroendocrine dopaminergic (NEDA) system by an altered intracellular or intraterminal synthesis and/or distribution of hypophysiotropic DA. Our findings show that 10 minutes after SAL injection, parallel to the rapid and marked elevation in plasma PRL, there is a significant increase in cAMP concentration of the AL. However, we assume that this rapid increase at the level of cAMP of the AL is related to the initiation of the secretory burst of pituitary mammatropes caused by SAL.

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ÉVA VINCZE (2004)

## The presence of PACAP and other regulatory peptides in the gastric glands of different species under physiological and experimental conditions

*Supervisor: Dr. Katalin Köves*

Pituitary adenylate cyclase-activating polypeptide (PACAP) which was isolated in 1989 is a pleiotropic neuropeptide that belongs to the secretin/glucagon/VIP family. It is widely distributed in plants, animals and human organs as well. PACAP has a lot of regulatory functions as a hypothalamic hormone, neurotransmitter, neuromodulator, neurotrophic factor, as a local regulatory peptide. Its structure has been remarkably conserved during evolution, which can be related to its important physiological functions. A lot of data have been acquired in the recent years on the presence and functions of PACAP in the gastrointestinal tract: in certain conditions it can have an effect on both motility and secretion in various place of the tract including stomach.

In our work PACAP immunoreactivity has been demonstrated in human, cat and rat parietal cells of the stomach oxyntic mucosa. In human samples under electron microscope PACAP immunoreactivity was associated with the surface of the intracellular canaliculi, which is the place of H<sup>+</sup> secretion. Our observation that PACAP mRNA is present by RT-PCR in rat gastric mucosa can confirm the existence of local synthesis. PACAP could be released by cultured parietal cells when we studied by cell immunoblot assay, so it may act on neighbouring cells in a paracrine or autocrine manner. The role of PACAP in parietal cells was studied in our physiological experiments. The changes in PACAP immunoreactivity were examined in rat gastric mucosa influenced by gastric acid secretion through acute and chronic treatments and by different adrenal steroid status. A relationship could be observed between changes in gastric acid secretion or adrenal steroid status and the intensity and localization of PACAP immunoreactivity in the parietal cells. In human fetal stomach the dynamism of the appearance of PACAP and other regulatory peptides were investigated during ontogenesis using immunohistochemical approach. Our experimental results suggest the role of PACAP as a local regulator in different acidity conditions, during adaptation in stress situations and in growth of the gastric glands during ontogenesis.

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Computational approach to the functional organization of the hippocampal formation	Péter ÉRDI
Electrophysiological study of neocortical mechanisms of sensory processes	György KARMOS
Relation between neocortical gamma oscillation and perceptual processes - intracortical multielectrode study in animal models	György KARMOS
Nonconventional effects of monoamine uptake blocker-type antidepressants in the central nervous system: common properties of monoamine transporters and ligand-gated ion channels	János KISS
Analysis of cognitive evoked potentials and nonlinear features of the EEG in stroke patients	Márk MOLNÁR
The role of ATP and adenosine in the signaling of the nervous system	Beáta SPERLÁGH
Identification of new target sites in the treatment of neuropsychiatric diseases (ischemia, Parkinson's disease, drug's abuse)	Beáta SPERLÁGH
The role of GABA signaling in development of the nervous system and other organs	Gábor SZABÓ
The role of GABA signaling in embryonic development	Gábor SZABÓ
Genetic modification of different neuronal cell types and circuits in transgenic mice	Gábor SZABÓ
Investigation of generator mechanisms of evoked potentials and pathologic activity in patients with epilepsy, and in animal models	István ULBERT
Models of brain diseases established by drug-induced alterations in neural networks of the peripheral and central nervous system.	E. Szilveszter VIZI
Nonsynaptic action mechanisms of neuroactive substances involved in chemical transmission in animal and in isolated human (obtained from surgical biopsy) tissues	E. Szilveszter VIZI
Function of ion channels and ionotropic receptors as revealed by optical and electrophysiological methods	E. Szilveszter VIZI
Action mechanisms underlying the effects of monoamine uptake inhibitors	E. Szilveszter VIZI

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**HENNINGS, ESTEBAN CARLOS (2003)****Non-conventional effects of nicotonic agonists and monoamine uptake blockers in the central nervous system***Supervisor: Dr. Szilveszter Vizi E.*

Nicotinic acetylcholine receptors have been linked to several important functions of the central nervous system including memory. Their pharmacological manipulation therefore provides future methods of treatment. In our previous experiments we have shown that dimethylphenylpyperazine (DMPP) has dual effect not only acting directly on the nicotinic receptor but also on the uptake transporter. Therefore the first part of this thesis investigated if other nicotinic agonists have some effect on the uptake transporter by using [3H]noradrenaline [3H]NA release from rat hippocampal slices. Our data indicate that the majority of nicotinic agonists (nicotine, epibatidine, anatoxin A, cytisine) increase the release of [3H]NA exclusively via stimulation of nicotinic acetylcholine receptors (nAChRs). DMPP, in addition to the stimulation of nAChRs, also evokes a carrier-mediated release. Lobeline has no stimulatory effect on nAChRs, induces a carrier-mediated release and has a further action of unidentified mechanism. Our results suggest that special caution is required for the interpretation of data, when DMPP or lobeline are used as nicotinic agonists. During these studies we observed that monoamine uptake inhibitors are able to block not only the uptake but also the nAChRs. The second part of the thesis focused on this phenomenon. We found that monoamine uptake blockers behave like non-competitive ion channel blocker-type antagonists and inhibit the nAChR-mediated response with the same efficacy than the most effective nAChR antagonist mecamylamine. This effect is independent of their chemical structure and substrate selectivity and seems to be a general feature of monoamine uptake transporters. Because these compounds are widely used in therapy (and abused in the case of cocaine), our finding may have great importance in the evaluation of their clinical effects and toxicology.

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ÁKOS GERENCSÉR (2005)

## Characterization of the mitochondrial calcium homeostasis in cultured rat brain capillary endothelial cells

Supervisor: Dr. Veronika Ádám

Mitochondria are not only metabolic powerhouses of the cells, but are important in intracellular signaling, performing a range of functions from physiological regulation to pathological mechanisms. The mitochondrial  $\text{Ca}^{2+}$  handling is crucial in each of these functions. The *in situ* approach – studying mitochondria within cells – revealed cellular and subcellular heterogeneity and specialization of mitochondrial  $\text{Ca}^{2+}$  handling, describing an increasingly complex scheme. However, so far, fine temporal and spatial resolution of the mitochondrial  $\text{Ca}^{2+}$  uptake dynamics, which is essential for the understanding intrinsic properties of the *in situ* mitochondrial  $\text{Ca}^{2+}$  handling have not been available.

We have developed a digital image processing technique of wide-field fluorescence microscopy images for the measurement of the mitochondrial  $\text{Ca}^{2+}$  concentration with high selectivity, simultaneously with the cytosolic  $\text{Ca}^{2+}$  concentration using a single, conventional  $\text{Ca}^{2+}$  dye (X-rhod-1). We have described the basic properties of mitochondrial  $\text{Ca}^{2+}$  handling of rat brain capillary endothelial cells in primary cultures, indicating fast mitochondrial  $\text{Ca}^{2+}$  transients upon purinergic stimulation of intact cells, and a dominant role of the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger in the removal of mitochondrial  $\text{Ca}^{2+}$ . To reveal heterogeneity of mitochondrial function on single mitochondrion level 1.) we have studied the spatiotemporal dynamics of the mitochondrial  $\text{Ca}^{2+}$  uptake using our novel image processing technique. This enabled the analysis of the mitochondrial  $\text{Ca}^{2+}$  transients in a higher temporal and spatial resolution than those available so far. We have demonstrated first time discrete sites – intramitochondrial hotspots – of  $\text{Ca}^{2+}$  uptake following  $\text{Ca}^{2+}$  release from intracellular stores, and the spreading of  $\text{Ca}^{2+}$  rise within the mitochondria. The findings indicate that  $\text{Ca}^{2+}$  diffuses laterally within the mitochondria, but that the diffusion is limited for shorter segments of the mitochondrial network. 2.) The mitochondrial membrane potential was investigated using the potentiometric dye TMRM. Irradiation-induced fluctuations of TMRM fluorescence showed synchronicity over large regions of the mitochondrial network, indicating that certain parts of this network are equipotential, forming electrical syncytia. Our data suggest that mitochondria form syncytia of electrical conductance while the passage of  $\text{Ca}^{2+}$  is restricted to the individual organelle.

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ZSUZSANNA PINCZE (2005)

## Investigation of auditory event-related potential features of preattentive cognitive processes on animal models

*Supervisor: Dr. György Karmos*

Due to their excellent temporal resolution the event-related potentials (ERP) accurately represent the rapid cognitive processes. ERP components reflecting these processes can be recorded also on animals, therefore in an adequately developed animal model the neural mechanisms underlying these components can be studied by invasive methods. In our experiments we investigated the location of generation, characteristics, and topographic relations of ERP components (N1 and mismatch negativity: MMN) related to the acoustic automatic (preattentive) information processing on animal models (cat, Rhesus macacus monkey) by the ERP technique (on the auditory cortex of cat, and on the surface of the brain of monkey).

At first, to find a well-defined starting point for our topographic studies we showed the frequency-dependent distribution of the early cortical component (P1) on 6 cats: the 1 kHz tone was represented usually on the transition area of AI and AII, or close to this area, while the 16 kHz showed representation on area corresponded to the AI. The negative ERP component in the latency range of 40-60 ms showed frequency dependent amplitude distribution on the auditory cortex similar to that of the P1. Moreover, we showed the intensity and ISI dependence of this component, therefore it can be concluded that this negativity corresponds to the supratemporal component of the human N1 wave. Its topography indicates that it is generated (at least) in part in the AI area. By varying the parameters of the oddball paradigm we showed similar changes of the MMN recorded from the auditory cortex of cat to those of the human MMN. The amplitude maximum of the MMN appeared always apart from those of the P1/N1, i.e. in the rostroventral part of the middle ectosylvian gyrus which corresponded to the AII area. On monkey two negative waves (at 39 and 85 ms) can be recorded: the N39 appeared on the temporal region and did not show frequency dependent distribution, while the amplitude maximum of the N85 was in the midline of the frontal region. It showed frequency dependence, therefore this negativity corresponds to the human N1. The probability dependence of the MMN recorded from monkey can be shown similarly to that of the cat. Maximum of the N85 was slightly more anterior related to that of the MMN, that indicates the different equivalent dipoles of the two components.

In summary, both the characteristics and topography of the N1 and MMN were similar to those of the human components therefore both the cat and the monkey can serve as an adequate model of the human N1 wave and frequency MMN.

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ERNŐ SÁNTHA (2003)

## The function of carrier proteins in the release of chemical neurotransmitters

*Supervisor: Dr. Szilveszter Vizi E.*

The focus of our investigations, that based this thesis, was to find out and characterize the function of carrier proteins located in the plasma membrane. The normal and pathophysiological functions of the carriers were studied and modeled in the central and autonomic nervous system. In details, one of the major objects of the present work was the analysis of hippocampal neural interactions with spe-

cial respect to the actions of selective agonists and antagonists on the neural nicotinic receptors to release serotonin from raphe-hippocampal axons.

Among the nicotinic agonists used in our experiments dimethyl-phenyl-piperazinium (DMPP) and lobeline were found to be able to release serotonin from hippocampal slices in an external  $\text{Ca}^{2+}$ -insensitive manner. The later observation prompted us to investigate the possible involvement of other transmitter releasing mechanisms different from the classical nicotinic receptor activation. At low temperature we observed opposite modulations: while the effect of DMPP was substantially potentiated by cold the action of lobeline was completely inhibited at 7.°C. In conclusion, here was described the heterogeneity of nicotinic receptor mediated serotonin releasing mechanisms in the hippocampus that may be linked to the activity of membrane transporter mechanisms, and the actual state of membrane ion channels.

Because of the surprising nature of lobeline-induced effects, we further studied the multiple effect of lobeline in various nervous tissues. We have tried to understand the cellular mechanisms underlying the complex effects of lobeline on [ $^3\text{H}$ ]norepinephrine ([ $^3\text{H}$ ]NE) release in guinea-pig was deferred. Lobeline increased the basal release of [ $^3\text{H}$ ]NE. In contrast, electrical stimulation-evoked release was [ $\text{Ca}^{2+}$ ] $_0$ -independent, insensitive to mecamylamine, a nicotinic acetylcholine receptor antagonist, and to desipramine, a noradrenaline uptake inhibitor. However, lobeline-induced release was temperature-dependent: at low temperature (12.°C), when the membrane carrier proteins are inhibited, lobeline failed to increase the basal release. Lobeline dose-dependently inhibited the uptake of [ $^3\text{H}$ ]NE into rat hippocampal synaptic vesicles and purified synaptosomes. Furthermore, lobeline inhibited cellular  $\text{Ca}^{2+}$ -influx both pre- and postjunctional side. Our data can be best explained as a reversal of the monoamine uptake by lobeline that is facilitated by the increased intracellular NE level after lobeline blocks vesicular uptake. Furthermore lobeline acts as a non-selective  $\text{Ca}^{2+}$  channel antagonist blocking pre- and postjunctional  $\text{Ca}^{2+}$  channels.

The target of our further experiments was to find out membrane carrier mediated mechanisms used by 1-phenylephrine (1-PE) an  $\alpha_1$ -adrenoceptor agonist. 1PE (10, 100 $\mu\text{M}$ ) enhanced both the basal and the stimulation-evoked release of tritium from guinea-pig was deferred. Both effects of 1-PE on basal release was independent on extracellular  $\text{Ca}^{2+}$  concentration [ $\text{Ca}^{2+}$ ] $_0$  and  $\alpha_1$ -adrenoceptor blockade. These results suggest that a non-receptorial and direct carrier-mediated mechanism were involved in NE releasing effect of 1-PE.

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ILDIKÓ SIPOS (2003)

## Nucleotide receptor characterization on primary rat brain capillary endothelial cells and formation of reactive oxygen species in situ mitochondria of neuronal origin

Supervisor: Dr. Veronika Ádám

We adapted and modified the rat brain capillary endothelial cell (RBCEC) culturing method, originally described by Abbott et al. (1992), to the requirements of fluorescence measurement techniques. The biological extracellular matrix (ECM) –coated glass coverslips have much less background fluorescence than the commonly used collagen-coated plastic surfaces. Moreover, RBCE cells grown on ECM produced by corneal endothelial cells grow more rapidly and uniformly to confluence, and show a more uniform spindle-shaped cell morphology characteristic of differentiated brain endothelial cells. We demonstrated pharmacologically the presence of P2Y<sub>2</sub> and P2Y<sub>1</sub>-like nucleotide receptors in RBCE cells, and in addition the presence of an A1-type adenosine receptor. The agonist-in-

duced  $[Ca^{2+}]_i$  changes were significantly higher when cells were grown on ECM, than in identical culture conditions without ECM, which highlights the role of ECM in the regulation of pharmacological phenotype of brain endothelial cells. Reactive oxygen species (ROS) generated in the respiratory chain was measured and the quantitative relationship between inhibition of the respiratory chain complexes and ROS formation was investigated in isolated nerve terminals. Inactivation of complex I to a small extent ( $16 \pm 2\%$ ) resulted in a significant increase in ROS formation, while 71% threshold inhibition were detected for complex III and IV, above that substantial aconitase inactivation was observed, indicating an enhanced ROS generation. This was confirmed by direct detection of  $H_2O_2$  under similar conditions. Dissipation of  $\Delta\psi_m$  by FCCP or DNP had no effect on the  $H_2O_2$  production induced by rotenone or antimycin. When antimycin was applied together with oligomycin,  $\Delta\psi_m$  was totally dissipated, but the ROS production decreased only by 15%. In the presence of rotenone, antimycin remained capable of inducing an enhanced ROS formation, though to a smaller extent than without inhibition of complex I. These experiments suggest that in *in situ* mitochondria of synaptosomes when complex I is completely inhibited electrons entering the respiratory chain distal from the rotenone site could fuel ROS formation.

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## 6/4. PROGRAM

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The role of serotonin in the regulation of the central nervous system. Clinical and experimental studies in psychiatric and neurological disorders and animal models	György BAGDY
Neurotransmitter-regulation and pharmacology of absence epilepsy	György BAGDY
Neurotransmitters and receptors in the regulation of vigilance	György BAGDY
Functional consequences of MDMA (Ecstasy)-induced neuronal damage	György BAGDY
Genetics, neurochemistry and pharmacotherapy of depression. Clinical and experimental studies	György BAGDY
Advances in neuropsychopharmacology	György BAGDY
Study of arousal responses and sleep inertia in somnambul patients	Péter HALÁSZ
Relationship between fine graded elements of sleep electrical activity and cognitive functions	Péter HALÁSZ
Study of spike genesis with surface and deep electrodes	Péter HALÁSZ
Relationship of sleep, memory and temporal lobe epileptic spike activity	Péter HALÁSZ
Study of transcranial magnetic/electric stimulation as diagnostic and therapeutic tool in epilepsy	Péter HALÁSZ

West syndrome (clinical, electrophysiological and therapeutical study)	Rozália KÁLMÁNCHEY
Seizures semiological and EEG studies in children with epilepsy	Rozália KÁLMÁNCHEY
Electrophysiological studies in speech disturbances in children	Rozália KÁLMÁNCHEY
Molecular pathology of conformational neurodegenerative diseases	Gábor KOVÁCS
Neuropsychopharmacology and drug development	György LÉVAY
Studies on mechanisms of ischaemic neuronal lesions in stroke models	Zoltán NAGY
Anti-apoptosis gene therapy using adenovirus construct in stroke models	Zoltán NAGY
Studies on new neuroprotective molecules in cell culture system	Zoltán NAGY
Neuronal plasticity and GAP-43 expression	Zoltán NAGY
Stem cells and brain plasticity	Zoltán NAGY
Studies on mechanisms of ultrasound assisted increased thrombolysis	Zoltán NAGY
Studies on „active-plaque” after carotid surgery	Zoltán NAGY
Neuroprotective effect of SSRIs after stroke	Zoltán NAGY
Neuroendocrinology of brain aging: role of glucocorticoids and gonadal steroids in neurodegeneration	Csaba NYAKAS
Neuronal adjustment and plasticity: brain maldevelopment and pathological brain aging	Csaba NYAKAS

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DIMITRULA ARABADZIS (2003)

## Permanent morphological and electrophysiological alterations in the rat hippocampus one year after global forebrain ischemia

*Supervisor: Dr. Zoltán Nagy*

A short interruption of blood flow due to cardiac arrest or occlusion of a blood vessel causes ischemia, a condition resulting in the brain in complex tissue damage due to energy deficiency and the accumulation of toxic metabolites. Investigations of long-lasting consequences of these injuries are important in understanding the pathophysiological mechanisms of ischemic brain damage and in order to find compensatory mechanisms, the support of which may lead to significant increase in the level of wellness of patients. The four-vessel occlusion (4VO) global forebrain ischemia is an animal model of cardiac arrest or interrupted blood flow that might occur during bypass surgery in humans and gives an ideal tool for investigating different aspects of cerebral damage occurring in these pathological situations. In this dissertation, we investigated long-term morphological and functional consequences of ischemia in one of the most susceptible region of the brain, the hippocampus.

Two groups of animals were distinguished on the basis of the damage that four-vessel occlusion ischemia induced in the hippocampus. In the non-sclerotic ischemic (NSI) group no apparent cell loss was found, while in the sclerotic ischemic (SI) group a spiny interneuron population, involved in feedback and feed forward inhibition at the first two stages of the hippocampal trisynaptic loop, was permanently lost and no other neuron population took over their function according connectivity and neurochemical markers. The large majority of pyramidal cells (80%) in the area CA1/CA2 were also killed by global forebrain ischemia in the SI group and there was not any aberrant connection found between the CA3 area and the subiculum or the entorhinal cortex that might have compensated for the lost relay function of the area CA1. Interestingly, Schaffer collaterals and interneurons in the stratum radiatum of CA1 did not degenerate even though the majority of their postsynaptic targets were lost. The loss of inhibitory interneurons at the first two stages of the trisynaptic loop coupled with a well-preserved excitatory connectivity among subfields suggests that hyperexcitability might occur in the hippocampus even one year after the ischemic impact. However, no mossy fiber sprouting, that is hypothesized to play important role in seizure generation and maintenance, has been observed in the ischemic hippocampus. This might be one of the reasons of the low incidence of epileptiform activity following global forebrain ischemia and can be considered as a hallmark for distinguishing ischemic hippocampal sclerosis from the epilepsy induced damage.

In spite of the striking difference in neuronal survival between NSI and SI groups, similar functional changes were observed in CA1/CA2 pyramidal cells that remained one year after the ischemic insult. A decreased excitability was observed in neurons from both groups, as shown by significant prolongation of inter-spike intervals (ISI) of evoked action potentials and by increased amplitude of fast after-hyperpolarization (fAHP) of both spontaneous and evoked action potentials. This reduced excitability might be another reason for the low incidence of seizures in global forebrain ischemia. There was a considerable difference between the two groups in the number of surviving CA1/CA2 pyramidal cells and in the ratio of calbindin-positive pyramidal cells of the surviving CA1/CA2. In NSI animals, the number of surviving CA1/CA2 pyramidal cells did not differ considerably from control, but the ratio of calbindin-positive CA1/CA2 pyramidal cells decreased substantially to 33% from the 60% of the control ratio. In SI animals, only about one fifth of the CA1 pyramidal cells survive ischemia, only 8% of which are immunoreactive for calbindin. These results suggest that decreased excitability of CA1/CA2 pyramidal cells represents a protective mechanism against ischemia-induced neurodegeneration and might be related to decreased calbindin expression. Furthermore, these findings indicate that calcium buffering by calbindin does not influence susceptibility to neurodegeneration. Rather, changes in Ca<sup>2+</sup>-buffering capacity are likely to result in altered excitability and thereby might be neuroprotective if they correspond to a decreased propensity to fire high-frequency trains of APs, as demonstrated here following global forebrain ischemia.

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ZSUZSA ASZALÓS (2004)

## Budapest Stroke Databank. A 10-year follow-up of 500 stroke patients

Supervisor: Dr. Zoltán Nagy

In Hungary, the annual number of new stroke cases is about 400/100 000 inhabitants, and about 18 000 stroke patients die in a year. Among younger than aged 50 years male patients 60 / 100 000, among female patients 40 / 100 000 inhabitants die annually in the acute phase of stroke. This rate in the countries of European Union is as high as 8 – 10. At about 32-42 % of survivors would be inappropriate in the every day life. Data of five hundred consecutive, unselected acute – subacute stroke cases were analyzed on the bases of the National Institute of Neurological and Communicative Disorders and Stroke Data Bank questionnaire, and the patients were followed up to maximum 10 years. We analyzed about 1200 basic data / patient, including previous history, neurological and functional status, laboratory evidences, CT, angiographic or neuropathological examinations, occasionally repeatedly. Subtypes studied were hemorrhage, atherosclerotic stroke, lacunar stroke, carcinogenic embolia, and “others”. Among our patients the rate of risk factors and cumulative risks were very high. Smokers’ age at the first stroke was 10 years lower than it was at the nonsmokers, and the smokers’ rate was the same in the first or recurrent stroke groups. Moderate alcohol drinking had a better influence on surviving but the rate of cortical atrophy was significant higher among them than among abstainers. The principal predictors of death in the first 24 hours were location in the posterior area and hemorrhage. The further predictors for 28-day mortality were older age, higher volume of the lesion, more serious clinical picture, inside lower level of consciousness, ischemic heart disease, atrial fibrillation, higher blood glucose at the admission, lower platelet count, and the right hemispheric lesion. The outcome of strokes of the subdominant hemisphere was less favorable, and the case fatality rate was higher than in the strokes of the dominant hemisphere. Leukoaraiosis is associated with worse prognosis, and it suggests an underlying generalized vascular disorder. Post-stroke depression is expected by more sever clinical picture and higher volume of the lesion. At about 65 % of the post-stroke depression cases arrives during the hospitalization.

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ZSÓFIA CLEMENS (2005)

## Temporal lobe epilepsy and sleep: focus on interictal spikes and memory consolidation

*Supervisor: Dr. Péter Halász*

Mechanisms underlying sleep, epilepsy and memory are interrelated through several structural and functional factors. Our aim was to investigate some aspects of this interrelationship. In the first study we examined distribution of temporal lobe epilepsy spikes across different vigilance states recorded via simultaneous intracranial foramen ovale and scalp electrodes. We found that distribution of foramen ovale spikes and scalp spikes is discrepant, the former ones tend to increase during light NREM sleep, while the latter during deep NREM. The second study was aimed to investigate the influencing role of different clinical factors on spiking in temporal lobe epilepsy patients. We found that spiking rates during most states and spiking stability across vigilance states increase with epilepsy duration, while relative spiking rates during deep NREM sleep increase with age at epilepsy onset. Moreover, spiking rates during REM were higher in the presence of hippocampal sclerosis, while spiking during waking eyes closed was higher if a patient had secondarily generalised tonic clonic seizures. The third study was designed to examine whether different epilepsy syndromes interfere with memory consolidation during sleep. There was no significant difference between right temporal lobe, left temporal lobe and idiopathic generalized epilepsy groups in evening measures, but left temporal patients showed impaired overnight verbal retention and both temporal groups were impaired on overnight visual retention.

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ATTILA CSÁNYI (2003)

## Validation and application of intima-media thickness measurement method in the quantitative analysis of determinant factors of arterosclerosis

*Supervisor: Dr. Zoltán Nagy*

The aim of this study was to improve the knowledge of epidemiology and pathogenesis of arteriosclerosis. High-resolution ultrasound methods were used to assess the arteriosclerotic lesions of cervical arteries and the obtained results were compared with the risk factors, ethnicity and the biochemical markers of arteriosclerosis. Firstly, the detailed validation of the accuracy of used methodology was performed, comparing to „gold standards” of angiography and histology. Next the reproducibility of methods, the inter- and interrater variability were examined. On the basis of my results the used ultrasound methods are suited for further application.

Secondly, in a cross-sectional study the physiological yearly progression of intima-media thickness (IMT) was estimated. It was justified that the predicted yearly increase in the thickness is lower than the generally conceded value ( $[0,00542 \times \text{age in years}] + 0,301$ ). Simultaneously it was shown that the first stage of the thickening of IMT is not only a compensatory (nonatherosclerotic) adaptation to arterial hypertension because smoking as single risk factor can induce the increase of IMT. In a group

of persons suffering from only single risk factors, the risk factors accounted for 51.8% of the variability of IMT. This fact emphasizes the importance of interactions between risk factors.

Thirdly, the correlations between the IMT, other morphological markers of arteriosclerosis, plaque score (PS) and stenosis score (SS), and the vascular risk factors were studied in Caucasians and Asians. In Asian persons, besides other characteristics, the race proved to be an independent, positive risk factor of arteriosclerosis. For both races, the correlation was closer between the risk factors and IMT than between the risk factors and PS/SS. Also for both races, the correlation between IMT and PS/SS was much weaker than between PS and SS. Fourthly, in a cooperative study I participated in the confirmation, by the application of the measurements of IMT, PS and SS in control and diabetic patients, that the semicarbazide-sensitive amine oxydase (SSAO) is a potent biochemical marker of arteriosclerosis.

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**GABRIELLA JUHÁSZ (2004)**

## The role of the serotonin and CGRP in migraine: Genetic and neurochemical studies

*Supervisor: Dr. György Bagdy*

The aim of the present study was to evaluate the role of the CGRP and the serotonergic system in the development of migraine. With this approach, I investigated the genetic and neurochemical risk factors of migraine, and studied how these neurochemical parameters change during a migraine attack. Our data support that the platelet serotonin concentrations are significantly lower in migraine patients without aura in the headache free period compared to controls. By examining the genetic background of the association between platelet serotonin concentrations and migraine we demonstrated that the frequency of the S allele of the serotonin transporter gene 5-HTTLPR functional polymorphism is significantly higher in migraineurs than in controls. However, the lower platelet serotonin concentration in migraine patients is not a consequence of the differing allele frequency of the 5-HTTLPR polymorphism. The higher anxiety level of the migraineurs suggested that the "stress sensitive" S allele of the serotonin transporter gene may express a greater susceptibility to migraine, by itself. Furthermore, we supported that the 5-HT<sub>2A</sub> receptor gene has no effect on the development of migraine.

The NO donor nitroglycerin induced migraine attack is the most commonly used human migraine model. Our results support that the lower platelet serotonin concentration and higher basal CGRP concentration in a headache free period are risk factors for migraine that express greater susceptibility to develop both spontaneous and NO-induced migraine attacks. According to our results the plasma CGRP concentration is a dynamically changing trait marker for the migraine-induced pain that increases with the pain. On the other hand, the 5-HT<sub>1B/1D</sub> receptor agonist sumatriptan causes a decrease in the plasma CGRP concentration in parallel with the ease of the migraine attack. Furthermore, the plasma CGRP concentrations failed to change during the immediate, non migraine-type, mild headache induced by nitroglycerin. With these results we could present human evidence that the CGRP plays a causative role in the development of the migraine.

Finally we can state that in the nitroglycerin-induced migraine model the serotonin release from platelets does not causally relate to the migraine attack, on the contrary, it may prevent or alleviate the migraine pain.

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## KRISZTIÁN KAPINYA (2004)

# Induction of ischemic tolerance by volatile anesthetics the brain

Supervisor: Dr. Zoltán Nagy

Volatile anesthetics have a well documented acute protective effect on the brain if applied during ischemic insults. It is also known that volatile anesthetics can induce the expression of certain transcription factors and therefore might have long-lasting effects in the brain. We tested, whether volatile anesthetics induce neuroprotection which is maintained for a prolonged time.

Using a permanent and a transient focal ischemia model in vivo we demonstrated that pretreatment with isoflurane or halothane provides a prolonged neuroprotection for at least 24 hours after pretreatment. These results were confirmed in cortical neuron-enriched cultures using oxygen glucose deprivation, which is commonly used as a model of ischemia in vitro. For this novel phenomenon the term "anesthetic preconditioning" was coined. To establish a hypothesis for the mechanism of AP, first the results from transcription factor analysis made in the gerbil model of ischemic tolerance induction were considered. The activation profile of the AP-1 transcription factor binding in the sham operated and ischemic experimental groups suggested, that AP-1 activation might be involved in ischemic tolerance induction, but AP-1 could also be induced by anesthesia. To confirm these results, we looked at c-JUN protein, an essential component of AP-1. Although with a different kinetic as expected, we found a delayed c-Jun-NH<sub>2</sub>-terminal kinase (JNK) activation and c-JUN phosphorylation after AP. The deleterious influence of the low oxygenation status and the usage of the antioxidant desferrioxamine in the mouse experiments support the hypothesis that redox-sensitive components, such as c-JUN and JNK might be involved in AP. Finally, we looked at possible effector candidates for AP and identified iNOS as a major contributor: iNOS was induced in the cerebral cortex after AP and pharmacologic inhibition of its activity abolished the delayed protection.

Our observations point to prolonged CNS effects of volatile anesthetics, which may not only account for protective, but also for unwanted delayed effects of general anesthesia, such as the frequent phenomenon of perioperative CNS dysfunction. According to our data the involvement of iNOS in AP is very likely, but the elucidation of the underlying signaling cascades certainly needs further study. It is tempting to speculate that anesthetics may be used to induce neuroprotection in cases in which damage to the CNS is anticipated.

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VENDEL KEMÉNY (2005)

## Microemboli detection by transcranial Doppler ultrasound

Supervisor: Dr. Zoltán Nagy

*Background.* Cerebral embolism is the main cause of ischemic stroke. Emboli generated from the chambers or valves of the heart or from atherosclerotic plaques in the arteries of the neck are variable in their size and consistency. Embolus detection using transcranial Doppler ultrasound (TCD) allows for the identification of active embolic sources in stroke prone individuals and the selection of high-risk patients for appropriate treatment. Cerebral embolus monitoring systems suitable for routine clinical use must have the ability to automatically recognize high intensity transient signals and differentiate between artifacts and emboli. Additional information is needed about microembolic signals (MES), mainly on their size, composition and nature (solid or gaseous). *Aims of these theses.* In this thesis, the technical background and possibilities of semi-automated and automated methods for identifying true embolic signals and approaches established for solid-gas differentiation were studied to answer the following questions: -how reliable are some of the "on market" automated/semiautomated TCD systems on MES evaluations? -is the newly developed automated off-line TCD system capable for differentiate artifacts and true MES? -whether our new TCD system is capable also to differentiate solid MES and gaseous MES? Is oxygen inhalation is capable to define the composition of MES in different circumstances? *Methods and materials.* In these works 53 control persons and a total of 158 patients with potential source of embolism (32 patients with mechanical heart valve, 120 with arterial source of emboli and 4 with patent foramen ovale, respectively) were investigated. The healthy subjects served as negative control and also for testing the provoked artifacts. The monitorings were performed uni/ or bilateral over middle cerebral arteries or posterior cerebral arteries with three different type of TCD devices. *Results.* The TC4040 TCD system achieved a sensitivity of 91.9% and the observer-software agreement on MES was 7.8% in the valve patients, and 77.7% and 7.5% in the carotid artery diseased patients, respectively. The artifact rejection rate was 62%. The neural network yielded an artifact rejection rate of 85% and revealed a sensitivity of 73.4% and a positive predictive value of 56.7. With the combination of the peak frequency of signals of interest and the time delay our new offline system achieved on the validation set a sensitivity of 97%, a specificity of 98%, a positive predictive value of 99% and a negative predictive value of 94% for separating artifacts from emboli. For solid-gas differentiation our system using the peak frequency, the relative power and the envelope symmetry of signals achieved a sensitivity of 89%, a specificity of 86%, and a positive predictive value of 89% and negative predictive value of 89%, where positive refers for solid emboli. And finally we found a significant decline of MES under oxygen inhalation in the patients with mechanical prosthetic cardiac valves (144 MES without versus 63 MES with oxygen) but not in the patients with arterial embolic sources (145 MES without versus 135 MES with oxygen). In the controls no MES were found. *Conclusions.* According to the findings of our researches, the four-gated technique revealed to be a relevant step forwards to semi-automated MES detection. Automatic MES detection using the neural network revealed that the verification of the signals by the investigator is still mandatory. The new off-line automatic system developed by our research team combining a time frequency technique and dual-gate TCD using two characteristic features achieved an excellent correct classification rate for artifact rejection and MES identification. Although the results of solid-gaseous discrimination are also promising, this performance should still be improved, but it seems evident, that this task is much more difficult than the separation of emboli from artifacts. However, in distinct patients, as in patients with mechanical prosthetic cardiac valves the solid-gas discrimination was quite successful by inhalation of pure oxygen.

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ANNA SZÚCS (2003)

## Neurological aspects of some sleep disorders

*Supervisor: Dr. Zoltán Nagy*

My aim is to examine the relation between some sleep disorders and neurological diseases; to analyse their mutual interactions in order to achieve new practical data for clinical use. In the theoretical part we summarise some main points of sleep physiology concentrating on the associations of sleep regulation and neurological diseases. In our examinations, besides clinical methods, the most important tools used are sleep analyses performed by polysomnography and MESEM IV as well as brain imaging methods. To assess clinical state of my stroke patients I utilised NIH Stroke Scale.

We found pathological sleep apnoe frequency in more than half of the patients in any type (bleeding/infarction) of acute stroke. In a prospective study, sleep apnoe parameters remain permanent during 3 months in the ischaemic group; on the other hand, sleep apnoe improves during follow up after brain haemorrhages. We showed pathological sleep apnoe frequency in myasthenia gravis among male patients without daytime respiration complaints.

We looked for the link between the mechanism of the sleep disorder and the underlying organic lesion in two cases. In this analyses we took into account the function of the affected structure in sleep regulation. We found a basal forebrain tumour, affecting sleep regulating centres underlying severe insomnia and we suggest a neuro-vascular compression of the lateral preoptic area of the hypothalamus being the reason of sleep related painful erection, a parasomnia of unknown origin.

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PÉTER VÁRADY (2004)

## Applications of bone morphogenetic protein gene therapy in experimental neurosurgery

*Supervisor: Dr. Zoltán Nagy*

*Objective.* In vivo gene therapy with the use of adenoviral vectors and bone morphogenetic protein (Ad-BMP) genes provides a local supply of osteoinductive molecules and promotes bone neof ormation which can be utilized for patients with bone defects, spinal and skull diseases. In our studies we aim to analyze the histological and ultrastructural changes that occur over time during BMP-9 gene therapy-induced osteoneogenesis in rodents. The other goal of our studies was to determine whether neurosurgical clinical imaging techniques could be used and compared to observe the process of osteogenesis induced by Ad-BMP-2 gene therapy in rodents. *Methods.* Replication defective human adenovirus vector containing the BMP-9 or the BMP-2 gene was used in these studies. Light microscopic and electron microscopic analysis of the rodent tissues injected with Ad-BMP-9 gene therapy was performed at certain timepoints. At various intervals after vector injection containing the BMP-2 gene, animals underwent morphologic and functional imaging, including x-ray, CT and isotope scintigraphy of the areas injected with the gene therapeutic vector. *Results.* Both histological analysis and radiologic studies clearly demonstrated ectopic bone formation at the Ad-BMP-transduced injection sites. The morphological dynamics of the osteogenesis induced by BMP-9 gene therapy are presented. The changes are evaluated by multimodality clinical imaging during the process of osteoneogenesis after BMP-2 gene therapy. *Conclusion.* It is becoming clear that

BMP gene therapy can be successfully used as an osteoinductive agent in vivo, although vector and promoter modification will certainly improve its efficacy. In our studies we presented and evaluated the process induced by in vivo gene therapy by using the diagnostic modalities of human clinical medicine, thereby advancing this novel technique towards clinical neurosurgical use.

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## 6/5. PROGRAM

### CLINICAL NEUROLOGICAL INVESTIGATIONS

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Transcranial motor evoked potential studies in the examination of motor pathways	Zsuzsanna ARÁNYI
Functional neuroimaging in the diagnosis of neurological disorders	Csaba JUHÁSZ
Electrophysiological examination of rhythmic motor actions in movement disorders	Anita KAMONDI
Examination of cognitive functions using electrophysiological and neuropsychological methods	Anita KAMONDI
Event related desynchronisation in Parkinson's disease and other movement disorder	Anita KAMONDI
Analysis of EEG alpha activity during cognitive processes and in neurological diseases	Anita KAMONDI
Changes of the cognitive event-related potentials and perception related gamma activity in cognitive disorders	György KARMOS
Clinicomorphological correlations in degenerative diseases of the nervous system	Tibor KOVÁCS
Histopathological investigation of systemic degenerations of the nervous system	Mátyás PAPP
Histology of prion diseases	Mátyás PAPP
Disorders of cognition, behaviour and speech in cerebrovascular diseases	Imre SZIRMAI
Changes of rheology / blood coagulation parameters in acutestage and in the course of cerebral ischemia	Imre SZIRMAI
Etiology and clinical aspects of cerebral ischemia in young patients	Imre SZIRMAI
Optokinetic nystagmus in neurological disorders	Imre SZIRMAI

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a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

**ZSUZSANNA ARÁNYI (2003)****The role of transcranial magnetic stimulation in the investigation of the human motor system**

*Supervisor: Dr. Anita Kamondi*

Transcranial magnetic stimulation (TMS), introduced by Barker et al. in 1985, is a method that allows non-invasive, painless excitation of the human cortex and other neural structures inaccessible to conventional electric stimulation techniques. Using TMS, we investigated the volitional motor control of proximal and distal arm muscles in healthy subjects, to gain further insight into the physiology of central motor control of these functionally different muscle groups. Three sets of experiments were performed. In the first, excitability of the motor pathway controlling the deltoid and the abductor digiti minimi muscle was examined under different conditions; in the second, uncrossed and transcallosal projections were examined in the same muscles; finally we investigated modulation of the transcallosal response under conditions when mirror movements occur (fatigue). Based on our results, the following conclusions are drawn: 1. There is a greater synaptic efficacy of corticospinal projections to distal muscles than to proximal muscles. 2. Proximal muscles have a greater reserve to activate additional motor units. 3. The mechanism of force gradation differs for proximal and distal muscles. 4. Uncrossed projections are functionally not relevant. 5. There exists a slow-conducting bilateral outflow to proximal muscles of probably subcortical origin. 6. Transcallosal inhibitory influence between the motor cortices is almost absent for proximal arm muscles, as opposed to hand muscles. 7. Transcallosal inhibition decreases during an effortful contraction, paralleled by the appearance of mirror movements. We have also investigated the utility of TMS in the assessment of corticospinal tract and facial nerve dysfunction in patients with neurological disorders. We found that TMS is a sensitive but non-specific method to detect corticospinal dysfunction. It provides diagnostic help mainly when corticospinal tract dysfunction is suspected but clinical signs are lacking; and in patients where diseases of the peripheral nervous system mask corticospinal involvement. It proved especially useful in the diagnosis of compressive myelopathy secondary to cervical spondylosis. Facial neurography combined with TMS is able to assess the intracranial portion of the nerve. It is the only method that provides a positive diagnostic criterion in Bell's palsy. The method is also able to detect subclinical dysfunction of the nerve and to differentiate between peripheral facial palsies due to lesions in the brainstem or to lesions more distal.

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## CSABA ERTSEY (2005)

## New observations about the clinical characteristics and pathomechanism of cluster headache

*Supervisor: Dr. Imre Szirmai*

We investigated the clinical characteristics and pathomechanism of cluster headache (CH). We described its characteristic symptomatology on a large sample of patients and determined the frequency of CH subtypes. Examining the changes of CH patients' gender ratio we found no evidence to suggest an increasing female proportion in the subsequent decades. We suggested that meteorological factors might influence the timing and characteristics of the attack. We observed that the abortive drug sumatriptan was effective for the long-term treatment of attacks, and was also preferred by the patients, regardless of its possible side-effects.

Assessing the effect of cluster headache on the individual, we were the first to compare the quality of life in CH patients, migraineurs and healthy controls. We found that CH severely affected the quality of life of the sufferer, and its effects were at least as severe as those of migraine and of many severe non-neurological conditions. We investigated the factors that determine quality of life in these headache disorders. During our examinations into the pathomechanism of cluster headache, we used a combination of transcranial doppler ultrasound and cranial SPECT to determine vascular changes during the attack. Our results underscored that the flow changes were secondary phenomena caused by trigeminovascular activation. Examining the habituation of cortical auditory evoked potentials we demonstrated the dysfunction of the raphe-cortical serotonergic pathways in the cluster period, which may play a role in the genesis of the attacks.

We were the first to study plasma nociceptin levels in primary headaches. Using radioimmunoassay techniques we found decreased nociceptin concentrations during the cluster period which normalised in the remission phase. Plasma nociceptin concentrations of headache-free migraineurs were also lower than those of matched controls. Lower nociceptin levels may predispose the individuals to head pain transmitted by the trigeminovascular system. This observation may suggest a new therapeutic approach for these primary headaches.

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## CSABA JUHÁSZ (2003)

## Glucose and flumazenil positron emission tomography in intractable epilepsy

*Supervisor: Dr. Anita Kamondi*

The success of cortical resection for intractable epilepsy of neocortical origin is highly dependent on the accurate presurgical delineation of the regions responsible for generating seizures. In addition to EEG and structural imaging studies, functional neuroimaging such as positron emission tomography (PET) can assist lateralization and localization of epileptogenic cortical areas. In the presented studies, objectively delineated focal PET abnormalities have been analyzed in patients (mostly children) with intractable epilepsy, using two different tracers: 2-deoxy-2-[<sup>18</sup>F]fluoro-D-glucose (FDG), that measures regional brain glucose metabolism, and [<sup>11</sup>C]flumazenil (FMZ), that binds to GABAA receptors. The PET abnormalities were correlated with scalp and intracranial EEG findings, structural brain abnormalities, as well as surgical outcome data. In patients with extratemporal foci and no le-

sion on MRI, FMZ PET was more sensitive than FDG PET for identification of the seizure onset zone defined by intracranial EEG monitoring. In contrast, seizures commonly originated from the border of hypometabolic cortex detected by FDG PET suggesting that such areas are most likely epileptogenic, and should be addressed if subdural EEG is applied to delineate epileptic cortex. In patients with cortical lesions, perilesional cortex with decreased FMZ binding was significantly smaller than corresponding areas of glucose hypometabolism, and correlated well with spiking cortex. Extent of perilesional hypometabolism, on the other hand, showed a correlation with the life-time number of seizures suggesting a seizure-related progression of brain dysfunction. FMZ PET proved to be also very sensitive for detection of dual pathology (coexistence of an epileptogenic cortical lesion and hippocampal sclerosis). This has a major clinical importance since resection of both the cortical lesion and the atrophic hippocampus is required to achieve optimal surgical results. Finally, we demonstrated that in patients with neocortical epilepsy, FDG PET abnormalities correctly regionalize the epileptogenic area, but their size is not related to the extent of epileptogenic tissue to be removed. In contrast, complete resection of cortex with decreased FMZ binding predicts good surgical outcome suggesting that application of FMZ PET can improve surgical results in selected patients with intractable epilepsy of neocortical origin.

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## GERTRÚD TAMÁS (2005)

### Electrophysiological investigation of Parkinsonian and essential tremor

*Supervisor: Dr. Anita Kamondi*

Post-movement beta synchronisation (PMBS) is a physiological indicator of the activity of movement related neural networks. Our aim was to investigate the pathomechanism of parkinsonian (PT) and essential tremor (ET) by studying the correlation between tremor severity and movement related beta rhythm changes of the human EEG.

We examined the characteristics of PMBS over the supplementary and primary motor area in 10 patients with unilateral tremor-dominant Parkinson's disease (PT) and in 8 control subjects. In the PT group, PMBS decreased significantly after the movement of the tremulous hand. In the same hemisphere, PMBS was higher after the movement of the non-affected hand, than after the movement of the tremulous hand. This proves that PMBS in PT is affected by the activity of tremor related neural networks, suggesting that both cortical and subcortical sources are responsible for its generation.

We also examined the power and latency of PMBS in ten ET, ten PT and ten control subjects. In ET tremor severity did not influence the amplitude of PMBS, however it was significantly delayed after the movement of the more tremulous hand. More pronounced PT decreased the amplitude of PMBS, but did not delay it. In controls the side of movement did not affect the power and latency of PMBS. The results suggest that neuronal mechanisms underlying PMBS generation are differently affected by ET and PT. Investigation of PMBS might be used for the differential diagnosis of essential tremor and Parkinson's disease.

While voluntary movement suppresses Parkinsonian tremor, essential tremor is enforced by postural and/or kinetic action. We measured the changes of tremor peak frequency power after flash signal, flash triggered (FM) and self-paced (SPM) movement of the contralateral hand in 9 PT and 7 ET patients using accelerometry. PT significantly decreased both during FM and SPM tasks, suggesting that it is generated by a constant subcortico-cortical network, which includes higher order motor areas. Intensity of ET showed a remarkable intra- and interindividual variability both during FM and SPM reflecting a different generator circuitry with variable functional connections.

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## 6/6. PROGRAM

### BIOLOGICAL PSYCHIATRY

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Clinical and neurocognitive predictors of pharmacotherapeutical responsiveness in schizophrenia and affective disorders	György BARTKÓ
Screening and follow up of cognitive dysfunction in neuro psychiatric disorders	György BARTKÓ
Psychobiology and genetic of depression spectrum diseases and anxiety disorders	Gábor FALUDI
Genetic of suicide and neurocognitive characteristics in affective and schizophrenia spectrum diseases	Gábor FALUDI
Polydiagnostic nosologic methods in psychiatry	Péter GASZNER
Measure of the symptoms during mania treatment	Péter GASZNER
Psychotropic drug treatments and metabolic syndrome	István KARÁDI
Serotonin gene polymorphisms in major psychiatric disorders	András LÁSZIK
Dopamine receptor polymorphisms and their association to psychiatric disorders	Mária SASVÁRI
Biogenic amines and nociceptin system in depression and anxiety disorders	Kornélia TEKES

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**Ph.D. graduates**

Hedvig Lukács

na

Ede Frecska

na

**Supervisors**

Gábor Faludi

Gábor Faludi

a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

HEDVIG LUKÁCS (2003)

## The role of hCG/LH receptors of the brain in the regulation of behavior and stress reaction

*Supervisor: Dr. Gábor Faludi*

Recently, a relationship between the brain and the reproductive system was showed by the fact of psychological vulnerability of women is related hormonal changes. The hypothalamic-hypophysigonadal axis (HPG-axis) gives the endocrine background of this relationship. One of the gonadotrop hormones of this axis is the luteinizing hormone (LH) synthesized by the hypophysis. Its peripheral counterpart is the human chorionic gonadotropin (hCG) synthesized by the trophoblast cells. The structure of the two hormones is almost identical and they bind to the same receptor, the hCG/LH receptor. It was shown, that these receptors are localized not only in the ovaries, but in many other nongonadal tissues of the human body, especially in the brain. The highest density of of the hCG/LH receptors can be found in the hippocampus either the human or the rat brain. The discovery of the hCG/LH receptors in the central nervous system raised the question, what functional significance have these gonadotropin receptors in the brain? The aim of the present study was, to characterize the effect of the hCG administration on function of the central nervous system (CNS). Specific behavioral patterns were tested on the base of specific anatomical regions of the brain, where these receptors were found. Behavioral patterns were studied: anxiety, maternal interest, maternal behavior, by using hCG given intracerebroventricularly(icv) to rats. The other part of interest was to decline whether hCG administration has any effect on the prostaglandin metabolism in the brain. In these experiments both in vitro molecular biological methods and in vivo behavioral methods were used such as activity and diurnal rhythm. The effect of hCG administration of these behavioral patterns was investigated in the presence and absence of prostaglandin metabolism inhibitor (indomethacine). Our aim was also to search for hCG/LH receptors in the brain areas yet not studied and investigate whether that are in relationship with emotions and motivation. We looked for the answer whether the hCG has any effect on physical damages (stress ulcer) caused by cold-restraint stress condition (combined physical and psychological stressor). And finally, our aim was to decide whether the icv. administered hCG does exert its' effect via the central hCG/LH receptors. For this experiments antisense hCG/LH receptor oligonucleotide pretreatment was used. The followed results were found: 1. The hCG treatment resulted in a decrease of CNS arousal, justified by EEG. 2. Several behavioral parameters were affected by centrally administered hCG (decreased activity and anxiety), the time spent sleeping was also decreased, and the social + maternal interest were facilitated). These behavioral parameters are related to the function of hippocampus, MPOA and olfactory bulb. 3. The presence of hCG/LH receptors was justified in several regions of the brain, so in olfactory bulb too. 4. The effect of the hCG on the diurnal rhythm is modulated by the prostaglandin pathway in the CNS. 5. The decrease in the stress induced gastric ulcers after central hCG treatment indicates the central stress protective role of the hormone. 6. The central administration of hCG is mediated by the hCG/LH receptors of the brain as it is justified by the results of receptor antisense oligonucleotide experiments. 7. The emotional, behavioral and somatic changes observed in the animal experiments might be in association with the cyclic changes parallel with the menstrual cycle. These changes resemble to those ones observed during human pregnancy, too. The results suggest, that the hCG/LH receptors, and LH like peptide occurring within several regions of the central nervous system, serve as a novel relationship between the brain and reproductive system. This relationship may have a role in the psychological disturbances related to the sexual cycle, maternal interest after delivery and sexual dimorphism of stress reaction can be explained.

EDE FRECSKA (2005)

## Investigation of endogenous opioid reactivity with fentanyl challenge in major depression and self-injurious behavior

*Supervisor: Dr. Gábor Faludi*

Some observations support the notion that regulation of the endogenous opiate system is deficient in major depression and sleep deprivation might exert its antidepressant properties via opioid mediation. In another psychiatric condition, namely stereotypic movement disorder, the clinical use of opiate antagonists have shown to diminish self-injurious behavior supporting involvement of opiate mechanisms in this pathological condition. The overall aim of presented studies was to elucidate the role of the endogenous opiate system in the pathomechanism of major depression, self-injurious behavior and in the antidepressant action of partial sleep deprivation. The main method applied was the use of the selective mu-receptor agonist fentanyl, as a challenging agent for testing endogenous opiate sensitivity by monitoring neuroendocrine responses to the drug, particularly measuring fentanyl-induced plasma levels of prolactin. The author had studies focusing on dose-response relationships in healthy volunteers and diurnal changes of opiate sensitivity. His subsequent studies utilized these results and addressed opioid mechanism in depression, self-injurious behavior and after partial sleep deprivation. Based on data obtained by fentanyl challenge tests, it seems possible that endogenous opiates play some role in the conditions investigated. The author of these short publications wishes to avoid oversimplification and is careful about drawing conclusions for treatment strategies from these findings. The role of the endogenous opiate system in psychiatric illnesses and pathological behaviors is not simple and can be best explained in interaction with other neurotransmitter and signal processing mechanisms.

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- *Frecska E, Arato M (2002) Opiate sensitivity test in patients with stereotypic movement disorder and trichotillomania. Prog Neuropsychopharmacol Biol Psychiatry 26:909-1012.*
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## 7. PH.D. SCHOOL OF MOLECULAR MEDICAL SCIENCES

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The postgraduate school of molecular medical sciences serves for both biomedical basic research and primary training of researchers starting their careers in the fields of clinical research. One of the shortages of biomedical research is that there is no efficient connection between the basic and clinical research. Therefore, five main Programs involve applied theoretical knowledge together with clinical research.

### **7/1. PROGRAM**

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*Aim:* The Program provides opportunity to receive training in the field of physiology. The professors of the Program offer courses and individual training for the Ph.D. students on their respective scientific research areas. Training courses include continuous basic methodical and scientific training for small groups of students. Individual training focuses on research under the supervision of training advisors aimed to understand physiological regulatory mechanisms on a cellular level using electrophysiological, molecular biological, biochemical, cell biological and physiological methods.

##### *Sub-programs*

Interaction of cytosolic and mitochondrial Ca<sup>++</sup> signal  
Molecular mechanism of chemoreception of carotid body  
Free radical formation in steroid producing cells  
Mode of action of calcium mobilizing hormones and neurotransmitters  
Molecular basis of angiotensin receptor function  
Regulation of G-protein coupled receptors  
The role of reversible protein phosphorylation in the control of cell function  
Interactions of plasma membranes and intracellular organelles  
Molecular physiology of hepatocytes  
Analysis of proteins involved in osteoclast differentiation and function

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*Sub-programs*

Intracellular signal transduction in immunodeficient (knockout) mouse

The role and regulation of Rho GTPase-activating proteins

The role of NADPH oxidase in antimicrobial defence

Reactive oxygen species-producing enzymes in mammalian cells

Methods in gene technology

Receptors and signal transduction in the immune system

Signalling in the cell nucleus

Molecular chaperones and cellular networks

Regulation of 2P type potassium channel

Structure and function of 2P type potassium channel

The role of ion channels in glomerulosacells

Modern biochemical separation techniques and in vivo bioanalytical methods

Developmental potential of neural stem cells implanted into the brain

In vitro cell technology

In vitro investigation of neuronal development

Receptor and signal transduction in the immune system

id3413876 Electron microscopy in cellular and molecular research

Functional cytology

Molecular biological experimental strategies in the cellular physiological research

Application of isotope techniques in cellular physiology

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a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

TAMÁS KARDON (2002)

## Regulation of bilirubin UDP-glucuronosyl transferase in different pathological conditions

Supervisor: Dr. József Mandl

Glucuronidation is quantitatively the most important reaction in the 2<sup>nd</sup> phase of biotransformation. Glucuronide formation is catalyzed by uridine diphosphate glucuronosyltransferases (UGTs; EC 2.4.1.17). The various UGT isoenzymes are membrane-integrated proteins of the endoplasmic reticulum (ER) with their active site located in the lumen of the ER. The capacity of glucuronidation is altered in some pathophysiological states, such as diabetes type 1, starvation, alcoholism. In these conditions the serum concentration of bilirubin, the toxic product of heme catabolism, is elevated.

The aim of our work was to investigate the changes in bilirubin-glucuronidation in the above-mentioned pathophysiological states. Spontaneously diabetic BB/Wor rats, starving rats as well as rats treated with acetone or ethanol were used as experimental system. Our more important findings are as follows: (1) In diabetes mellitus type 1, in starvation and after acetone and chronic ethanol treatment the rat liver microsomes showed (a) an elevated bilirubin glucuronosyl transferase activity, and (b) an elevated level of UGT1A1 protein, the most significant UGT isoenzyme for bilirubin glucuronidation. (2) In diabetes, starvation, and after chronic ethanol treatment the induction was detected on the level of transcription, as mRNA levels were elevated as well. This change in the mRNA level, however, was not detectable after acetone treatment, from which we inferred that acetone does not play a role in UGT1A1 induction in diabetes mellitus or in starvation.

Induction of UGT1A1 by chronic ethanol treatment was not observed in rats co-treated with gadolinium-chlorid. This compound leads to a depletion of Kupffer cells, so we concluded that extrahepatocellular processes may also play a role in UGT1A1 induction.

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JUDIT MAKARA (2003)

## Inwardly rectifying chloride current in astrocytes and adrenal glomerulosa cells

Supervisor: Dr. András Spät

Anion channels play an important role in Cl<sup>-</sup> transport and in the regulation of membrane potential, cell volume, intra- and extracellular pH and proliferation. We used the whole-cell patch-clamp method to investigate the resting anion conductance of cortical and hippocampal astrocytes as well as adrenocortical glomerulosa cells. After pharmacological elimination of the K<sup>+</sup> conductance, an inwardly rectifying Cl<sup>-</sup> current was observed in rat cortical astrocytes co-cultured with neurons. The current activated slowly at potentials negative to -40 mV, did not show inactivation, and could be inhibited by known Cl<sup>-</sup> channel blockers. The current was sensitive to physiological changes in extracellular pH (pH 6,9-7,9): acidosis activated, whereas alkalosis inhibited the conductance. Patch-clamp experiments conducted in situ in cortex and hippocampus of mouse acute brain slices revealed the expression (albeit with markedly lower density) of a very similar inward current in a subtype of EGFP-expressing astrocytes, namely the complex astrocytes. The current was abolished in

a 'knock-out' mouse line where the *ClC-2* Cl<sup>-</sup> channel gene was inactivated, indicating that the *ClC-2* channel was responsible for generating the conductance. In situ EGFP-expressing reactive astrocytes exhibited small or not detectable Cl<sup>-</sup> current component. An inwardly rectifying current with similar characteristics to that observed in astrocytes was detected in cultured rat glomerulosa cells as well. The current in glomerulosa cells could be also activated with acidosis (pH 6,9). In astrocytes we propose a role for this anion current in Cl<sup>-</sup> transport accompanying K<sup>+</sup> buffering. In glomerulosa cells, to our knowledge this is the first observation of an anion conductance in resting cells. The current may play a role in regulation of steroid synthesis in glomerulosa cells.

Cell swelling induced by extracellular hyposmosis increased current amplitude through T and L type voltage-gated Ca<sup>2+</sup> channels in glomerulosa cells. Using microfluorimetric technique we showed that this effect can influence the [Ca<sup>2+</sup>]<sub>i</sub> signal evoked by physiological elevation of extracellular [K<sup>+</sup>]: the [Ca<sup>2+</sup>]<sub>i</sub> signal induced by 5 mM K<sup>+</sup> increased significantly in hyposmotic environment (250 mOsm) compared to that of under isosmotic conditions (290 mOsm). Augmented responsiveness by cell swelling may be an important element of the extreme sensitivity of glomerulosa cells to physiological elevation of [K<sup>+</sup>]<sub>e</sub>.

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**BALÁZS MIHALIK (2003)**

## Role of the carboxyl-terminal region of the AT1A angiotensin receptor in its endocytosis

*Supervisor: Dr. László Hunyady*

The type 1 angiotensin II receptor (AT1 receptor) is a G protein-coupled receptor, which mediates the major physiological effects of the octapeptide hormone, angiotensin II. Agonist-induced activation of the AT1 receptor causes phosphorylation, desensitization and internalization of the receptor. Our results demonstrate that elimination of the serine/threonine-rich region of the carboxyl-terminal (C-terminal) tail of the receptor abolished its agonist-activated phosphorylation. Truncation of the receptor tail at distinct sites localized the major phosphorylation site of the receptor to a 11 amino-acid segment. The impairment of receptor phosphorylation was correlated with the attenuation of agonist-induced internalization rates of these mutants and the magnitude of inositol phosphate production normalized to an equal number of receptors. These results suggest that agonist-induced internalization and desensitization of the AT1 receptor is associated with phosphorylation of this serine/threonine-rich region.

In yeast, hyperphosphorylation of the alpha-factor pheromone receptor regulates endocytosis of the receptor by monoubiquitylation of its cytoplasmic tail on lysine residues, which process is regulated by phosphorylation of adjacent serine and threonine residues. The role of receptor ubiquitylation in AT1 receptor internalization at subnanomolar angiotensin II concentrations was evaluated by deletion or replacement of lysine residues in its C-terminal serine/threonine-rich region, which is the major site of agonist-induced phosphorylation. Expression of the mutant receptors in CHO cells showed that these modifications had no detectable effect on the angiotensin II-induced endocytosis of the AT1 receptor. Furthermore, fusion of ubiquitin in-frame to an internalization-deficient AT1 receptor mutant with a truncated carboxyl-terminal tail did not restore the endocytosis of the resulting chimeric receptor. Substitution of all lysine residues in the serine/threonine-rich region also did not inhibit the internalization of the receptor at saturating angiotensin II concentrations, where endocytosis occurs by a beta-arrestin and dynamin independent mechanism. Taken together, these data demonstrate that ubiquitylation of the cytoplasmic serine/threonine-rich region of the AT1 receptor on lysine residues is not required for its agonist-induced internalization, and suggest that endocytosis of

mammalian G protein-coupled receptors occur by a different mechanism than that of yeast G protein-coupled pheromone receptors.

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**GERGELY ZOLTÁN MOLNÁR (2002)**

## Detection and characterization of Rac-GTPase-activating proteins in neutrophil granulocytes

Supervisor: Dr. Erzsébet Ligeti

Rac is a Rho family small G protein. It has a role in cytoskeleton rearrangements (e. g. membrane ruffling) and is the regulatory subunit of the NADPH oxidase of granulocytes. As a small GTPase, Rac is able to bind and hydrolyze GTP, therefore it can act as molecular switch shuttling between active GTP-bound and inactive GDP-bound state. The activatory GDP-GTP exchange is catalyzed by exchange factors (GEFs). Negative regulators of small G proteins are the GTPase activating proteins (GAPs) which accelerate GTP hydrolysis and the guanine nucleotide dissociation inhibitors (GDIs) that can lock the bound GDP to the small GTPase. We investigated the intracellular localization and molecular identity of Rac-GTPase-activating proteins (Rac-GAPs) in neutrophils. Immunoblot analysis detected the presence of both p190RhoGAP and Bcr mainly in the cytosol. An overlay assay performed with [ $\gamma$ - $^{32}$ P]GTP-bound Rac revealed dominant GAP activity related to a 50 kD protein both in the membrane and cytosol. This activity could be identified by Western blotting and immunoprecipitation with specific antibody directed against the GAP domain of p50RhoGAP. Also the role of prenylation, the carboxy terminal lipid modification of small G proteins, was investigated in the interaction of Rho family small GTPases with their GAPs. Prenylated and nonprenylated small GTPases were expressed in *Sf9* cells and *E. coli*, respectively. Nucleotide binding to and hydrolysis by prenylated and nonprenylated proteins were identical but three major differences were observed in their reactions with GAPs. (1) Membrane-associated GAPs accelerate GTP hydrolysis only on prenylated Rac1 and RhoA but they are inactive on the non-prenylated form of these proteins. In contrast to this, non-prenylated Cdc42 is able to interact with membrane-localized GAPs. The difference is independent of the presence of detergents. (2) Full-length p50RhoGAP and p190RhoGAP react less intensely with non-prenylated Rac1 than with the prenylated protein, whereas no difference was observed in the reaction of isolated GAP domains of either p50RhoGAP or Bcr with the different types of Rac1. (3) Fluoride exerts inhibitory effect only on the interaction of prenylated Rac1 with the isolated GAP domains of p50RhoGAP or Bcr. This inhibition is almost absent in the case of nonprenylated Rac. The effect of fluoride is not influenced by addition or chelation of  $Al^{3+}$ . Our results clearly suggest that prenylation in addition to influencing interactions of proteins with membranes, also determines protein-protein interactions.

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ZOLTÁN MOLNÁR (2004)

## Functional study of chloride currents of the rat carotid body

Supervisor: Dr. András Spät

Hypoxia, hypercapnia and pH are known to activate the chemoreceptor cell of the carotid body by inducing membrane depolarisation. It is widely accepted that stimulus-induced depolarisation is due to the inhibition of K<sup>+</sup> currents. Besides these cation currents that undoubtedly play significant role in the chemotransduction process, chemoreceptor cells also possess anion currents, which functions are hardly known. We aimed to study the functional role of the anion currents of rat chemoreceptor cell: the swelling-activated Cl<sup>-</sup> current and also the ClC-2-like Cl<sup>-</sup> current which latter one was previously characterised in the chemoreceptor cell by our group.

Experiments were performed on primary cultures of chemoreceptor cells isolated from rats. Ionic currents were monitored by patch-clamp technique using the whole-cell mode, while of cytoplasmic Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]<sub>c</sub>) and pH (pH<sub>c</sub>) were detected by fluorescent technique with Indo-1 and carboxy-SNARF, respectively.

In the first set of experiment we verified by patch-clamp measurements that hyposmotic challenge activates the swelling-activated Cl<sup>-</sup> current in our cell culture, as well. In the subsequent fluorescent measurements we examined the effect of hyposmotically induced cell-swelling on resting [Ca<sup>2+</sup>]<sub>c</sub>. Decreasing osmolality by 50 mosmol/kg caused elevation of [Ca<sup>2+</sup>]<sub>c</sub> in CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup> buffered media, and more moderate decreases in osmolality (25 and 15 mosmol/kg) were capable to induce Ca<sup>2+</sup> response. By using Ca<sup>2+</sup>-free medium and nifedipine, a blocker of the L-type Ca<sup>2+</sup> channel, we proved that hiposmosis-induced Ca<sup>2+</sup> response is due to the opening of high-voltage activated Ca<sup>2+</sup> channels that are activated by the swelling-induced depolarisation. This depolarisation is mediated by the swelling-activated Cl<sup>-</sup> current indeed, since niflumic acid, an inhibitor of the current, abolished the hiposmosis-induced Ca<sup>2+</sup> response. The cellular mechanism that is likely to underlie cytoplasmic Cl<sup>-</sup> accumulation which accounts for the Cl<sup>-</sup>-mediated depolarisation was tested by the withdrawal of CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup> buffer. In our further investigations on the function the other, ClC-2-like Cl<sup>-</sup> current, we found that inhibition of current, induced slow, reversible cytoplasmic alkalinisation. Our results demonstrate that activation of the swelling-activated Cl<sup>-</sup> current results in the activation of the cell and this current underlies the osmotic sensitivity of the chemoreceptor cell. The phenomenon of Cl<sup>-</sup> mediated depolarisation was reported by us and this finding re-evaluates the significance of Cl<sup>-</sup> currents in the signal transduction process of the cell. Furthermore we demonstrated that the ClC-2-like Cl<sup>-</sup> current contributes to the adjustment of resting cytoplasmic pH which supports the functional importance of the conductance.

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PATRYK PAWEL MOSKWA (2005)

## Possible regulatory mechanisms of the neutrophil NADPH oxidase: Role of GTPase activating proteins and lipids

Supervisor: Dr. Erzsébet Ligeti

The superoxide (O<sub>2</sub><sup>-</sup>) producing enzyme NADPH oxidase is composed of the membrane associated catalytic subunit cytochrome b558 and the cytosolic regulators p47phox and p67phox. The cytosolic components translocate to the phagosomal membrane to establish a catalytically active complex with cytochrome b558. In this process the small GTPase Rac and lipids are of particular importance

but their precise role is poorly understood. Rac, like the entire subfamily of Rho GTPases, cycles between the biologically active GTP bound and inactive GDP bound state and undergoes posttranslational prenylation. The endogenous GTPase activity of the small GTPases controls the duration of biological activity and is highly accelerated by GTPase activating proteins (GAP). In the present work, I investigated the role of biologically active RacGTP in the regulation of the catalytic enzyme activity and possible factors that control the GTP bound state of Rac. I also studied the effect of glucocerebroside, a lipid that is accumulated in phagocytes of Gaucher patients. I found that: 1. RacGTP is absolutely required for sustained O<sub>2</sub>- production. 2. p67phox, the cytosolic subunit of the NADPH oxidase effectively inhibits its conversion to the GDP bound state. 3. Once the active NADPH oxidase complex has assembled, the soluble cytosolic GAP proteins are unable to access RacGTP but the membrane-associated ones are still capable of downregulating the O<sub>2</sub>- production. 4. The p50RhoGAP occurs in closed conformation, in which two N-terminal stretches (1-48 and 169-197AA) restrict the accessibility to its C-terminal GAP domain. Interaction with the prenyl tail of Rac opens this conformation. 5. Glucocerebroside counteracts the assembly of the active oxidase complex by interfering with p47phox and p67phox. In conclusion, we propose RacGTP and the membrane-associated GAP proteins as a possible mechanism controlling the duration of O<sub>2</sub>- production and interference of glucocerebroside with the cytosolic subunits p47phox and p67phox as the molecular basis of decreased O<sub>2</sub>- production in Gaucher phagocytes.

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JÁNOS GYÖRGY PITTER (2005)

## Mitochondrial calcium metabolism and regulation of pyridine nucleotide redox state in endocrine cell types

Supervisor: Dr. András Spät

The cytoplasmic Ca<sup>2+</sup> signal is transferred to the mitochondrial matrix and activates mitochondrial dehydrogenases. The requirement for supramicromolar [Ca<sup>2+</sup>]<sub>c</sub> in perimitochondrial microdomains in this response has been suggested. In the first part of our work, we studied the mitochondrial calcium homeostasis in digitonin-permeabilized rat glomerulosa, insulinoma (INS-1/EK3) and human osteosarcoma (143B) cells, preventing the formation of high [Ca<sup>2+</sup>]<sub>c</sub> microdomains. Changes in [Ca<sup>2+</sup>]<sub>m</sub> were monitored using either the fluorescent dye rhod-2 or the bioluminescent probe aequorin. To compare the results obtained by these two methods, we applied in situ calibration for the signals and found that mt-aequorin luminescence in these cells shows one – one and a half order of magnitude higher calcium sensitivity, compared to the calibration equations obtained in vitro and used extensively in the literature. Using our in situ calibration curve, the two methods yielded compatible results. In all three cell types, step-by-step elevations of [Ca<sup>2+</sup>]<sub>c</sub> in the submicromolar range were followed by higher and higher steady-state [Ca<sup>2+</sup>]<sub>m</sub> elevations. In rat glomerulosa cells, the biological significance of the observed [Ca<sup>2+</sup>]<sub>m</sub> elevations was indicated by the parallel increase in mitochondrial reduced pyridine nucleotide (NAD(P)H) autofluorescence. In the second part of our work, and ATP on rat osteosarcoma studied the effect of two calcium mobilizing agonist, PGF<sub>2</sub> luteal cells. Our initial finding was that although these agonists led to evoked significantly larger a similar global cytoplasmic calcium signal, PGF<sub>2</sub> increase in mitochondrial NAD(P)H. Similar results were found in the presence of the respiratory chain inhibitor rotenon. Using confocal microscopy and a custom algorithm evaluating the mean response of all the mitochondria measured, we could not find difference in the propagation into the mitochondrial matrix of and ATP. To study the non-[Ca<sup>2+</sup>]<sub>m</sub> mediated effects αCa<sup>2+</sup> signals evoked by PGF<sub>2</sub> of the agonist, we compared the mitochondrial NAD(P)H elevations in calcium-ionophore treated cells at identical, permissive [Ca<sup>2+</sup>]<sub>c</sub> elevations in the presence or absence of the agonists, and found that in addition to the calcium-dependent activation of

mitochondrial dehydrogenases shared by both potentiates the  $\text{Ca}^{2+}$ -induced generation of NAD(P)H by  $\alpha$ 2agonists, PGF non- $\text{Ca}^{2+}$  mediated mechanism.

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## BALÁZS RADA (2004)

# Role of the NADPH oxidase in calcium metabolism and bacterial killing of human neutrophils

Supervisor: Dr. Erzsébet Ligeti

Important members of the body's immune system are the neutrophilic granulocytes which arrive first at the sites of infection and participate in the destruction of invading microorganisms. An essential enzyme of this process is the NADPH oxidase which produces superoxide anions, the proforms of reactive oxygen radicals. The electron transport of the enzyme causes the membrane to depolarize, thereby altering the electric force for ions. During activation of the cells the membrane depolarization inhibits the calcium influx through the capacitative channels. In differentiated wild-type granulocyte-like cells a high level of superoxide production was associated with significant membrane depolarization and an inhibition of capacitative  $\text{Ca}^{2+}$  entry. These changes were not observed in cells lacking the oxidase or transfected with a non-functional mutant of the enzyme. Membrane depolarization and inhibition of the capacitative calcium influx reappeared in cells retransfected with a wild-type oxidase. The chemotactic peptide induced calcium signal was higher in model cells lacking the oxidase or in neutrophils treated with the oxidase inhibitor (DPI) as in the wild-type or untreated cells. Our experiments showed a clear correlation between the membrane depolarization induced by the NADPH oxidase and the inhibition of the capacitative calcium entry. According to the classical model of bacterial killing, the reactive oxygen species produced by the oxidase are responsible for the destruction of pathogens. Novel studies, however, say that the membrane depolarization induced by the NADPH oxidase drives  $\text{K}^{+}$  ions into the phagosome which release the inactive proteases and those attack the invaders. To understand the role of the oxidase induced superoxide production and depolarization deeper, we measured the effect of DPI on the killing of bacteria by neutrophils using a new, controlled, validated, semi-automated method developed by us for 96-well plates. The correlation between  $\text{K}^{+}$ -release and membrane potential was linear, whereas that between the latter and superoxide production turned out to be non-linear. At lower concentrations of DPI the superoxide production was only 10-20 %, but the extent of depolarization still 80% of the uninhibited. Under these conditions killing of *Staphylococcus aureus* impaired seriously which points to the important role of reactive oxygen species in the killing of bacteria. Reaching the highest concentration of DPI, superoxide production decreased only by some percent, but membrane depolarization was reduced to 50% of the uninhibited. The killing of *S. aureus* in this range was impaired significantly, as well, pointing to the relevant function of membrane potential changes in the process of bacterial killing. The proton channel inhibitor zinc increased and accelerated the depolarization, reduced the superoxide production, but the killing of bacteria remained unchanged. Thus, both the superoxide production and membrane depolarization accompanying the activation of the NADPH oxidase are important in the digestion of phagocytosed microbes.

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MÁRTA SZASZÁK (2003)

## The role of dynamin in the internalisation of the AT1 angiotensin receptor

Supervisor: Dr. László Hunyady

The renin-angiotensin system has a role in the pathogenesis of several cardiovascular diseases. Drugs that selectively block the AT1 receptor have important roles in the treatment of hypertension. AT1 receptor belongs to the superfamily of G-protein-coupled receptors, which contain seven transmembrane regions. Elucidation of the molecular mechanisms that control the receptor function has a high importance in regard to the use of receptor antagonist and agonist compounds and for the understanding of the mechanism of endocytosis. Endocytosis of the angiotensin II receptor influences the signal transduction, causes resensitization of the receptor after its desensitisation and has a role in the long-term regulation of the number of receptors. The aim of our study was to examine the role of dynamin2 in the clathrin-mediated internalization of the AT1 receptor. Dynamin2 is a GTP-ase protein that is ubiquitously expressed in mammalian cells and can interact with lipids and several proteins. The internalization of the AT1 receptor was inhibited by generating a pointmutation in the PH domain of dynamin, which can bind to phospholipids and by deletion of the proline-rich region that can interact with SH3 domain containing proteins. In addition the deletion of the proline-rich region has inhibited the plasmamembrane localization of dynamin as it was confirmed by confocal microscopic studies. Coexpression of the SH3 domain of amphiphysin and endophilin impeded the localization of dynamin in a similar way. These SH3 domains also inhibited the internalization of the receptor, which was overcome by the coexpression of dynamin suggesting that the specific interaction of dynamin with these SH3 domain containing proteins has a role in the endocytosis of the AT1 receptor. Our experiments show that the non-neuronal isoform of dynamin, amphiphysin and endophilin has a similar role during receptor endocytosis as it was described by their neuronal isoforms in the recycling of the synaptic vesicles. In addition, we concluded that the proline-rich domain of dynamin has a role in its localization, whereas the PH domain most probably regulates its function.

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**7/2. PROGRAM**


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Aim: The pathobiochemistry showed a remarkable dynamic progress in the past decades. The Program has two roles: a.) it outlines the aetiology and pathogenesis of different pathological conditions, b.) it wants to help the detailed knowledge of certain important fields of pathology. In planning the program the following viewpoints were considered: in diseases different mechanisms of pathological regulation can develop reflecting changes in extracellular signals or signal transduction.

*Sub-programs*

The role hypoxic pathway in expression of oxygen sensitive genes  
 Regulation of glucuronidation  
 Transport systems of endoplasmic reticulum  
 The role of Vitamin C in antioxidant defence  
 The role of protein phosphorylation in regulation of cell functions  
 Comparative analysis of signalling pathways involved in cell scatterings  
 Investigation of signal transduction pathway of tyrosine kinase receptors  
 Role of Vav2 exchange factor in the regulation of small GTPases  
 Investigation of the role of ABC transporter proteins in xenobiotic resistance and drug metabolism  
 The role of Ca<sup>+2</sup> in the mechanism of cell death  
 The role of ABC transporter proteins in hereditary diseases  
 Investigation of calcium transport systems in healthy and pathological conditions  
 Molecular genetic studies in immunology  
 Design of drug molecules based on the structure of SHP-2 and SHP-1 tyrosine phosphatase  
 Design and synthesis of kinase inhibitory molecules based on QSAR and combinatorial chemistry  
 Structure – activity relationship studies with kinase inhibitors  
 Rational drug design and virtual screening  
 Utilization of chromatographic parameters in QSAR  
 Investigation and application of length polymorphism in psychogenetic studies  
 Association studies of the dopamine D4 receptor gene (DRD4) polymorphisms in child psychiatric disorders  
 Application of capillary electrophoresis in medicine

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*Sub-programs*

Medical importance of nucleotide metabolism, in inherited metabolic disorders, enzymopathies: in molecular targeted cancer chemotherapy: in anti-viral therapy and in immunosuppression

Fate of glucuronides in the endoplasmic reticulum

Biochemistry of the 90 kDa heat shock protein (Hsp 90)

Chaperone overload in eukaryotic cells

Functional changes of chaperones in pathological states

Biosynthesis of nitric oxide, its relation to oxidative stress and their roles in the pathobiochemistry of human placenta

Pathobiochemistry of pancreatic enzymes

Membrane transport processes in diabetes mellitus

Isolation and characterisation of specific kinase inhibitory molecules

Separation and techniques: analytical methods

Characterisation of ABCG transporter family

Biological activity measurements of compounds with various techniques

Production of natural ligands with antitumor activity with biotechnological methods

Ascorbic acid transport of endoplasmic reticulum in diabetes mellitus

Gene polymorphism of estrogen receptor and lipoproteins

Investigation of tyrosine kinase inhibitory molecules in pathogenesis of atherosclerosis

Determination of particle specific analytical and physicochemical parameters of drugs and drug-candidate molecules with capillary electrophoresis

Role of kinases in signal transduction

Pathobiochemistry of the haemostasis system

Signal transduction therapy

Molecular mechanism of UV light induced tumour in skin

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AMERE SUBBARAO SREEDHAR (2004)

## Hsp90 inhibitors: their effects on redox status and on the cytoarchitecture

*Supervisor: Dr. Péter Csermely*

The 90 kDa heat shock protein (Hsp90) is a highly abundant molecular chaperone. Hsp90 stabilizes the structure of several important signaling molecules suggesting a key role for this chaperone in cell proliferation and survival. In recent years Hsp90 inhibitors gained more and more attention as promising novel anticancer agents. Hsp90 inhibitors act at the N- and C-terminus of Hsp90. Although inhibition of Hsp90 may promote tumor cell death either by cytostasis or apoptosis, the mechanism behind this phenomenon needs to be further explored. Hence I measured the effect of Hsp90 inhibitors for the partial lysis of the Jurkat T lymphoid cell line. We were also interested, if the middle, charged linker region of Hsp90 is an active component in substrate binding or not. My major findings are the following: 1. Hsp90 inhibitors enhance the lysis of Jurkat cells by mild detergent treatment, by hypoxia or by the complement system. 2. Hsp90 inhibitors do not affect the lysis of bacteria, while they have a smaller effect on yeast cells, than on T lymphocytes. 3. The enhancement of the cell lysis has two components: it is partially derived from the increased amount of superoxides after the addition of Hsp90 inhibitors, while the rest of the effect comes from the inhibition of Hsp90. 4. Hsp90 inhibitors (especially geldanamycin) induce superoxide production, uneven lipid peroxidation of the plasma membrane and a change in membrane fluidity. 5. Hsp90 can be successfully inhibited by the specific hammerhead ribozyme we constructed. 6. Analyzing the binding of neuropeptide Y (NPY) to Hsp90 I showed that the binding of NPY to Hsp90 is grossly dependent on the ionic strength of the buffer, which gave a further evidence for the electrostatic nature of NPY/Hsp90 interactions, further supporting the role of the charged linker region in NPY binding. My results uncovered an important role of Hsp90 in the maintenance of the cellular integrity, showed a novel element of the antitumor mechanism of Hsp90 drugs (which entered to phase II clinical trials recently) and helped to establish a functional role of the charged, linker region of Hsp90 by giving additional support for the identification of this site as the third peptide binding site of Hsp90. Moreover, my studies gave the first example for the successful use of anti-Hsp90 ribozymes for the inhibition of Hsp90 function. Further experiments are on the way to explore the mechanism of the effects observed either by proving a role for Hsp90 in the maintenance of the cytoarchitecture or by establishing a role of Hsp90 in raft-dependent membrane stabilization.

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HAJNALKA ANDRIKOVICS (2004)

## Molecular genetic testing of haemochromatosis and haemophilia A

*Supervisor: Dr. Balázs Sarkadi*

Molecular genetics is a rapidly advancing field of laboratory medicine. In the current work, I illustrate the application fields of molecular genetics in laboratory medicine by using two examples of hereditary diseases: hereditary haemochromatosis (HH) and haemophilia A (HA). HH is a disease of

the iron metabolism with an autosomal recessive inheritance. In 64 to 100% of the cases, the disease is caused by homozygous C282Y, less frequently C282Y/H63D compound heterozygous mutation of the HFE gene. The aim of our work was to define the Hungarian allele frequency data for the two point mutations of the HFE gene. By testing a healthy control population, we found an allele frequency of  $3.8 \pm 0.8\%$  ( $n=1273$ ) for the C282Y point mutation, and  $13.2 \pm 2.1\%$  ( $n=517$ ) for the H63D mutation. In a patient group of myelodysplastic patients, the combined frequency (52%) of the C282Y and H63D mutations was significantly higher than in the control group (31%). The allele frequency data for the normal Hungarian population indicate that, HH is a frequent disease in Hungary. The mutation detection represents a new, non-invasive technique that becomes integral in HH-diagnostics. HA with an X-linked, recessive mode of inheritance is a bleeding disorder caused by the deficiency of the factor VIII (FVIII) coagulation protein. Approximately 50% of severe HA-cases are caused by the inversions of intron 22 and 1 of FVIII gene. The remaining 50% of severe HA-cases, as well as all moderate and mild HA-cases are caused by mutations randomly located on all 26 exons of the FVIII gene. By using the easily executable direct techniques (detection of inversions of introns 22 and 1) and indirect techniques (BclI-RFLP; IVS13CA and p39CA), the carrier state was excluded in cases of 54 potential carriers while this status was confirmed in cases of 28 women. We identified the presence of gene inversion or the existence of at least one informative indirect marker in cases of 129 carrier women. Our diagnostic strategy proved to be informative in 129/130 (99.2%) carriers. Prenatal diagnoses were completed in 13 male fetuses. The genetic diagnostic strategy introduced in our laboratory proved to be informative, reliable and cost effective for the carrier and prenatal diagnostics of HA.

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**ESZTER DURA (2003)**

## **Fundamental and clinical investigations in diabetology: experiments in isolated islets and clinical studies on diabetic retinopathy**

*Supervisor: Dr. László Romics*

(1) At a concentration of D-glucose comparable to that found in the rat plasma postprandially (8.3 mM), a mixture of 20 amino acids at their physiological concentrations supplemented together with citrulline, ornithine and taurine augments by about 50% the output of insulin in islets prepared from fed rats. Our metabolic, cationic and secretory findings extrapolated to the situation found *in vivo* document an impressive role of circulating amino acids in the normal control of insulin release. Simulating physiological conditions, our results were considered as compatible with both the role of certain amino acids as nutrients (e.g. L-glutamine) and the accumulation of other amino acids (e.g. L-arginine, L-lysine) as positively charged molecules in the islet cells.

(2) Cytochalasin B (21  $\mu$ M) enhanced insulin release evoked by D-glucose and non-glucidic secretagogues (2-ketoisocaproate, L-leucine and L-glutamine), despite inhibiting D-glucose uptake and D-glucose metabolism in pancreatic islets. Cytochalasin D (20  $\mu$ M), which failed to affect D-glucose uptake and metabolism by isolated islets, also augmented glucose-stimulated insulin release, but unexpectedly to a lesser extent than cytochalasin B. However, in islets stimulated by non-glucidic nutrients cytochalasin D was as potent as cytochalasin B in potentiating the secretory response. This situation coincided with the fact that cytochalasin B inhibited more severely D-glu-

cose metabolism in non-B, as distinct from B islet cells and, in the former case, caused a relatively greater inhibition of hexose catabolism at 2.8 mM than at 16.7 mM D-glucose. These findings suggest a so-far-unidentified interference of cytochalasin B with the B-cell glucose-sensing device.

(3) The copper-containing semicarbazide-sensitive amine oxidase enzyme (SSAO) catalyses the conversion of certain endogenous monoamines, like methylamine into cytotoxic aldehydes, hydrogen peroxide and ammonia. We demonstrated that the physiological activity of serum SSAO enzyme is significantly higher in Type 2 diabetic patients with high-risk proliferative diabetic retinopathy compared to those without retinopathy. Our clinical results support the hypothesis that elevated SSAO activity may be involved in the pathogenesis of microvascular diabetic late complications, such as retinopathy. The pharmacological manipulation of SSAO activity might be an interesting new concept for prevention and treatment of diabetic retinopathy.

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## ANNAMÁRIA GUJDÁR (2005)

# Signalling pathways involved in cell-scattering: the role of protein kinase C

Supervisor: Dr. Anna Faragó

We have demonstrated that protein kinase C (PKC) plays antagonistic regulatory roles in the scattering of HepG2 cells induced by hepatocyte growth factor (HGF)/scatter factor or phorbol ester. Though the activity of PKC accounts for the phorbol ester-induced motility, the HGF-induced migration of HepG2 cells is decreased by PKC.

We have observed that phorbol ester-induced migration of HepG2 cells is accompanied by intensive actin stress fiber formation. The polymerisation of actin stimulated by phorbol ester-activated PKC is not prevented by the inhibition of phosphatidylinositol 3-kinase (PI 3-kinase) indicating that PI 3-kinase is not involved in the initiation of this process. The phorbol ester-induced migration is not prevented by the inhibition of Src tyrosine kinase, either. We have demonstrated that p21-activated protein kinase (PAK) is activated in phorbol ester treated cells, and this effect is also not prevented by the inhibition of PI 3-kinase or Src kinase. PAK 1 is transiently down-regulated during the migration. We have demonstrated that the migration of HepG2 cells is accompanied by enhanced  $\alpha 2$  and  $\alpha 1$  integrin expression. Phorbol ester-activated PKC enhances the integrin expression, while in HGF-induced cells PKC decreases the intensity of integrin expression. The activity of PI 3-kinase is essential for the growth factor-induced integrin expression. We have demonstrated that the duration of PI 3-kinase activation in HGF-treated cells increases when PKC is inhibited. In the EGF signalling system PKC has no similar effect on PI 3-kinase. Our observations suggest that the molecular background of the negative modulatory effect of PKC on the HGF-induced migration is the termination of PI 3-kinase activation. Phorbol ester-activated PKC seems to be able to stimulate the migration of HepG2 cells via another signalling route, which does not require the activity of PI 3-kinase.

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ERIKA GYÓRFFY (2002)

## The analysis of a macromolecular drug carrier, its conjugates and multidrug resistance reversal peptides by high-performance separation techniques

*Supervisor: Dr. György Kéri*

The aims of my research has been to analyse a macromolecular drug carrier and its tyrosin kinase inhibitor-containing conjugates, as well as particular multidrug resistance reversetargeted hydrophobic peptides by high-performance separation techniques. Free zone capillary electrophoresis, micellar electrokinetic chromatographic and gel permeation chromatographic methods have been elaborated and optimised for the separation of the poly-(N-vinylpyrrolidone-co-maleic acid) drug carrier macromolecule and its conjugates. As a result, the presence of extremely strong carboxyl groups on the polymer chain has been demonstrated. A series of carboxyl groups with a wide range of acidity has also been recognised on the polymer chain. These carboxyl groups can influence the behaviour of the free drug carrier and its conjugates in organisms, and the conjugation process itself. Polydispersity of all the investigated macromolecules has also been proved. Micellar electrokinetic chromatographic and reversed phase high performance liquid chromatographic methods have been developed and optimised for the analysis of potential multidrug resistance reversal hydrophobic peptides and for the characterisation of their hydrophobicity. A relationship has been recognised between their hydrophobicity and their biological activity. This relationship can be used in the prescreening of multidrug resistance reversal capacity of entire molecule libraries, before biological testing. The behaviour of the  $k'$  normalised retention factor and its applicability to estimate the hydrophobicity have also been investigated based on the micellar electrokinetic chromatographic experimental data of two homolog series from the literature and of the multidrug resistance reversal hydrophobic peptides. This investigation contributed to the development of the theoretical background of micellar electrokinetic chromatography.

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MIKLÓS HORVÁTH (2003)

## New observations on the anatomy of the olivocochlear system and auditory pathway

*Supervisor: Dr. Ottó Ribári*

Extensive data link the growth associated protein-43 (GAP-43) to axonal elongation and synapse formation during development and in plastic responses of nervous tissue. We have studied the changing levels of GAP-43 expression in the auditory brainstem nuclei of the developing rat, applying immunocytochemical techniques. By the first postnatal day, GAP-43 was expressed at high concentrations in all subdivisions of the cochlear nuclear complex and superior olivary complex. The staining intensity markedly reduced in the following days, but a distinct level of staining persisted into adulthood in all of them, indicating that a relevant potential for plasticity is retained in these auditory structures. We also studied localization and time course of the re-expression of GAP-43 following deafening through cochlear ablation. As a consequence of unilateral cochlear lesioning, a sub-

stantial increase in the expression of GAP-43 was observed in the neuropil of the ipsilateral ventral cochlear nucleus and in cell bodies of the ipsilateral lateral superior olive. This re-expression of GAP-43 indicates that the sudden loss of spiral ganglion cells leads to a reactive synaptogenesis in complex patterns across several auditory brainstem nuclei. The olivocochlear neurons reside in the superior olivary complex and project to the inner and outer hair cell receptors in the cochlea. By injecting the fluorescent retrograde axonal tracers diamidino yellow and fast blue into the cochlea and ventral cochlear nucleus, we studied the distribution and number of olivocochlear neurons with and without axon collaterals into the ventral cochlear nucleus of the rat. We found that lateral olivocochlear neurons residing in the lateral superior olive do not send collaterals to the cochlear nucleus, while the majority of medial olivocochlear cells residing in the ventral nucleus of the trapezoid body do have such collaterals. These cells may thus affect processing in the cochlea and cochlear nucleus as well. In another experimental setting pseudorabies virus was used to label transneuronally descending auditory projections following intracochlear injections. Initially, virus-infected cells were detected immunohistochemically in the olivocochlear cells. At longer postoperative times, labeled cells were found in higher order auditory brainstem nuclei, in the auditory cortex, as well as in the locus coeruleus and pontine dorsal raphe. Viral transneuronal labeling in the auditory cortex after intracochlear application confirms the existence of a continuous descending chain of neurons from the auditory cortex to the cochlea.

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**GERGELY KESZLER (2005)**

## The potential role of deoxycytidine kinase activation in DNA repair and in apoptosis induction

*Supervisor: Dr. Mária Staub*

Deoxycytidine kinase (dCK) plays a central role in the deoxynucleoside salvage processes. Moreover, based on its surprisingly broad substrate specificity, this enzyme is responsible for the bioactivation of several nucleoside analogues of therapeutical importance, influencing the sensitivity of malignant tissues towards chemotherapy. The prevailing view suggested that the activity of dCK is not cell cycle-regulated and seemingly constant. In sharp contrast to this assumption, dCK activity was found to be elevated several fold upon short-term treatments of normal primary lymphocytes with 2-chloro-2'-deoxyadenosine, which might be a potentially important phenomenon with respect to the clinical practice, too. Further investigations showed that the activity of dCK could be augmented equally well both in normal and in leukaemic peripheral lymphocytes, but huge differences were revealed in the extent of stimulation in the malignant cell lines tested. Potentiation of enzyme activity was also elicited by a number of purine and pyrimidine substrate analogues as well as by DNA-damaging agents including the DNA polymerase inhibitor aphidicolin, the topoisomerase II inhibitor etoposide and  $\gamma$ -irradiation. These findings indicated that the main trigger of activation could be the damaged DNA itself, and the biological relevance might be to supply the dNTPs for the enhanced DNA repair, monitored by the Comet assay. Activation of dCK was paralleled by specifically elevated levels of intracellular dATP, raising the possibility that dCK activation might be linked to apoptosis induction. With regard to the mechanism of enzyme activation, no changes were found in the protein levels of dCK upon stimulation. The protein phosphatase inhibitor calyculin A enhanced the activity of dCK, while opposite results were obtained with BAPTA-AM, a cell-permeant calcium chelator, demonstrating that the activation process is calcium dependent and comprises a protein phosphorylation step. A positive correlation was found between the enzymatic activity and the na-

tive immunoreactivity of dCK, strongly arguing that dCK undergoes a conformational change during activation that results in the formation of a catalytically more active steric structure.

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**GÁBOR NARDAI (2003)**

## Effects of diabetic redox changes on stress protein

Supervisor: Dr. Péter Csermely

Stress response is an important and highly conserved phenomenon of cellular defense. Stress proteins were discovered 40 years ago, since then a variety of new members of this family were identified and characterized. As further studies unraveling the increasing number of specific functions of these proteins not just upon stress but also under normal physiological conditions, stress proteins ought to believe to play a central role in cellular environment. There is also a growing interest on these proteins during pathophysiological conditions emphasis made on stress protein injury in various acute tissue injuries and diseases. However, very little is known about the changes of stress proteins in diseases associated with chronic cellular stress. Hence, I have chosen a model system, diabetes mellitus, a chronic and widespread disease causes massive extra and intra-cellular oxidative injuries, which influence the structure and function of stress proteins in the cytoplasm and endoplasmic reticulum. I focussed my study on the possible oxidoreductive enzymatic activities of the cytoplasmic 90-kDa heat shock protein (Hsp90) and examined how environmental oxidative changes influence the Hsp90 chaperone activity. Using animal models I studied the diabetic redox changes of the endoplasmic reticulum and its effect on the structure and function of luminal stress proteins with respect to members of the redox folding processes. My experiments prove that Hsp90 cystein-thiol groups are reactive, reduce cytochrome c, but not protein disulfide bridges. Further experiments prove that the redox environment influences Hsp90 activity, oxidative changes of the environment decrease the passive chaperone activity of the protein. We also showed that the effect of the ascorbate/dehydroascorbate redox system on the in vitro function of Hsp90. Both oxidative stress and the changes of the ascorbate/dehydroascorbate system are characteristic of diabetes mellitus, so our results indicate a possible alteration of Hsp90 function in this disease.

Our experiments on diabetic animals verified that the environment of the endoplasmic reticulum changes to more reducing state. The reductive changes of the lumen influence the functional redox state of the stress proteins catalyzing oxidative disulfide bond formation in proteins (protein disulfide isomerases). The reductive shift of the protein-thiols was reoxidizable by glutathione-disulfide and dehydroascorbate on isolated diabetic rat microsomes. These results indicate that beside the well-known glutathione redox system the oxidative protein folding can be influenced by the ascorbate/dehydroascorbate redox system.

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ZSÓFIA NEMODA (2003)

## Investigation and psychological associations of the polymorphisms in the serotonin transporter and the dopamine D4 receptor gene

Supervisor: Dr. Mária Sasvári

The search for genetic risk factors of complex inherited disorders, including personality traits and psychiatric syndromes, has been attracting more attention recently. In our laboratory we have started studying the polymorphisms of the dopamine D4 receptor (DRD4) and the serotonin transporter (5HTT) gene. We have genotyped 334 healthy Hungarian individuals for the VNTR (Variable Number of Tandem Repeats) in the promoter region of the 5HTT gene (5HTTLPR). The obtained genotype frequencies were in Hardy-Weinberg equilibrium, verifying our genotyping method. In addition, we have developed a direct haplotype determining method for two adjacent polymorphisms in the 5' non-coding region of the DRD4 gene, namely for the -521C/T SNP and the 120 bp duplication. We have verified this method by obtaining haplotypes with a family-based approach.

In our psychogenetic association studies we have searched for genetic components of temperament and attachment behavior in infants. The phenotype was determined by Dr. Judit Gervai and her colleagues (Institute of Psychology, Hungarian Academy of Sciences) using a videotaped laboratory procedure, the Ainsworth Strange Situation Test. Studying the temperament dimensions, we have found an association between the 5HTTLPR s/s genotype and the infants' observed responses to a novel social and an anxiety-provoking stimulus (the appearance and approach by a stranger). We have also shown an interaction between the 5HTTLPR and the DRD4 48 bp VNTR: the s/s 7<sup>+</sup> group spent significantly more time in the anxious state in the presence of the stranger, than the l/l+1/s 7<sup>+</sup> group ( $p=0.043$ ). Regarding the attachment behavior: we have found that the 7-repeat allele of the DRD4 48 bp VNTR is over represented in the disorganized attachment group ( $p<0.005$ ). The -521T allele has strengthened the effect of the 7-repeat form on the disorganized attachment behavior (for CT genotype:  $p=0.015$ , for TT genotype:  $p=0.024$ ). In the presence of both risk alleles the odds ratio for disorganized attachment is 12-fold. As far as we know, this was the first report on specific genetic risk factors for disorganized attachment behavior.

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KRISZTINA NÉMETH (2003)

## The role of neutrophil granulocytas in tissue damage

Supervisor: Dr. Balázs Sarkadi

Neutrophil granulocytes are one of the elements of the aspecific immune system. They play a key role in the host's defense and in the elimination of tissue debris during acute inflammation concomitant with tissue injury. Since the reactive oxygen intermediates and enzymes released by neutrophil granulocytes are non-specific for the particles to be eliminated the surrounding tissues can be damaged as well. Formyl-Met-Leu-Phe (fMLP) induced ROI production of rat neutrophils commonly used as models in studying the role of neutrophil activation in tissue injury was measured by

luminol-dependent chemiluminescence (LDCL). According to our findings the LDCL signal is biphasic, and in the first phase luminol is oxidized by the hydroxyl radicals, while in the second one peroxidation of luminol by hydrogen peroxide is catalyzed by myeloperoxidase. In contrast to that found with the human neutrophils in case of rat neutrophils both phases of LDCL signal were of extracellular origin.

Neutrophils migrate towards the site of inflammation against an increasing concentration gradient of chemotactic agents. This situation was modeled by activating cells by consecutive fMLP stimuli. ROI production, degranulation and changes in intracellular  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_i$ ) concentration of human polymorphonuclear leukocytes (PMNLs) upon the first and second stimuli were studied. The fMLP-concentration dependence of the IC  $\text{Ca}^{2+}$  signal was different from that one found for ROI production. The lack of correlation between the intensities of these two signals shows that changes in  $[\text{Ca}^{2+}]_i$  are not proportional to and not exclusively determinative for the extent of ROI production. In addition these two functions were desensitized by different fMLP concentrations suggesting that ROI production and changes in  $[\text{Ca}^{2+}]_i$  are modulated in a different manner. The degranulation of PMNLs was determined as the amount of  $\beta$ -glucuronidase (GLU) and myeloperoxidase (MPO) released from azurophil granules. According to our results no more than about 36% of GLU or MPO can be mobilized either by a single or by consecutive fMLP stimuli. In case of single fMLP stimulus the ratio of GLU/MPO released decreases with the increasing concentration of fMLP. ROI production and degranulation were measured in blood samples obtained from various groups of clinically ill patients in order to reveal the role of neutrophils in aggravating tissue injury and to find parameter(s) of neutrophil function possibly being predictive for patients' outcome. Reperfusion during coronary bypass surgery resulted in a decrease of PMNL counts and an elevation of ROI production of the single cells between the aortic and sinus coronarius blood samples. These PMNL-parameters were correlated with first-postoperative-day concentration of troponin T of serum characteristic to the heart muscle injury. Furthermore, the concentration of troponin T in the serum reaches its maximum on the first postoperative day. According to our measurements the tendency of changes in APACHE II scores of acut pancreatic patients could be predicted from the intensity of the fMLP induced ROI production of PMNLs' measured on the previous day. In a previous study it was found that TP4 treatment of traumatic patients resulted in a reduction of nosocomial infections and acceleration of convalescence. We found, that changes in the neutrophil counts, in the ROI producing activity of the whole blood and in the serum elastase concentration of patients suffering from hip fracture and treated with TP4 were more balanced, compared to those of treated with placebo. Thus, we considered TP4 as to be a good candidate for keeping the inflammatory response between rational limits.

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**RITA RÉKA PADÁNYI (2004)**

## **Studies on the auto-inhibitory interactions of the plasma membrane $\text{Ca}^{2+}$ ATPase**

*Supervisor: Dr. Balázs Sarkadi*

The plasma membrane calcium pump (PMCA) is the sole  $\text{Ca}^{2+}$  extrusion system in many cells that is responsible for maintaining the low cytosolic calcium concentration critical to cell function. The role of this protein is to eject  $\text{Ca}^{2+}$  from the cytosol to the extracellular space using the energy of ATP. The carboxyl terminus of PMCA contains a high affinity calmodulin-binding sequence that interacts with the catalytic core and inhibits the activity of the pump in resting cells. In activated cells binding of  $\text{Ca}^{2+}$ -calmodulin to the calmodulin-binding sequence frees the catalytic core from the

auto-inhibition and the pump becomes activated. In addition to the changes between the activated and inhibited states, the conformation of the pump also changes between the high (E1) and low (E2) Ca<sup>2+</sup> affinity states during the reaction cycle. In this study we utilized limited proteolysis and site directed mutagenesis to monitor the regions involved in the auto-inhibitory interactions of the ubiquitous PMCA4b. We used three different proteases that all cleaved the protein at its carboxyl terminus upstream (caspase-3) or downstream (chymotrypsin and calpain) of the calmodulin-binding sequence. We found that the fragmentation highly depended on the conformational state. When the pump was in the auto-inhibited state and the E1-E2 equilibrium was shifted more towards the E2 conformation (in the absence of Ca<sup>2+</sup>) the C-terminal region was resistant to proteolysis because of tight intra-molecular interactions. When the equilibrium was shifted more towards the E1 conformation (in the presence of Ca<sup>2+</sup>) the cleavage sites became more exposed and the pump was partially degraded suggesting that the interaction between the C-terminal region and the catalytic core was weaker. Binding of Ca<sup>2+</sup>-calmodulin to the binding site made the carboxyl terminus more flexible and the fragmentation more intensive. Our experiments show that the conformational changes in the C-terminal region induced by Ca<sup>2+</sup> or by Ca<sup>2+</sup>-calmodulin extend to the regions both upstream and downstream of the calmodulin-binding sequence. In contrast, replacing Trp1093 (a key residue within the calmodulin-binding sequence) to an alanine exposed only proteolytic sites within the calmodulin-binding sequence. We also showed that an aspartate residue (Asp1080, the target of caspase-3 cleavage), although, located outside of the calmodulin-binding sequence greatly contributes to the stabilization of the auto-inhibited conformation of the pump.

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## ISTVÁN REIBER (2003)

### Investigations of postprandial lipaemia and genes mutations in familial dyslipidemia

Supervisor: Dr. Albert Császár

We have investigated the postprandial lipemia in several risk factor groups for cardiovascular disease, two mutations in the exon region of lipoprotein lipase gene, the polymorphism of apolipoprotein E, the T-1131C polymorphism of apolipoprotein AV and their frequencies and roles in familial combined hyperlipidemia (FCH).

We found normal fasting triglyceride levels and abnormal higher and extended postprandial lipemia in higher risk groups for vascular diseases and in a part of healthy FCH family members. The distributions of PvuII and HindIII polymorphisms in FCH were different compared to normolipidemic controls. At the same time we did not find any associations between these lipoprotein lipase mutations and fasting serum lipid levels. In patients with type 2 diabetes and moderate dyslipidemia did not exhibit the PvuII polymorphism of lipoprotein lipase typical features. We found significantly higher incidence of apo e4 allele in metabolic syndrome and FCH. We demonstrated higher and extended postprandial lipemia in FCH subjects with apo E4/3 genotype. In Hungarian FCH group investigated by us the minor allele of apolipoprotein AV T/C polymorphism were seen more frequently. The carrying status of the minor allele accompanied with higher fasting lipid levels. We investigated first the association between apolipoprotein AV T/C polymorphism and postprandial lipemia in FCH. Our results suggest a decreased and extended catabolism of the remnants in FCH caused by apoAV T/C promoter variation that seems to have a more direct effect on the postprandial status than that of apoE 3/3-4/3 polymorphism.

The knowledge of characteristics of postprandial lipemia influenced by mutations of genes described by us are more useful as only the fasting triglyceride level, and it is as effective as HDL-cholesterol value in the measuring of prognosis of development of vascular disease with athero-thrombotic origin.

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**ANDRÁS SZARKA (2003)**

## Role of antioxidants in the formation of protein disulfide bonds

*Supervisor: Dr. Gábor Bánhegyi*

The effect of certain antioxidants in the electron transfer process of the protein disulfide bond formation was investigated in rat microsomal vesicles. Our most important new states are the the followings: - The ER membrane owns a cytoplasmic orientated ascorbate oxidase activity. The activity presumably belongs to a copper contained metalloprotein. - The microsomal ascorbate oxidase activity – in the presence of ascorbate – maintains permanent ascorbyl free radical and dehydroascorbate levels. - The microsomal ascorbate oxidase activity is required for the ascorbate dependent disulfide bond formation. - a-tocopherol content of the microsomal membrane plays an intermembrane electron transfer role between the intraluminal ascorbate and the extravesicular reactive oxygen containing species, contributing to the formation of disulfide bonds. The ascorbate is oxidized to dehydroascorbate – through ascorbyl free radical intermediate – by a microsomal metalloenzyme on the outer surface of the endoplasmic reticulum. The dehydroascorbate is transported into the lumen of endoplasmic reticulum by its transporter. Reactive oxygen species are generated during the oxidation of ascorbate. These compounds can react with vitamin E directly or indirectly through membrane lipids. The result of these reactions is a-tocopheryl radical. It can rereduce on the expense of intraluminal ascorbate, form further amount of dehydroascorbate. In the lumen dehydroascorbate can oxidize the thiol groups of target proteins, in the process mediated by protein disulfide isomerase or other proteins, capable the catalysation of thiol-disulfide exchange. The formation of protein disulfide bridges can be explained by our system in vitro, however the situation in vivo is not known yet. It is a thorny question that the pathway hallmarked by ascorbate is the main route to disulfid bond formation.

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**PÉTER TAMÁS (2003)**

## **Mechanism of EGF regulation of Vav2, a guanine nucleotide exchange factor for Rac**

*Supervisor: Dr. László Buday*

Vav2 is a member of the Vav family that serves as guanine nucleotide exchange factors (GEFs) for the Rho family of Ras-related GTP-ases. Unlike Vav1, whose expression is restricted to cells of hematopoietic origin, Vav2 is broadly expressed. Vav2 was identified as a substrate for EGF receptor, however the mechanism by which Vav2 is activated in EGF-treated cells remained unclear. In our experiments we characterized the mechanism of Vav2 activation in the EGF receptor signaling pathway. By the means of an *in vitro* protein kinase assay, we found, that purified and activated EGF receptor phosphorylated Vav2 exclusively on its N terminal domain.

Furthermore, EGF receptor phosphorylated Vav2 on all three possible phosphorylation sites, Tyr-142, Tyr-159, Tyr-172. The EGF dependent phosphorylation of these Tyr residues was also verified in intact cells. We also showed that that Vav2 associated with the activated EGF receptor in an SH2 domain dependent manner. Using a phosphopeptide competition assay we found that Vav2 SH2 domain binded preferentially to autophosphorylation sites pTyr- 992 and 1148 of EGF receptor. According to our two independent experiments this association was prerequisite for phosphorylation of Vav2 by EGF receptor. To investigate the regulation of Vav2 exchange activity, we measured Rac activation by a PAK pull down assay in EGF treated cells overexpressing different mutant proteins. Treatment of cells with EGF resulted in stimulation of exchange activity of Vav2, however the intensity of the exchange activity did not show any correlation with the phosphorylation level of our different tyrosine mutant proteins. Introducing a point mutation into the Vav2 PH domain or treatment of cells with the PI- 3 kinase inhibitor LY294002 prior to EGF stimulation inhibited Vav2 exchange activity. We investigated the effect of our mutants on the actin cytoskeleton structure of cells by fluorescent microscopy.

Although phosphorylation mutants of Vav2 could readily induce actin rearrangement in Cos7 cells, PH domain mutant did not stimulate membrane ruffling. These results suggest that EGF regulates Vav2 activity basically through PI 3- kinase activation, whereas tyrosine phosphorylation of Vav2 may rather be necessary for mediating protein - protein interactions.

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**SÁNDOR VALENT (2004)**

## **Role of tetrahydrobiopterin in the regulation of NO synthase of human placenta and its chemical stabilization by ascorbate**

*Supervisor: Dr. Miklós Tóth*

**Introduction:** The eNOS activity plays important roles in the regulation of the placental circulation and the pathogenesis of the preeclampsia. It forms NO from arginine in the presence of tetrahydrobiopterin (BH4). Because of rapid BH4 autooxidation, the factors preserving BH4 concentration may be important from the aspect of NO production. BH4 is stabilized by ascorbate but the mechanism of this has not been clarified. **Methods:** The eNOS activity was measured by the conversion of 3H- and 14C-arginine to citrulline, the BH4 level by HPLC analysis, and the ascorbate concentration and the oxidation of BH4 and ascorbate mol/l BH4 the by spectrophotometry. **Results:** In the

presence of 20 nmol/l - 1 eNOS activity is increased 2.5 fold in first trimester placenta. Placental BH4 concentration decreases to 30 % during pregnancy. The EC50 value is 79 nmol/l, thus the eNOS activity is 70 and 41 % of the possible maximal activity in first trimester and term pregnancies, respectively. In contrast with normal (n=4) and BH4 responsive preeclamptic (n=3) groups physiological BH4 concentrations do not increase the eNOS activity in the BH4 resistant preeclamptic group (n=7). In the mol/l ascorbate increases the eNOS $\mu$ microsomal fractions of human placenta 100 activity. The oxidation of ascorbate is accelerated in the presence of BH4. The oxidation rate is suppressed by catalase but still remains about 2 fold higher than without BH4. Ascorbate decreases the rate of BH4 autooxidation. The apparent second-order rate constant of BH4 oxidation is decreased by ascorbate in 22 - 37 °C range to  $64 \pm 0.77$  and  $51 \pm 0.67$  % (mean  $\pm$  SEM, n=12) in the absence and the presence of catalase, respectively. The calculated Arrhenius activation energies are nearly identical. Discussion: The decrease of BH4 level until term and its individual variations may cause significant differences in eNOS activity during pregnancy. In certain preeclamptic cases a decreased BH4 activation of eNOS activity can be observed. Supposedly, in these patients a defect of the BH4 binding to eNOS contributes to the increased formation of superoxide, hydrogenperoxide and peroxynitrite and to the oxidative stress. The stabilization of BH4 level is important in the maintenance of foeto- and maternoplacental circulations. Ascorbate may contribute to this by the direct reduction of quinonoid BH2 to BH4.

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- Tóth M, Kukor Z, Valent S (2002) *Chemical stabilization of tetrahydrobiopterin by L-ascorbic acid: contribution to placental nitric oxide synthase III activity.* *Mol Human Reprod* 8:271-280.
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### 7/3. PROGRAM

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## EMBRYOLOGY, THEORETICAL, EXPERIMENTAL AND CLINICAL DEVELOPMENTAL BIOLOGY

### *Coordinator*

**Imre OLÁH**

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The program deals with the progressive changes of the vertebrate organisms from the primordial germ cells through the fertilization up to the organogenesis. During this progressive development primary tissues (epithelial, supporting, muscle and nervous tissues) are formed from different stem cells or progenitors, what is timely and spatially strictly regulated at genetic and molecular level. The Program focuses on the early formation of lymphoid organs including the epithelio-mesenchymal interactions (thymus and bursa of Fabricii), formation of hemopoietic sites, cardiac anlage, accessory dendritic cells of the immune system and the effect of different environmental agents on the ontogeny. The ontogeny of the visual system and its relationship with the circadian rhythms (pineal body) is a significant and progressive topic of the Program. The endocytosis of the monocyte-macrophage system is also a rapidly expanding area of the Program. The methods used by the Program incorporate wide range of different techniques like light- and electron microscopy, immunocytochemistry, immunofluorescence combined with confocal microscopy, monoclonal antibody production, tissue culture, embryo manipulation (ablation and transplantation of embryonic organ rudiments, chimaerism, parabiosis), Western blotting.

*Sub-programs*

Development of color-specific cones in the retina		Ágoston SZÉL
Pre- and postembryonic development of epithalamus and pineal body		Béla VÍGH
Development of lymphoepitheloid tissues and organs		Imre OLÁH
Entoderm and the development of branchial derivatives		Imre OLÁH
Peripheral blood fibrocytes in the organogenesis		Imre OLÁH
Prenatal stress and the neuroendocrine system		Ágnes NEMESKÉRI
Membrane transport processes (endocytosis, exocytosis). Signal transduction in the development of the immune cells	Signal	Anna L. KISS

*Supervisors***Ph.D. students**

	<b>p</b>	<b>Supervisors</b>
Éva Bíró	ft	Imre Oláh
Erzsébet Botos	ft	Anna L. Kiss
Csaba Dávid	ft (a)	Béla Vígh
Zoltán Hajdú	ft	Imre Oláh
Botond Zoltán Igyártó	ft (a)	Attila Magyar
Arnold Szabó	ft	Ágoston Szél
Vanda Olga Szlávik	ft	Gábor Varga
Miklós Tóth	ft (a)	Imre Oláh

**No Ph.D. candidates in this program at the moment****Ph.D. graduates**

		<b>Supervisors</b>
Ákos Lukáts	ft	Ágoston Szél
Krisztina Minkó	ft	Imre Oláh

a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

**ÁKOS LUKÁTS (2004)****Photopigment coexpression in mammals: comparative and developmental aspects**

*Supervisor: Dr. Ágoston Szél*

In mammals, up to the recent past, each cone photoreceptor had been thought to contain only a single type of photopigment. It was not before the early 1990s that the first case of photopigment coexpression (dual cones) was reported, and even today, the phenomenon remains rather mysterious. In some species, such as the house mouse, the distribution of color sensitive cones shows a characteristic division (divided retinas). Whereas in the upper retinal field the ratio of short wave sensitive (S) to middle-to-long wave sensitive (M/L) cones falls in the usual range (1:10), in the ventral retinal field M/L-pigment expression is completely missing. In the transitional zone of the two fields, dual cones are visible in great number (spatial photopigment coexpression). In some other species without retinal division dual cones appear during development. In these latter cases M/L-cones probably develop from S-cones, with dual elements representing a transitory stage in M/L-cone differentiation, and disappearing with retinal maturation (transitory photopigment coexpression). These two reported patterns seem to be mutually exclusive, only either of them being present in individual species. In this thesis both coexpression patterns are examined. The retinal cone distribution of eight rodent species is reported in the comparative part. Two cases are discussed in detail, where dual cones appear in adult specimen without retinal division, proving that the phenomenon is much more common, than previously thought. Dual elements either occupy the dorsal peripheral retina, or - in two species, the Siberian hamster and the pouched mouse - they even make up the entire cone popula-

tion. This is the first observation in the literature showing all cones of the retina to be dual cones. This finding makes these species good models for the study of molecular control mechanisms of opsin expression and renders them suitable as sources of dual cones for future investigations on the role and neural connections of this peculiar cone type. In the developmental part the retinal maturation of two species - the tree shrew and the common rabbit - is examined in detail to prove or to exclude the presence of transitory photopigment coexpression. In both species S-pigment expression precedes M/L-pigment, but while in tree shrew dual cones could be identified in a small number during retinal development, they are completely missing from the developing rabbit retina. These results exclude a common mechanism for M/L-cone maturation: they either develop from S-cones with transdifferentiation or independently from them.

- Lukáts Á, Dkhissi-Benyahya O, Szepessy Zs, Röhlich P, Vigh B, Bennett NC, Cooper HM, Szél Á (2002) Visual pigment coexpression in all cones of two rodents, the Siberian hamster and the pouched mouse. *Invest Ophthalmol Vis Sci* 43:2468-2473.
- Szepessy Zs, Lukáts Á, Fekete T, Barsi Á, Röhlich P, Szél Á (2000) Cone differentiation with no photopigment coexpression. *Invest Ophthalmol Vis Sci* 41:3171-3175.
- Szél Á, Lukáts Á, Fekete T, Szepessy Zs, Röhlich P (2000) Photoreceptor distribution in the retinas of subprimate mammals. *J Opt Soc Am A* 17:568-579.

**KRISZTINA MINKÓ (2005)**

## **Molecular aspects of haematopoietic stem cell emergence in the avian embryo**

*Supervisor: Dr. Imre Oláh*

The detailed comparative expression patterns of key transcription factors in haematopoiesis (such as SCL, Lmo2 and GATA factors) have been established during avian embryonic haematopoiesis. These factors were chosen because molecular studies have established they work together in multimeric transcription factor complexes. In order to study the gene expression pattern of Lmo2, its chicken ortholog has been isolated and characterised. Two distinct expression patterns for GATA-2 were found in the yolk sac. One low, associated to the lateral-posterior mesoderm, and a second high, blood island-specific. This latter is contemporary with GATA-1 expression and post-dates SCL and Lmo2 in the hemangioblast population. Such a bimodal GATA-2 expression has not been reported before on other vertebrate models. According to the expression patterns, it seems that Lmo2 is equally important in the development of both the endothelial and the haematopoietic lineages, in contrast to SCL. The molecular patterns revealed in the developing allantois mesoderm lead us to conclude that both angioblasts and haematopoietic progenitors, or hemangioblasts are derived in situ from the mesoderm prior to the establishment of vascular connections between the allantois and the embryo. Additionally, two GATA factors were detected in the endodermal layer. GATA-3 expression indicated the presumptive area of allantoic endoderm, and was followed by the switching on of GATA-2. The established expression patterns in the yolk sac and allantois mesoderm refers predominantly to the development of the erythroid lineage. In contrast, the expression patterns in the intra-aortic haematopoietic clusters indicate progenitor emergence, with the presence of early haematopoietic marker genes -such as SCL, GATA-2, 3 and Lmo2- and the absence of the lineage specific GATA-1. The haematopoietic potential of the E3 haematopoietic cluster-containing dorsal aorta has been demonstrated in vivo, in quail-chick chimera experiments. Grafting was performed one day earlier than that was previously reported. The unexpected finding of these experiments is that the implanted cells of the aorta region were found preferentially in the thymus of the host embryos.

- Caprioli A, Minkó K, Drevon C, Eichmann A, Dieterlen-Lièvre F, Jaffredo T (2001) Hemangioblast commitment in the avian allantois: cellular and molecular aspects. *Dev Biol* 238:64-78.
- Minkó K, Oláh I (1996) Expression of intermediate filaments and N-cadherin adhesion molecule in the thymus of domesticated birds. *Acta Biol Hung* 47:323-340.
- Jaffredo T, Alais S, Bollerot K., Drevon C, Gautier R., Guezguez B, Minkó K., Vigneron P, Dunon D (2003) Avian HSC emergence, migration, and commitment toward the T cell lineage. *FEMS Immunol Med Microbiol* 1635:1-8.

**7/4. PROGRAM****HUMAN MOLECULAR GENETICS AND GENE DIAGNOSTICS****Coordinator****András FALUS**

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Aim: To provide an overview on various fields of human medical and molecular genetics, genomics, including theory and methodology.

*Sub-programs*

Bioinformatic analysis of expression gene chip data  
 Gene expression analysis of murine mast cell differentiation  
 Genomic investigation of the pathomechanism of allergy and asthma  
 Molecular studies on oncohaematology  
 Molecular studies on coagulopathology  
 Cardiac differentiation in genetically histamine free transgenic model animals  
 Analysis of tumor progression and histamine production in transgenic model animal  
 ALL genomics  
 Molecular biology of histamine and cytokineresearch  
 Prenatal molecular genetic diagnosis for prevention of inherited monogenic disorders  
 Genetic diagnostic methods in clinical pediatrics  
 Molecular genetic approach in forensic medicine  
 Forensic DNA criminal profiling  
 Genetics of lipid metabolism  
 LDL receptor, ApoB, ApoE and ACE genetic polymorphisms in early atherosclerosis  
 Genetic polymorphism of proteins and receptors involved in lipid metabolism in different complement factor (C4A, C4B, Bf, C3) allotype carriers in atherosclerosis and liver cirrhosis  
 Corticosteroid receptors, p53 and bcl 2 in corticosteroid induced apoptosis and in acute lymphoid leukemia  
 Molecular medicine  
 Role of growth factors in progression of chronic liver diseases. Role of proteoglycans in lymphoproliferative processes. Analysis of tumor-stroma interactions. Genetic diagnosis in pathology.  
 Northern- and Wester blot analysis of the expression of Waf 1, p53, PCNA, cyclins, and cdk's in different phases of the cell cycle.

*Supervisors*

Csaba SZALAI  
 Zoltán WIENER  
 Csaba SZALAI  
 Imre BODÓ  
 Imre BODÓ  
 Sára TÓTH  
 András FALUS  
 András FALUS  
 András FALUS  
 Zoltán PAPP,  
 Margit NÉMETHI  
 György FEKETE,  
 AndrásVÁRADI  
 Péter SÓTONYI  
 Péter SÓTONYI  
 László ROMICS  
 László ROMICS  
 György FÜST,  
 László ROMICS  
 Béla SZENDE  
 József BOCSI  
 László KOPPER  
 László KOPPER,  
 Ilona KOVALSZKY  
 Zoltán MARCSEK

*Sub-programs*

Analysis of tumor suppressor gene cluster on short arm of human chromosome 3. Expression of classical and novel 3p21 genes in tumor and in non transformed cell lines in vitro, analysis of tumor suppressor genes localized to these loci.

Analysis and isolation of genes expressed differentially in different phases of a model cell line in vitro.

Molecular oncology

Apoptosis

Mapping unknown gene sequences in healthy and pathologic human cells.

Regulation of proliferation and gene expression of epithelial glands.

Molecular biology of exocrine glands in normal and pathological conditions.

Molecular endocrinology

Molecular genetic analysis of pathodermatological mechanisms

Clinical and molecular biological analysis in disorders of skin keratinization.

In vitro examination of melanoma cell lines according to their chemotactic ability and HDC activity

Regulation of HDC gene expression in physiological and pathological processes.

Bikological and clinical aspects of chemotaxis

Connections between histamine and sexual steroid metabolism – studies on a transgenic mouse model.

Molecular biology of malignant haematological diseases

Investigation of the pathomechanism of rheumatology diseases

Prognostic significance of cytogenetic analysis in childhood acute, and in adult chronic leukemia.

Analysis of nucleotide binding domains of human MRP1 ABC transporter and investigation of the catalytic cycle of the protein.

Role of diabetes in the apoptotic process following cerebral ischaemia

Umbilical cord stem cells in leukemia therapy

Molecular biological studies in muscular dystrophy

Molecular biologic and genetic investigations of the pathomechanism of allergy and asthma

*Supervisors*

Zoltán MARCSEK

Zoltán MARCSEK

Zoltán MARCSEK  
Béla SZENDE  
Antal CZINNER

Gábor VARGA

Gábor VARGA

Gábor VARGA  
Sarolta KÁRPÁTI  
Sarolta KÁRPÁTI

Zsuzsa DARVAS

Zsuzsa DARVAS

László KÓHIDAI  
Erna PAP

Edit BUZÁS  
Edit BUZÁS  
György FEKETE

András VÁRADI

András VÁRADI

Sára TÓTH,  
Zoltán WIENER  
Veronika KARCAGI  
Csaba SZALAI

***Ph.D. students***

Réka Benkő ft

Dániel Erdélyi ft (a)

Orsolya Hegyesi pt

Judit Horváth ft (a)

Zsuzsanna Horváth ft

Ivett Jelinek ft

Márk Keresztesi pt

Zoltán Kornseé ft

László Losonczi ft

***Supervisors***

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Gábor Varga

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Valéria László

Zsuzsa Darvas

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György Fekete

Ilona Milánkovic	pt	Veronika Karcagi
Marianna Murányi	pt	András Váradi
Krisztián Németh	ft	Sarolta Kárpáti
Zsuzsanna Ortutay	ft (a)	Edit Buzás
Mária Pásztói	ft	Edit Buzás
Barbara Patócs (Zentainé)	ft	György Fekete
Henriett Pikó	pt (a)	Veronika Karcagi
Petra Mária Scharek	pt	András Falus
Gergely Tölgyesi	ft	Csaba Szalai
Krisztina Török	pt	László Kóhidai

**Ph.D. candidates**

Gusztáv Bélteki	i
Irén Haltrich*	pt
Éva Pállinger	na
Emőke Rácz	ft

(\*Defended after Nov. 2005)

**Ph.D. graduates**

Márta Csikós	ft
Szilvia Désaknai	pt
Miklós Ivor Garami	na
Krisztina Hegyi	ft
Erika Héninger	pt
Marianna Csilla Holub	pt
László Keresztury	pt
Tibor Gergely Kozma	ft
Gergely Kriván	pt
Zsolt Melczer	na
Adrienne Nagy	pt
Zoltán Pócs	pt
Gábor Zoltán Rácz	pt

**Supervisors**

András Falus
György Fekete
András Falus
Sarolta Kárpáti

**Supervisors**

Sarolta Kárpáti
Géza Sáfrány
György Fekete
Sára Tóth
András Falus
András Falus
András Falus
Csaba Szalai
András Falus
András Falus
Csaba Szalai
Sára Tóth
Gábor Varga

a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

**MÁRTA CSIKÓS (2004)****Molecular genetic studies in epidermolytic genodermatoses***Supervisor: Dr. Sarolta Kárpáti*

Clinical and molecular genetical studies in different epidermolytic genodermatoses are presented. These inheritable skin diseases are characterized by blister formation of the skin and mucous membranes induced by mechanical stress. In some cases abnormal keratinisation is also present. This study presents several novel mutations in the *KRT9*, *KRT5/14* and *COL7A1* genes based on the strategy of heteroduplex analysis introduced as a novel method in our laboratory. In terms of phenotypes, clinical and ultrastructural features of epidermolytic palmoplantar keratoderma (EPPK), epidermolysis bullosa simplex (EBS) as well as dominant and recessive forms of dystrophic epidermolysis bullosa (EBD) are discussed.

In one large family with EPPK the novel 479A→T, N160I mutation of the *KRT9* gene was identified. The first molecular genetic studies from Hungary on the *KRT5/14* genes are also presented. In two patients with the most severe form of EBS (Dowling-Meara) two novel missense mutations (369T→A, N123K and 373C→G, R125G) were detected in the evolutionary conserved aminoterminal end of the *KRT14* gene. In the milder Weber-Cockayne variant form of EBS the novel 397G→T, V133L mutation

was found in *KRT14*. In families affected by different subtypes of EBD many novel mutations were identified in the large *COL7A1* gene. One of them, the 425A→G, K142R splice site mutation, was present in 12.8% of the Central European EBD population. This observation may change the of *COL7A1* mutation strategy screening in this geographical area. Various cutaneous and extracutaneous complications in patients carrying *COL7A1* mutations, such as secondary reactive pulmonary amyloidosis, described by our group in EBD, are also included. These informations are of importance for clinicians taking care of EB patients.

- Csikós M, Szalai Z, Becker K, Sebok B, Schneider I, Horváth A, Kárpáti S (2004) Novel Keratin 14 Gene Mutations in Hungarian Patients with Epidermolysis Bullosa Simplex. *Exp Dermatol* 13:185-191.
- Csikós M, Holló P, Becker K, Rác E, Horváth A, Kárpáti S (2003) Novel N160I mutation of keratin 9 in a large pedigree from Hungary with epidermolytic palmoplantar keratoderma. *Acta Derm Venereol* 83:303-305.
- Csikós M, Orosz Zs, Bottlik Gy, Szócs H, Szalai Z, Rozgonyi Z, Hársing J, Török É, Bruckner-Tuderman L, Horváth A, Kárpáti S (2003) Dystrophic epidermolysis bullosa complicated by cutaneous squamous cell carcinoma and pulmonary and renal amyloidosis. *Clin Exp Dermatol* 28:163-166.

### SZILVIA DÉSAKNAI (2003)

## Treatment of gliomas with cytokine-producing cancer cell vaccines and gene directed enzyme pro-drug therapy in murine tumor model

Supervisor: Dr. Géza Sáfrány

The aim of our study was to investigate the effect of gene therapeutic methods in the treatment of gliomas, which constitute a considerable part of malignant intracranial tumors. The therapeutic effect of cytokine producing cancer cell vaccines and gene directed enzyme pro-drug therapy was studied under in vitro circumstances and in a mouse tumor model. Adenovirus vectors were used to transduce the tumor cells with the therapeutic genes. Both methods were combined with local irradiation.

First, the effect of cytokine producing cancer cell vaccines was investigated. According to our findings the cytokine level produced by the vaccines is linearly related to the number of viral particles per cell, thus it is easy to alter the cytokine level secreted by the gene-modified cancer cells. Our data suggested that the anticancer effect strongly depended on the concentration of cytokines, which indicated that the determination of the optimal cytokine level was essential. Vaccines producing IL-2, IL-4, IL-12, GM-CSF cured 20-40% of the mice. CD4+ lymphocytes were involved in the antitumor response of the vaccines, GM-CSF induced CD8+ infiltration of the tumors, as well. Combining the cytokine vaccination with local tumor irradiation increased the survival up to 80-100%.

Next, the effect of gene directed enzyme pro-drug therapy was studied by combining the thymidine kinase/ganciclovir and the uracil phosphoribosyltransferase/5-fluorouracil systems. Our data indicated the synergistic effect of the two different drug sensitising system: The combined treatment with both 5-fluorouracil and ganciclovir enhanced the survival of brain tumor bearing mice more efficiently (60-80%), than the single agent treatment either with 5-fluorouracil (10-20%) or with ganciclovir (40-50%) alone. Combination with irradiation increased further the anticancer effect: 90-100% of the animals survived.

Our results suggest that the cytokine producing cancer cell vaccines and gene directed enzyme pro-drug therapy are very effective in experimental brain tumor model, especially applied with local irradiation. Gene therapy combined with existing anticancer modalities might open a new potential on the treatment of malignant tumors.

- Désaknai S, Lumniczky K, Ésik O, Hamada H, Sáfrány G (2003) Local tumour irradiation enhances the anti-tumour effect of a double-suicide gene therapy system in a murine glioma model. *J Gene Med* 5:377-385.

- Désaknai S, Lumniczky K, Hidvégi EJ, Hamada H, Sáfrány G (2001) Treatment of brain tumors with IL-2 and IL-12 producing autologous cancer cell vaccines. *Adv Exp Med Biol* 495:369-372.
- Lumniczky K, Désaknai S, Mangel L, Szende B, Hamada H, Hidvégi EJ, Sáfrány G (2002) Local tumor irradiation augments the antitumor effect of cytokine-producing autologous cancer cell vaccines in a murine glioma model. *Cancer Gene Ther* 9:44-52.

**MIKLÓS IVOR GARAMI (2005)**

## **Genetic and epidemiological analysis in cystic fibrosis, congenital adrenal hyperplasia caused by 21-hydroxylase deficiency and atrial natriuretic gene expression**

*Supervisor: Dr. György Fekete*

The presented theses include investigations of genetic (mutation and polymorphism) analyses in four genes: cystic fibrosis (CF, CFTR) gene, 21-(steroid)-hydroxylase (21-OHD, CYP21) gene and human natriuretic peptide genes (hANP, hBNP). In CF and CAH disorders the correlation between the genetic observation and the clinical symptoms were also investigated. Cystic fibrosis is one of the most common autosomal recessive disorders in the Caucasian population. A nationwide, standardized clinical questionnaire form has been used to determine and follow the severity of the disease. Hungarian Cystic Fibrosis Database was established and analyzed, in order to determine the phenotype variety and the genotype spectrum of the Hungarian CF patients. We analyzed 276 Hungarian patients' clinical (demographic data; pulmonary - and gastrointestinal (GI) manifestations; emotional, social and gender developments) and genetic data. Congenital adrenal hyperplasia is a group of autosomal recessive disorders, causing impaired secretion of cortisol and aldosterone from the adrenal cortex with subsequent overproduction of adrenal androgens. The most common enzyme defect causing CAH is steroid 21-hydroxylase deficiency which is due to the mutation of CYP21 gene. The importance of genetic screening is increased by the fact that virilization of the newborn can be prevented by prenatal steroid treatment. Genotype and phenotype correlation has been determined (167 patients). The natriuretic peptides are a potent diuretic, natriuretic, and vasorelaxant hormones which are expressed early in ventricular hypertrophy. The ANP gene is expressed predominantly although not exclusively in the myocardium. We have examined in vitro and in vivo (50 patients) the hANP and hBNP gene expression determining factors. Our work is significantly contributed to better understanding the regulations of natriuretic peptides.

- Garami M, Kincs J, Horváth E, Szabolcs J, Kovács GT, Müller J, Schuler D (2001) Elevated ANP level may be a sensitive indicator of cardiotoxicity in a pediatric population treated with anthracyclins. *Blood* 98:199b
- Garami M, Gardner DG (1996) An E-box Motif Conveys Inhibitory Activity on the Atrial Natriuretic Peptide Gene. *Hypertension* 28:315-319.
- Chen S, Garami M, Gardner DG (1999) Doxorubicin Selectively Inhibits Brain versus Atrial Natriuretic Peptide Gene Expression in Cultured Neonatal Rat Atrial Myocytes. *Hypertension* 34:1223-1231.

KRISZTINA HEGYI (2004)

## Studying leptin, leptin receptor and other adipokines in histidine decarboxylase gene targeted mice

*Supervisor: Dr. Sára Tóth*

The role of histamine in leptin production and effects has not been well established yet. We explored how the lack of histamine influences serum and white adipose tissue leptin levels in histidine-decarboxylase gene targeted (HDC KO) Balb/c mice. We measured leptin levels in both males and females feeding them either with normal or histamine-free nutrition. We found an almost 4-fold elevation in serum leptin levels in mice kept on histamine free diet, but the difference diminished on normal nutrition in both genders. We also studied leptin levels at different ages and observed an increase both in genotype and a tendency for KO mice to have higher leptin levels at any age. Comparing the effect of normal and histamine free diet there was no difference in serum leptin levels in wild type mice in contrast to KO mice that showed a three-fold elevation upon administration of histamine free diet. The source of high leptin levels could be white adipose tissue, since its leptin content is also higher in KO mice. Because no fat accumulation was observed, the high leptin levels could be explained by the accelerated rate of synthesis. A possible molecular explanation to this can be a constitutive CREB activation in HDC KO primary fibroblasts (PF). We did not detect increased CREB mRNA expression (RT-PCR), but observed a reduced protein level in the cytoplasm and an elevated one in nuclear extracts (Western blot). Since CREB is involved in adipogenesis, we differentiated PF cells and found more adipocytes in KO cells. To evaluate the possibility of desensitisation as a response to hyperleptinemia, we studied the long isoform of the leptin receptor (OBR) and the main signal transducer STAT3. RT-PCR showed no difference in OBRlong in KO mice, nor did Western blot analysis in the amount and basal activity of STAT3, indicating that cells did not become insensitive to leptin. On the contrary, high leptin levels were accompanied by an elevated concentration of soluble leptin receptor, which can maintain normal levels of functionally available leptin in the sera and contribute to high leptin levels by increasing the half-life of leptin. These findings are further supported by the observed unchanged body weight, which also suggests that leptin can maintain normal body weight without histamine and that no change in leptin sensitivity has occurred. Studying whether histamine affects the production of other adipokines, we measured TNF $\alpha$  and adipocyte-complement-related-protein30 (Acrp30), which are also involved in body weight regulation. We observed only a very slight difference in serum levels when comparing the two genotypes, which was not significant. An unexpected finding was the observation of two yet unknown proteins with decreased basal tyrosine phosphorylation in KO mice. The change seemed to be diet and LPS inducible. These observations suggest that HDC KO mice can provide a model to understand the relationship between leptin and histamine, and can also contribute to an elucidation of the role of this adipocytokine in pathological conditions such as obesity, diabetes and osteoporosis.

- Hegyi K, Fülöp KA, Kovács KJ, Falus A, Tóth S (2004) High leptin level is accompanied with decreased long leptin receptor transcript in histamine deficient transgenic mice. *Immunol Lett* 92:193-197.
- Hegyi K, Fülöp K, Kovács K, Tóth S, Falus A (2004) Leptin-induced signal transduction pathways. *Cell Biol Int* 28:159-169.
- Hegyi K, Fülöp AK, Tóth S, Buzás E, Watanabe T, Ohtsu H, Ichikawa A, Nagy A, Falus A (2001) Histamine deficiency suppresses murine haptoglobin production and modifies hepatic protein tyrosine phosphorylation. *Cell Mol Life Sci* 58:850-854.

**ERIKA HÉNINGER (2003)**

## **Investigations of the roll and the effects of histamine and cytokines**

*Supervisor: Dr. András Falus*

The histamine is a biogen amine involved in physiological and pathological processes. Beside its classical functions and locations its new functions in the cell proliferation, differentiation and immune regulation have been revealed in the last decade. Data proved, that the histamine forming HDC enzyme is active in almost all proliferable cell types. Last years genomic methodology became also one of the new tools to investigate histamine metabolism and inflammatory processes.

In our experiments the role of intracellular histamine, the effect of antiproliferative interferons on HDC activity was investigated in tumour model system. The immunomodulatory effects of histamine on the IFN- $\gamma$ , TNF- $\alpha$  and IL-18 expressions in ex vivo peripheral blood mononuclear cell cultures derived from patients suffering from inflammatory diseases (type 1 diabetes (IDDM), preclinical IDDM, inflammatory bowel disease) were examined. In the third part of our experiments we analysed the genomics of the IL-18 in inflammatory diseases (IDDM, necrotizing enterocolitis (NEC), sepsis).

We found the (1) HDC enzyme to be active in melanomas. (2) The HDC activity was higher in primary melanomas. (3) IFN- $\gamma$  and  $\alpha$  decreased the HDC activity of the HT168 melanoma cell line. (4) Histamine decreased the TNF- $\alpha$  expression in all of the inflammatory models, like in the controls. (5) It has no significant effect on the IL-18 levels. (6) It decreased the IFN- $\gamma$  expression in all of the investigated groups, but its dual effect was found in the IDDM cultures. (7) The TNF- $\alpha$  and IL-18 levels were increased in preclinical IDDM compared to the control or to the IDDM groups. (9) Our genomic studies showed differences in the genotype frequencies of the IL-18 (-607) polymorphism in IDDM. (10) We found correlation between the most serious stage of the NEC (bowel perforation) and the IL-18 (-607)A allele.

Our investigations confirmed, that the histamine is one of the important elements of the regulation of the cell proliferation and the immune response. Our genetic analysis on the (-607) locus of the IL-18 gene suggested, that the polymorphism of this locus could be one of the genetic markers of the autoimmune processes of the IDDM and the inflammation of the intestinal mucosa in necrotising enterocolitis.

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- Héninger E, Falus A, Darvas Zs, Szalai C, Zsinkó M, Pósz Z, Hegyesi H (2000) Both interferon- $\alpha$  and interferon- $\gamma$  inhibit histidine decarboxylase expression in the HT 168 human melanoma cell line. *Inflamm Res* 49:393-397.

**MARIANNA CSILLA HOLUB (2004)**

## **Investigation the mechanism of generation of soluble IL-6 receptor and the effects of its presence**

*Supervisor: Dr. András Falus*

The receptors of the interleukin-6 (IL-6) family cytokines include a common signal transducing chain (gp130) and a ligand specific chain. The ligand specific part of the IL-6 receptor is found either in the cell membrane or in body fluids in soluble form. The soluble form can be generated by proteolytic cleavage of the membrane bound protein or by alternative splicing at the mRNA level. Although the soluble receptors are usually competitive with those bound in the membrane, and thus, they act as antagonists, however binding of soluble IL-6R (sIL-6R) to gp130 can induce an intra-

cellular signal. Since almost all nucleated cells express gp130, the sIL-6R plays a key role in the regulation of the IL-6 mediated cellular responses, and it often becomes the central factor of the IL-6 mediated local and systemic events. sIL-6R became as important factor in the effects of IL-6 as the cytokine itself. In our experiments we investigated the mechanism of the generation of the soluble IL-6R and we examined the effects of its presence. Our results are as follows: 1. We confirmed that sIL-6R can be generated also by alternative splicing. We established an RT-PCR method to detect alternatively spliced sIL-6R mRNA. 2. In the cell lines we used, we demonstrated that alternative splicing of the sIL-6R is influenced by the IL-6 ligand itself, and by oncostatin-M (OSM), which uses the same signal transducing pathway as IL-6. OSM, using gp130 for signal transduction, enhances expression of either membrane bound, or alternatively spliced soluble IL-6R expression in HepG2 human hepatoma and in MDA-MB435 human mammary carcinoma cell lines. 3. We showed that sIL-6R plays a role in the pathomechanism of inflammatory bowel disease and rheumatoid arthritis. 4. We first demonstrated the sIL-6R induced gene expression pattern by the novel macroarray method. We examined the expression of 580 human genes in HepG2 human hepatome cell line, in which we increased the endogenously expressed amount of IL-6R with additional exogenic sIL-6R. Our results showed that the increase in density of IL-6R is followed by characteristic changes in gene expression pattern of the cells. We drew the conclusion from our experiments that in the presence of sIL-6R is important in interactions among cytokines: meanwhile it widens the repertoire of IL-6 responsive cells, it changes their gene expression pattern at the same time.

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### LÁSZLÓ KERESZTURY (2003)

## Molecular examination of the MHC and application in parentage, population genetic survey and bone marrow transplantation

Supervisor: Dr. András Falus

The HLA-typing in paternity cases in applied and completed with examination of DNA polymorphisms of MHC at the DNA-level from the beginning of the nineties. These methods are in up-to-date laboratory-practice related to examination of donor-recipients pairs in bone marrow transplantation. The examination of HLA-A2 alleles of HLA-A locus from Class I HLA was carried out with health unrelated Hungarians. The identification of polymorphisms of HLA-C locus was set up with two geographically isolated Hungarian populations, and control group. In addition previously serologically HLA-ABDR matched bone marrow donor-recipient pairs was examined at HLA-C locus. The identification of DNS sequence-polymorphisms of TAP proteins was set up. The DNA content from nucleated cells of anticoagulant blood samples and buffy coat samples was extraction with salting-out method.

The HLA-A\*0201 (96.4%) and HLA-A\*0205 (5.4%) subtypes were identified from extremely polymorph allele A2 in our group. An additional population genetic survey would be reasonable consideration of functionally importance allele HLA-A2 and detectable polymorphisms in different ethnic groups. On the basis of our results: the allele frequencies of above mentioned populations merge with the average European results, in spite of the fact there were some minor alteration between groups. It was 87% difference with PCR-based method in HLA-C locus in serologically HLA-ABDR matched donor-recipients pairs. There were no null (blank) alleles; the heterozygosity was above 90%.

Two VNTR-loci examination were carried out and the data evaluation was accomplished.

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**TIBOR GERGELY KOZMA (2004)**

## **The genomic investigation of asthma (and atopic eczema dermatitis syndrome) with special regard to the immune modulation of histamine**

*Supervisor: Dr. Csaba Szalai*

The immune system of human beings at different geographical areas is challenged by various environmental factors, which might evoke allergic responses. The immune system can adjust itself to the environment milieu through different mediators. In the first part of my Ph.D. thesis we investigated some genetic variations of ligands and receptors taking part in the immune response. The objective of my particular study was to find out whether in children living in Budapest the prevalence of some common genetic polymorphism can be related to asthma, atopic eczema/dermatitis syndrome or some other (atopic) allergic diseases. We did not find any connection between the studied polymorphisms of RANTES, CCR5, TNF- $\alpha$  or high affinity IgE receptor and the abovementioned diseases. However, the -2518G allele of the MCP-1 promoter was associated with the susceptibility and the severity of asthma. It is assumed that the malfunction of the allele inherited in an autosomal dominant way takes place via increasing the number of the eosinophil granulocytes. There was clear correlation between the number of eosinophiles in the blood and the presence of this polymorphism. The results are especially important since this is the first demonstration and discovery that connection between one of the chemokine system's polymorphism and the susceptibility to asthma. In the second part of my Ph.D. thesis we aimed for a deeper analyse of histamine, a key mediator in the allergic response. We used an animal model to investigate the role the histamine in the late asthmatic response. A genetically modified mouse strain (HDC KO) was applied to check phenotype changes after sensitization and challenge in the absence of histamine in the animal. We concluded that in the late phase of asthma the total lack of the histamine does not prevent entirely the airway hyperresponsiveness and the allergic inflammation of the lung however it decreases them. Using cytological methods, we found that this finding can be explained by reduced number of the effector cells. A small number of cells elicited only a mild asthmatic response in the HDC KO mice. Furthermore, there were pronounced defects in the production of the allergen specific IgE, which, in turn can decrease the activation of these cells. Micro array (Super Array) analysis of the RNA expression profile revealed that the lack of histamine shifts the immune polarization balance toward a Th1 direction, which can protect against the development of asthma ab ovo and significantly reduces the expression of pro-inflammatory cytokines as well. Moreover in the absence of histamine the activation and chemo taxis of the eosinophil granulocytes were directly inhibited. Besides, it is probable that in HDC KO mice the pathomechanism of asthma is shifted to another way. Summarizing our results: histamine acts in vivo not only as an effector molecule, but also as a part of the "metabolom" it affects the entire immune system thus taking part in the pathomechanism of asthma.

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- Kozma GT, Falus A, Bojszko A, Krikovszky D, Szabó T, Nagy A, Szalai C (2002) *Lack of association between atopic eczema/dermatitis syndrome and polymorphisms in the promoter region of RANTES and regulatory region of MCP-1. Allergy 57:160-163.*
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**GERGELY KRIVÁN (2003)**

## **Haemopoetic stem cell transplantation in paediatric malignant and non-malignant disorders**

*Supervisor: Dr. András Falus*

The author has summarized the experience of 112 pediatric stem cell transplantations performed between January 12 and February 2003 in St. László Hospital, Budapest. He has compared and analyzed the data and survival of patients with severe aplastic anemia treated with stem cell transplantation and/or immunosuppressive treatment. The author was the first to transplant patients with several congenital and acquired malignant and non-malignant disorders (Fanconi anaemia, Diamond-Blackfan anaemia, mucopolipidosis type II, mucopolysaccharidosis type I, histiocytosis X, juvenile rheumatoid arthritis, X-adrenoleukodystrophy, infantile malignant osteopetrosis, juvenile chronic myeloid leukemia) in Hungary. He instituted and regularly performed stem cell transplantations with unrelated donors, CD34+ selections and allogeneic peripheral stem cells in children. In children the author was the first to apply non-myeloablative conditioning regimen during non-related stem cell transplantation and to use unrelated cord-blood as stem cell source in Hungary. From the several interesting observations in the course of transplant activity he has emphasized the possibility of cytomegalovirus-induced colitis without preceding or simultaneous CMV antigenemia and highlighted the emerging incidence and characteristics of Epstein-Barr virus induced posttransplant lymphoproliferative disorders. In his laboratory research work on neurodegenerative diseases the author has studied the action of an inflammatory cytokine, interleukin-6 on cell surface MHC II receptors. In isolated mouse induced microglial cells he has shown that IL-6 by itself had effect on IFN- $\gamma$ /MHC-II expression neither at mRNA nor at protein level. However, transient transfection with 5' deletional mutants of MHC-II IA $\alpha$  promoter linked to chloramphenicol acetyltransferase reporter gene (IA $\alpha$  CAT) demonstrated enhancing effect of IL-6 on a 456 bp long proximal region, while this effect was down-regulated by more upstream DNA sequences.

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**ZSOLT MELCZER (2003)**

## **Role of tumor necrosis factor system in pregnancy induced insulin resistance and in the pathogenesis of cancer of the uterine cervix**

*Supervisor: Dr. András Falus*

The role of TNF- $\alpha$ , soluble(s) TNFR-1, sTNFR-2 and leptin was studied in pregnancy-induced insulin resistance. Significantly elevated TNF- $\alpha$ , sTNFR-1, sTNFR-2 and leptin levels were found in the 3<sup>rd</sup> trimester of physiological pregnancy in correlation with higher fasting C-peptide concentration, C-peptide/Blood glucose ratio, body mass index (BMI) and dominant thigh circumference (DTC). Correlation among the components of the TNF system, leptin, BMI, DTC and indirect parameters of insulin resistance (fasting C-peptide level and C-peptide/Blood glucose ratio) may support the role of

increasing body adiposity and adipocytokines, mainly TNF- $\alpha$  and leptin in pregnancy-induced insulin resistance. Serum TNF- $\alpha$ , sTNFR-2 levels, mitogenic induced TNF- $\alpha$  production of isolated peripheral blood mononuclear cells (PBMNC) and the expression of erbB-2 oncoprotein was studied in patients with the cancer of the uterine cervix in correlation with the clinical course of the disease. Significantly lower serum TNF- $\alpha$ , sTNFR-2 levels and mitogenic induced TNF- $\alpha$  production of PBMNC were detected in cancer patients as compared to age-matched healthy individuals. A progressive decrease in these parameters was observed in more advanced stages of the disorder. The expression of the erbB-2 oncoprotein was frequently detected in the cancer tissue and correlated with poor prognosis of patients. Serum TNF- $\alpha$ , sTNFR-2 concentrations and mitogenic induced TNF- $\alpha$  production of PBMNC isolated from patients with erbB-2 positivity were significantly decreased as compared to those with erbB-2 negativity. Connection between the alteration of the TNF-system and erbB family of oncoproteins may contribute to the pathophysiology and progression of the cancer of the uterine cervix.

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**ADRIENNE NAGY (2004)**

## **The role of Chlamydia pneumoniae airway infection, allergy and certain genetic factors in the pathomechanism of childhood bronchial asthma**

*Supervisor: Dr. Csaba Szalai*

The aim of our research was to contribute to the modern diagnostics, differential diagnostics, targeted treatment and care of childhood bronchial asthma. In order to achieve our objectives, we presented the pathogenetic and differential diagnostic difficulties of Chlamydia pneumoniae infection, conducted genetic studies of asthmatic patients, and performed clinical research activities. The identification of new risk factors can enhance the possibilities for prevention, and improve both diagnostic and therapeutic methods. Our results can be summarised as follows:

1. We have established that the CCR5 $\Delta$ 32 mutation does not confer protection against the development of asthma. 2. MCP-1-2518 A/G polymorphism increases the susceptibility to the development of bronchial asthma. 3. MCP-1 plays an important role in the development of asthmatic inflammation. 4. Our results support the assumption, that there is an association between childhood bronchial asthma and chronic Chlamydia pneumoniae infection. a. We have found a significant association between bronchial asthma with an underlying allergy to inhaled allergens and chronic Chlamydia pneumoniae infection. b. We have shown that the simultaneous occurrence of MBL mutation and chronic Chlamydia pneumoniae infection promotes the development of bronchial asthma. So MBL mutation can have a predictive value.

Our results show the interaction of genetic and environmental factors involved in the development of bronchial asthma, a multifactorial disease. According to our conclusions of practical clinical importance, the genetic and infectological screening of asthmatic and/or allergic children may have long term effects in improving the quality of life of our patients.

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## ZOLTÁN PÓS (2005)

### Comparative in vivo progression profiling of transgenic melanoma variants with different levels of histamine production

Supervisor: Dr. Sára Tóth

In the present study, the impact of acquired neoplastic L-histidine decarboxylase (HDC) expression, and its direct consequence, the release of histamine in the tumor environment, was assessed on melanoma tumor progression. B16-F10 mouse melanoma cells were manipulated via stable transfection, and nine novel transgenic variants were generated in triplicates, constitutively expressing the full-length sense mouse HDC mRNA, a mock control, and an antisense HDC RNA segment, respectively. Establishing both primary skin tumors and lung metastases in C57BL/6 mice, the nine variants with different histamine releasing capacities were subjected to a comprehensive comparative progression profiling in vivo. Our analyses showed trends of markedly accelerated tumor growth ( $p < 0,001$ ), and moderately increased metastatic colony-forming potential ( $p = 0,010$ ) along with rising levels of local histamine production. Using RNase Protection Assay for screening of the melanoma progression profile, and Western blotting for subsequent result-validation, we looked for molecular progression markers affected by melanoma histamine secretion. Investigation of 21 functionally clustered markers associated with tumor proliferation, angiogenesis, invasivity, metastasis formation, local or systemic immunomodulation, and histamine signaling revealed positive correlations between histamine production, and tumor histamine H2 receptor, and rho-C expression ( $p < 0,001$ ,  $p = 0,002$ , respectively). These observations confirm the involvement of histamine in the molecular machinery of melanoma progression.

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- Falus A, Hegyesi H, Lázár-Molnár E, Pós Z, László V, Darvas Z (2001) Paracrine and autocrine interactions in melanoma: histamine is a relevant player in local regulation. *Trends Immunol* 22:648-652.

## GÁBOR ZOLTÁN RÁCZ (2005)

### Calcium-sensing receptors and aquaporins in human pancreas

Supervisor: Dr. Gábor Varga

Calcium-sensing receptor (CaR) plays a key role in the regulation of calcium homeostasis and is therefore expressed throughout the calcium homeostatic system. However, CaR is expressed outside the calcium homeostatic system as well, where it has several different roles. CaRs have been shown in rat pancreas. The exocrine pancreas secretes a copious amount of isotonic fluid, originating mainly from ducts. The role of aquaporins in this process is unknown. We studied the expression of CaR and aquaporins, and the possible physiological role of CaR in normal and diseased human pancreas, and

human pancreatic cancer cell lines. We detected the CaR in multiple cell types in human pancreas: ducts, acini, vessels, nerves and islets of Langerhans. The receptors may have multiple roles in the physiology of the gland: they may be involved in the regulation of ductal fluid secretion, cell proliferation and organ blood flow. The Capan-1 cell line is a promising model system for studying the physiological role of CaR in ductal pancreatic cells. AQP1 and AQP5 are abundantly expressed in the intercalated ducts of human exocrine pancreas. They colocalize with CFTR, a marker of ductal electrolyte secretion. Along the ductal tree, each of the three proteins is decreasingly expressed with distance from intercalated ducts. This implies that, in human pancreas, the major site of fluid secretion is probably the terminal branches of the ductal tree and AQP1 and AQP5 play major roles in coupling fluid movement to ion secretion. Based on our work and the results of others, we can propose a model wherein aquaporins and calcium-sensing receptors play key roles in a homeostatic mechanism operating in pancreatic ducts. Local increases in calcium level arising from the exocytosis of secretory granules from acini stimulates luminal calcium-sensing receptors in the initial segments of ducts. Stimulated receptors in turn activate signalling mechanisms that eventually increase bicarbonate secretion, and ions secreted into the lumen are passively followed by water along the osmotic gradient. Rapid transepithelial water movement is mediated by aquaporins expressed on the basolateral and luminal membranes of human pancreatic ducts.

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## 7/5. PROGRAM

### BASIC AND CLINICAL IMMUNOLOGY

#### *Coordinator*

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*Aim:* The recognition of immunology as an independent scientific discipline is recent, therefore, in many universities immunological research is carried out in various (e.g. biochemical etc) departments. Qualified immunologists are in a great demand in many areas, including clinical science. This project, completing the curriculum of undergraduate training, yields a perspective to qualify in several areas of immunology. The purpose of this Ph.D. Program is to train independent, reliable and competent research scientists. The Program stresses the importance of studying basic immunology and laboratory methods, both being prerequisites of any work in experimental and clinical immunology.

#### *Sub-programs*

Signal transduction and membrane transport in the immune system

Study of signal transduction processes in normal and pathologic blood cells. Study of calcium transport in immunocompetent cells

Study of ABC transporters (multidrug transporter and MHC I TAP1-TAP2 transporter)

Immunology of HIV infection

#### *Supervisors*

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Zsolt HOLLÓ

Attila HORVÁTH,

György FÜST

*Sub-programs*

Study of immunologic and genetic factors in inflammatory bowel diseases, autoimmune diseases and atherosclerosis

Proteins and heat shock proteins playing a role in storage of proteins of oligomeric structure

Study of humoral factors (antibodies, complement) facilitating the progression of HIV infection

Longitudinal study of CD4 antigen density on lymphocytes from HIV infected individuals

Study of the origin of false positive reactions during HIV antibody screening

Regulation of immune response in malignancy

Allogeneic and tumor cell recognition of human NK cells

Human NK cells in bone marrow transplantation

Significance of membrane marker (immunophenotyping) in human bone marrow transplantation

Molecular biological analysis of immunoglobulin specificity of tumor infiltrating B lymphocytes

Biological function of high affinity TNF-alpha receptors (TNF-RI, TNF-RII)

The role of chronic infections in oncogenesis

Expression of protooncogenes and tumor suppressor genes in trophoblast tumors in pregnancy and in normal placenta

Effect of bacterial products on the progression of HIV infection

Role of immune regulation and cytokines in the pathomechanism of autoimmune diseases

Immune pathomechanism and immune diagnosis of gastric diseases due to *Helicobacter pylori*

Prognostic factors in cutaneous lymphoma

Role of immune pathological processes in the pathomechanism of AIDS

Network regulation in normal and pathologic systems. Cytokines, receptors, signal transduction, and gene regulation in normal and pathologic immune regulation

Effects of exogenous and endogenous factors on immune activation and regulation

Immunostimulatory effects of sulfhydryl compounds

Autoimmune diseases

Immunologic effects of free radical scavengers

Pathogenetic factors of development of nasal polyps

Study of the properties, cellular interactions and clinical relevance of cholesterol antibodies (anticholesterol antibodies – ACHA)

Prognostic significance of expression of cytokines, chemokines and their receptors in tumor infiltrating leukocytes and malignant cells

DNA-binding protein microbead assay

*Supervisors*

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Zoltán Prohászka
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György Füst

a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

**ÁGOTA APÁTI (2003)**

## Studies on proliferation, survival and differentiation of early erythroid cells: Role of mitogen activated protein kinase ERK1/2

Supervisor: Dr. Balázs Sarkadi

Survival and proliferation of cells of a human myelo-erythroid CD34+ leukaemia cell line (TF-1) depend on the presence of GM-CSF or IL-3. Upon hormone withdrawal these cells stop proliferating and undergo an apoptotic process. In this work we demonstrate that a controlled increase in  $(Ca^{2+})_i$ , induced by CPA or ionomycin leads to the hormone-independent survival and proliferation of TF-1 cells. In both cases caspase-3 activity was reduced and Bcl-2 level was upregulated. Higher elevation of  $(Ca^{2+})_i$  by ionomycin induced MEK-dependent biphasic ERK1/2 activation, phosphorylation of the Elk-1 transcription factor, and consequently a substantial elevation of Egr-1 and c-Fos levels, as

well as AP-1 DNA binding. This activation of ERK1/2 was sufficient to shift the cells from G0/G1 to S/M phases and cell proliferation was initiated. On the other hand, moderate elevation of  $(Ca^{2+})_i$ , achieved by the addition of CPA, caused a delayed monophasic activation of ERK1/2 and Elk-1 that was accompanied only by a small increase of Egr-1 and c-Fos levels, and AP-1 DNA binding. We found that this moderate elevation of  $(Ca^{2+})_i$  protected TF-1 cells from apoptosis but did not cause cell proliferation. The specific MEK-1 kinase inhibitor, PD98059, inhibited all the effects caused by increased  $(Ca^{2+})_i$ , indicating that the MAPK/ERK pathway activation is essential for TF-1 cell survival and proliferation. Based on these results we suggest that the elevation of  $(Ca^{2+})_i$  may influence the cytokine dependence of hemopoietic progenitors and may contribute to pathological hematopoiesis. In TF-1 cells GM-CSF induced proliferation occurred parallel with the activation of STAT-5, ERK1/2, and the induction of c-Fos and Egr-1 expression. Although Epo treatment did not activate the STAT-5 and MAPK pathways or survival signals, it resulted in differentiation parallel with decreased c-Myb DNA binding activity, and the appearance of haemoglobin. Strikingly, simultaneously added Epo and GM-CSF, or Epo and CPA, abolished the differentiating effect of Epo and resulted in the activation of ERK1/2 MAP kinase. These results illustrate that ERK 1/2 activation inhibits Epo induced erythroid differentiation.

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## ADRIENN BÍRÓ (2005)

### Anti-cholesterol antibodies (ACHA): characteristics, cellular applications and clinical approaches

Supervisor: Dr. Béla Fekete

Experiments summarized in this work are focused on the characteristics of antibodies reacting with cholesterol, and their role in virus infections and atherosclerosis. The existence of anti-cholesterol antibodies (ACHA) was suggested early in the 1920s, but it was proved just after long years by Alving and his coworkers. The aim of our experiments was to measure the serum level of ACHA in patients with severe carotid stenosis and with Hepatitis-C (HCV) virus infection. Furthermore we aimed to produce monoclonal ACHA of IgG isotype (that was never published before) and to describe their interactions with lipoproteins and with different cell types. Our further aim was to study the lipoproteins' role in atherosclerosis in serum-free conditions. According to our results ACHA level is significantly lower in patients with carotid stenosis compared to healthy controls, and after carotid endarterectomy ACHA titer increases to control level. In Hepatitis C virus-infected patients ACHA level is significantly higher compared to healthy controls, and during IFN-therapy in the responder group of patients with the decrease of the virus load the ACHA level decreases either. We succeeded in producing IgG isotype monoclonal ACHA, that interacts with all types of lipoproteins and cholesterol-like sterols containing -OH-group. The monoclonal ACHA binds rather weakly to different intact human $\beta$ 3 and murine cells, but after papain digestion of cell membrane proteins the reactivity of the antibody notably increase and the level of the binding is correlated to the lipid raft content of the cell membranes. We studied the modified lipoproteins' induced complement activation in an in vitro system that makes possible to investigate the role of the influencing factors in serum free conditions. Measuring the activation of the C1 complex, that plays role in the initiation of the classical complement pathway, we found that native and oxidized LDL do not activate C1 directly, but the enzymatically modified LDL induces direct C1 activation. Upon the results described in the thesis we propose that antibodies reacting with cholesterol might play role in atherosclerosis and viral infec-

tions. Their contribution can be important in states with modified cell membrane cholesterol exposition, and with altered lipoprotein structure and distribution.

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**MÁRIA DERVADERICS (2004)**

## Screening of allergic airways diseases examination of some questions of the pathogenesis

*Supervisor: Dr. György Füst*

*Background:* allergic airway diseases mean global health problem, they affect 1-36% of population, and their prevalence is increasing. Objective to examine 1) the effect of cotton dust exposition on textile workers 2) the effect of increasing ragweed allergen (RA) exposition in industrial population.

*Methods:* After a screening questionnaire I have studied the data of 1.) 12 workers with byssinosis and 2.) 880 workers with rhinitis and/or asthma their history, symptoms, results of lung function and allergic tests, complement activation products, and the effect of SLIT were analysed. *Results:* Among the patients with byssinosis early- and late phase airways obstructions and granulocyte aggregation activation could be detected after cotton dust exposition. Among the workers of the power plant with rhinitis/asthma there was a significant difference between the immigrants and natives referring to RA sensitivity: the immigrants have high sensitivity tested by prick test and spec-IgE, and serious seasonal-symptoms occurred in 5 years after their immigrations. In contrary, low sensitivity could be observed without/mild symptoms among the natives. RA allergy seems to be robust with advanced age among atopy-prone families. RA causes in vivo complement activation, the degree of activation shows a significant correlation with the degree of symptoms. SLIT with RA extract significantly reduces symptoms-scores, drug-intakes and skin reactivity. It has no effect on the complement activation. Results of long-lasting study showed, that sublingual immunotherapy has a preventive effect on the development of asthma and allergic sensitisation. *Discussion:* both the cotton dust and ragweed pollen exposition can cause airways diseases among sensitive persons followed by complement activation. The onset and degree of symptoms showed correlation with the time and the degree of exposure. SLIT proved to be effective and preventive treatment. *Conclusion:* because of the prevalence – and the high costs - of allergic airway diseases, further investigations are of great importance.

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BERNADETTE HIDVÉGI (2004)

## Pathogenetic mechanisms of chronic urticaria

Supervisor: Dr. Péter Gergely

Chronic urticaria (CU) is a multietiologic disorder. Pseudoallergic reactions to foods and infections can be causative. Based on autologous skin test reactivity, CU is considered an IgE-mediated wheal-producing IgG-type autoantibodies against the Fc of an autoimmune disease in about one third to one half of patients. Recent reports raised the possibility that HP might play a role in CU. Clinical characteristics in autoimmune and non-autoimmune CU, cellular and humoral immune reactivity of patients were studied. Our main goal was to investigate whether Hp might be an etiologic or triggering factor in the pathogenesis of CU. 17/50 CU patients, who -antibodies by Western blotting, and had a positive IgE both had IgG-type anti-Fc AST response, were classified as autoimmune CU. Autoimmune and non-autoimmune CU cases are indistinguishable clinically. All patients with CU had higher lymphoproliferation to PHA, and significantly higher serum sIL-2R and tryptase levels, than healthy controls indicating T-cell and mast cell activation. Patients with higher sIL-2R levels also had higher tryptase levels, the strongest correlation was shown in the autoimmune group of patients. There was a tendency towards higher tryptase levels in the autoimmune subgroup, as compared to the non-autoimmune CU patients, while there was no significant difference in their sIL-2R levels. Thus, T-cell activation in CU may not be related to the presence of autoantibodies. There were significantly higher proliferative responses to various concentrations of Hp antigen in CU patients compared to healthy controls. We found a tendency to exhibit a higher proliferative response to either helicobacter antigens or mitogens in seropositive compared to seronegative patients. Conclusion: The significant correlation between sIL-2R and tryptase levels in patients with CU indicates that T cell activation is proportional to mast cell degranulation in these patients. The increased level of tryptase in autoimmune CU may suggest a more severe disease. The increased lymphocyte reactivity of CU patients, perhaps further enhanced by the presence of Hp, which, therefore, may play a trigger role in the pathogenesis of CU.

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GÁBOR ILLEI (2003)

## Clinical and immunological effect of interleukin-10 in patients with psoriatic arthritis

Supervisor: Dr. Péter Gergely

*Introduction:* Anti-inflammatory cytokines may play a beneficial role in the treatment of inflammatory diseases by restoring the immune-homeostasis. Interleukin-10 (IL-10) is one of the key anti-inflammatory cytokines, that was shown to be protective in animal models of inflammatory arthritides. We investigated the effects of recombinant human interleukin-10 (rhIL-10), in 29 patients with psoriatic arthritis (PsA), in a double-blind, placebo-controlled study. Patients were treated for 28 days with daily subcutaneous injections of 1, 5 or 10mg/kg rhIL-10 or placebo.

*Results:* Modest but significant clinical improvement in skin, but not articular disease activity scores with only minor adverse effects was observed. 'Type 1', but not 'type 2' T cell cytokine production in vitro was suppressed in rhIL-10 compared with placebo recipients. Similarly, monokine production in vitro was reduced, whereas serum sTNFRII levels were elevated, indicating suppression of

monocyte function. Decreased T cell and macrophage infiltration in synovial tissues was accompanied by reduced P-selectin expression. Moreover, suppressed synovial enhancement on MRI and 3 integrin expression on vWF+ vessels were observed. Discussion: Patients with psoriatic arthritis tolerated subcutaneous rhIL-10 treatment well. This study demonstrated that rhIL-10 has substantial immunomodulatory effects in vivo. These are mediated via various mechanisms, such as inhibition of monocyte and Th-1 lymphocyte function, and effects on endothelial activation and inhibition of angiogenesis.

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## JUDIT KOCSIS (2005)

# Heat shock protein antibody evaluation in cardiovascular disease and in chronic conditions involving the immune system

Supervisor: Dr. Zoltán Prohászka

In this work I summarize the results of antibody detection against 60- and 70 kDa heat shock proteins (Hsps) in some chronic human diseases. The existence of anti-heat shock protein antibodies is proved for a long time, however there is still lack of satisfactory data about regulation of the production and clinical role of these antibodies. For detecting antibodies against the 70 kDa heat shock protein I set up a solid phase ELISA assay. Then I evaluated the anti-Hsp70 and anti-Hsp60 antibody levels in certain human diseases. My aim was to find association between the humoral immunity against these hsps and the pathomechanism and clinical course of the disease. For better understanding of the regulation of anti-Hsp antibody formation, we evaluated these antibody levels in histamin deficient mice also. In a group of patients with severe coronary artery disease I found that anti-Hsp70 antibody level doesn't associate with the disease, unlike anti-Hsp60 and anti-Hsp65 antibodies. The opposite result was found among patients with HIV infection, another disease, where the immune system is proved to be exposed chronically to Hsp70. In this patient population anti-Hsp70 IgG level was significantly higher compared to healthy controls, and this level decreased markedly with highly active antiretroviral treatment. In patients with hematologic malignancies, anti-Hsp60 and anti-Hsp65 antibody levels were lower, compared to healthy subjects and this difference was significant in the subgroup of patients with lymphoproliferative disease. Long after hemopoietic bone marrow transplantation there was a trend toward faster reconstitution of natural autoantibody levels compared to total IgA levels. Detecting anti-Hsp70 and anti-Hsp60/65 antibody levels in histamine deficient mice we came to the conclusion that endogenous histamin plays a role in the regulation of natural autoantibody production, including anti-Hsp antibodies. My results support, that the regulation of anti-Hsp60 and anti-Hsp70 antibody production is different, and these antibodies have different physiologic and pathophysiologic roles in humans.

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**GYÖRGY NAGY (2003)**

## Laboratory activation markers of systemic lupus erythematosus

*Supervisor: Dr. Péter Gergely*

Systemic lupus erythematosus is a systemic autoimmune disease, with disease activity varying over time. There are some laboratory parameters, which help to assess disease activity, however, we still lack unequivocal disease activation markers. Our aim was to find new SLE activation markers, which display better characteristics than the existing methods. We found that protein-protein complexes produced upon complement activation are useful SLE activation markers. Alternative convertase enzyme level [C3B(Bb)P] had the highest correlation with disease activity ( $R_s=0.41$ ;  $P<0.001$ ), as well as it showed the highest difference between clinically active and inactive patients ( $P<0.001$ ), among the parameters measured. The soluble terminal complex SC5b-9 was less sensitive and less specific activation marker than C3B(Bb)P, however, it was still more useful than traditional complement determinations such as C3, C4, or CH50 measurement. Anticholesterol antibody level was found to be elevated in lupus patients, comparing with healthy individuals ( $P<0.001$ ), but there was no significant difference between clinically active and inactive patients and there was no significant correlation with clinical activity. Intracellular cytokine balance was also measured in lupus patients' lymphocytes. We did not find significant difference in interleukin-4 (IL-4) and interferon-gamma (IFN- $\gamma$ ) levels between either SLE patients and healthy individuals or active and inactive SLE patients. One patient with highly active disease showed a marked, however with decreasing disease activity, IL-4/IFN- $\gamma$  predominance over IFN- $\gamma$  increased. In contrast with the protein levels, IFN- $\gamma$  level decreased and IFN- and IL-10 mRNA were significantly increased, IL-4 mRNA was significantly decreased in SLE patients' peripheral blood lymphocytes. According to our results, it is possible to reach more information about clinical activity with the combination of more laboratory test results. We constructed a simple formula with logistic regression method which can be used in clinical laboratory. Using this formula Probability of Clinical Activity (PCA) can be calculated, which gives significantly better estimation of clinical activity than single tests.

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**ILONA UJFALUSSY (2004)**

## Measurement of disease activity and characteristics of the disease modifying antirheumatic drug treatment in psoriatic arthritis

*Supervisor: Dr. Péter Gergely*

Psoriatic arthritis (PsA) has been defined as an inflammatory arthritis, usually seronegative for rheumatoid arthritis, associated with psoriasis. The incidence of various forms of inflammatory polyarthritis in patients with psoriasis is reported to be about 10 percent. Its course and severity can be highly variable. It has been thought to be a benign arthropathy, with a better overall prognosis than for rheumatoid arthritis. At the same time in 25% of the patients develops an aggressive disease. Recently it has been shown, that at the end of a ten years follow up, 50 percent of the patients suf-

ferred from the polyarticular form of the disease, and 11 percent of the patients were severely restricted, in ARA functional class III-IV. To measure disease activity, single variables and composite indices were compared with the patient's and the physicians' global assessment of disease activity. ACR, EULAR and Clegg criteria were used to measure the changes in disease activity, and the efficacy of drug treatment. Good correlation was found between disease activity score (DAS) and the patients' and physicians' global assessment of disease activity. Response to drug treatment was characterised well with the EULAR response criteria, and the Clegg criteria. No correlation was found between the serum level of the cartilage oligomeric matrix protein (COMP) and the clinical and laboratory activity parameters of 37 patients with PsA. Due to these results we do not propose to use the serum COMP level to assess disease activity in PsA. 104 PsA and 102 RA patients were treated with traditional disease modifying antirheumatic drugs (DMARD). Significant difference was found in the two patient groups between the treatment duration of all DMARDs, and the treatment duration of aurothimalate and methotrexate. No difference was seen between the sulfasalazine treatment times. The shorter treatment duration was due to the frequent side effects in PsA. Due to the many dermatological side effects and elevation of liver enzymes the treatment interruptions occurred more frequently in PsA than in RA.

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ÁGNES TERÉZIA VATAY (2004)

## The major histocompatibility complex (MHC) and its role in autoimmune diseases

Supervisor: Dr. György Füst

Experiments summarised in this work are focused on the linkage disequilibria between the different MHC genes, and the relationship between the different autoimmune diseases (autoimmune diabetes, IBD) and some genes encoded in the MHC II and MHC III region. We described strong positive correlation between the presence of the TNF- $\alpha$  -308 A allele mono-S RCCX structure C4AQ0-C4B1 haplotype. The TNF- $\alpha$  -308 A allele, the C4Aqo haplotype and the monomodular RCCX structure with short C4B gene also take part of the 8.1 ancestral haplotype. In Caucasian populations the presence of the 8.1 ancestral haplotype is associated with an increased risk to develop a variety of diseases including autoimmune diseases. The ability of C4A for handling immune complexes is better than that of C4B, a carriage of the 8.1 AH may be associated with impaired handling of circulating immune complexes, and this could be one of the factors responsible for developing autoimmune diseases among 8.1 AH carriers. The relationship between the type 1 autoimmune diabetes and the genes encoded in the MHC II region (HLA-DR and DQ) is well known. However the relationship between the genes encoded in the MHC II region and the slowly form of type 1 autoimmune diabetes, the Latent Autoimmune Diabetes in Adult (LADA) is not clear. According to our results there is a strong positive association between the presence of the HLA-DR4/DQ8 haplotype and the development of both type 1 diabetes and LADA, while there are differences between the two disease entities in respect of the HLA-DR3/DQ2 haplotype and the tumour necrosis factor alpha promoter polymorphism. Among LADA patients the presence of the TNF- $\alpha$  -308 A allele (which is known to be associated with elevated TNF- $\alpha$  production) is significantly less frequent, and could be one of the factors responsible for the relatively slow progression. We found, that the HLA-DR3/DQ2/ TNF- $\alpha$  -308 A haplotype (designated the 8.1 ancestral haplotype, 8.1 AH) was more frequent among type 1 diabetes than LADA pa-

tients. According to our results the frequency of the TNF- $\alpha$  -308 A allele was significantly increased in inflammatory bowel disease (IBD). The median CRP levels were significantly higher in the active phase of the disease than in the inactive phase among the -308 A allele carriers, while this difference was not significant among those patients who not carried the mentioned allele. The decreased frequency of the TNF  $\alpha$  -308 A allele in IBD may modify the pathogenesis of this chronic inflammatory disease.

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### AMARILLA VERES (2003)

## Regulation of anti-heat shock protein antibody production and its clinical impact

Supervisor: Dr. György Füst

Experiments summarised in this work are focused on the induction and clinical role of humoral autoimmunity against heat shock proteins (hsp). The presence of self hsp60-reacting antibodies can only be partially explained by microbial infections and induction by bacterial hsp65 proteins, since important differences (including the epitope specificity and complement activating ability) between hsp60 and hsp65 reacting antibodies have been shown. According to our results, we found a strong association between IL-6 -174 gene polymorphism and anti-hsp60 antibody levels. Significant interactive effects of GM and IL-6 genotypes were noted for both anti-hsp60 and anti-hsp65 antibody levels. Several groups have reported high levels of antibodies against hsp60 is associated with coronary heart disease, and we investigated whether family risk is associated with high levels of anti-hsps antibodies. Concentrations of complement activating (CA) anti-hsp60 antibodies were significantly higher in children whose parents underwent early myocardial infarction than in children without known family history of CHD, - even after adjustment for classical risk factors. As a part of the Heart Outcomes Prevention Evaluation (HOPE) study we conducted a nested case-control study with and without cardiovascular (CV) events in a high risk population with a mean follow-up of 4.5 years. Our data showed that high levels of anti-hsp65 antibodies predicted CV events (incident myocardial infarction, stroke, or CV death). Anti-hsp60 antibodies did not predict any event type. Anti-hsp65 antibodies and fibrinogen, and high levels of anti-hsp65 and presence of cytomegalovirus antibodies had a strong joint effect in predicting the CV events.

We described associations between genetic polymorphisms and autoantibody levels in healthy subjects, and showed that high levels of CA autoantibodies against hsp60 can be considered as a novel independent family risk factor of CHD. This is the first evidence for genetic regulation of anti-hsp antibodies. In the HOPE study of high-risk patients anti-hsp65 antibodies were associated with subsequent CV events, independent of conventional CV risk factors and other inflammatory markers.

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## 8. PH.D. SCHOOL OF PATHOLOGY

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### *Head of School*

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The Doctoral School of Pathology includes four postgraduate teaching Programs as follows: Oncology, Experimental and Diagnostic Pathomorphology, Microbiology, Public Health and Health Sciences. Consequently the training covers a rather broad area of medical sciences involving both the etiopathogenesis of the most common human diseases (i.e. cardiovascular, cancer, infectious) and health education (nutrition, nursing). The training concentrates on individual research work guided by the tutors who makes proposal for the topic of the research, provides the facilities, warrant the technical/intellectual up-to-dateness and the progress of study. For the introduction to basic and applied pathology it is compulsory to attend the regular courses with final examinations. At present 34 Ph.D. students with diploma in medicine, pharmacy, or biology are holders of fellowship, in addition 46 as corresponding Ph.D. students are preparing their dissertation. The Ph.D. degree has been awarded to 75 students trained in the frame of Doctoral School of Pathology in the last ten years.

### **8/1. PROGRAM**

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#### **ONCOLOGY**

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The Program invites those who intended to learn and study tumor biology and basic science in experimental and clinical oncology. Special attention is given to the interactions between laboratory and clinical research activities. The Ph.D. students are also trained to apply updated techniques in cell biology, pathomorphology, biochemistry and recombinant gene technology. Participation at the following courses is mandatory: Experimental Oncology, Clinical Oncology, Molecular Oncology.

#### *Sub-programs*

Biochemistry and chemotherapy of tumors

Altered growth regulation in cancer

Molecular oncology

Gynecological oncology

i0Etiopathogenesis and clinicopathology of the tumors - early cancer

Control of cell birth and cell death (apoptosis)

Progression of malignant disease

#### *Supervisors*

András JENEY

László KOPPER

Ilona KOVALSZKY

Zoltán PAPP

Zsuzsa SCHAFF

Béla SZENDE

József TÍMÁR

#### *Further topics*

#### *Supervisors*

*Sub-programs*

PARP and apoptosis  
 Molecular markers in tumor progression  
 Effect of interstitial irradiation on brain tumors  
 Tumors of central nervous system  
 Role of growth factors and adhesion molecules in hepatocarcinogenesis, viral hepatitis and cirrhosis  
 Prediction of drug resistency and toxicity in colorectal cancer  
 Gene polymorphism in malignancies  
 Decisive factors in drug response  
 Experimental and clinical pharmacokinetics of antitumor agents  
 Polymorphism of thymidilate synthase and methylen tetrahydrofolate genes  
 Tumor immunology  
 Lymphoma transformation and DNA methylation  
 Molecular genetic changes in lymphomagenesis  
 Signaling pathways in receptor and metabolism mediated cell death  
 Hepatic stem cells in pathological processes and hepatocarcinogenesis  
 Radiotherapy of malignancies  
 Angiogenesis and metastases  
 New molecular targets by studying signaling pathways of death receptors  
 WT1 oncogenic biology  
 Pathological importance of TGFb signaling pathway in SLE  
 Study and restoration of TGFb induced apoptotic effect in lymphoma cells  
 Tumors of oral cavity  
 lphaProgression and extracellular matrix in oral squamous cell carcinoma  
 DNA methylation in transformation and progression of NHLs  
 Modeling and regulation of motility of human tumor cells

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ISTVÁN BABÓ (2005)

## Function and modification of matrix metalloproteinase-9 in malignant tumours

Supervisor: Dr. András Jeney

Matrix metalloproteinases (MMP, also designated as collagenases) have a crucial function in the degradation of ECM and exert an important role in tumour invasion, metastasis, and angiogenesis. However, the accumulated data indicate that the role of the MMPs is not confined to degradation of ECM, because they have other substrates as well. The complex functions of MMPs explain the difficulties of their utilization in the decision of tumor prognosis and more investigations are required to be considered them as molecular target for antitumor drugs. To collect further data concerning preferentially MMP-9 and MMP-2 - in tumor progression their gelatinase potency were correlated with the clinical-pathology state in human tumors originated either from clinical situation or from human tumor xenograft model system. Furthermore the cell growth and migration dependent MMPs activities were investigated in tissue culture to test the hypothesis that MMP participate in both pathobiological events of tumor progression. Finally the question has been addressed whether MMP could be considered as activator or target for antitumor drug action. Surgically removed human tumors and human tumor cell lines in tissue cultures were applied. Measurements on MMP-s activities by using gelatin zymography, morphological characterization of human tumours by using light microscopy, immunohistochemistry, flow-cytometry, human tumor xenograft model of basaloid squamous cell carcinoma from the oral cavity. The identification of histogenesis and of the human origin by applying assay for LDH isoenzymes. Determination of tumour cell growth (mitotic and apoptotic index in animal experiment and using MTT-test, DNA synthesis in vitro studies). Assays for the invasive growth of tumour cells in Boyden chamber and in three dimensional gel. Conclusions: (1) A close correlation has been observed between MMP-9 activity of the human colorectal adenocarcinoma samples and the 5 year life span of the patients. The analysis according to Kaplan-Meier indicated that the activity of collagenase IV enzymes was higher in the tumours of patients who had a short period of survival than in those who lived for a long time. This difference was significant only in the case of 92 kDa MMP-9 ( $P < 0.05$ ). (2) The microenvironment of the tumor influences the dominant tumour cell population of basaloid squamous cell carcinoma (BSCC). The basaloid and squamous tumour cell populations develop together in the orthotopic localization while in the heterotopic localization the basaloid component is dominant. (3) The microenvironment influences the malignant phenotype of BSCC: infiltrative growth was only observed in the orthotopic localization; the invasive growth of BSCC is accompanied by MMP-9 induction in the orthotopic localization. (4) The production of MMP-9 increases in the slow proliferative stage of HT1080 cell culture when the migrating capacity of the tumour cells has increased. (5) The elevated MMP activity of the tumour cells could be utilized in the activation of peptide cytostatic agents, provided the peptide contain collagenase cleavable site. (6) Antisense oligodeoxynucleotide to MMP-9 selectively inhibits the production of MMP-9 and reduces the migration of tumour cells.

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**TEODÓRA BÁNFALVI (2003)****Follow up of progression of malignant melanoma by tumour markers**

Supervisor: Dr. József Tímár

*Purposes of the study:* (1) Examination of S-1100B and 5-S-cysteinyldopa concentrations in several patients with malignant melanoma in all stages. (2) Comparison of serum concentration of S-100B protein, 5-S-cysteinyldopa and LDH in Stage III-IV melanoma patients. (3) Evaluation of heterogeneous S-100B protein expression in malignant melanoma and association with serum protein levels. 4. Investigation of expression and function of the AMF receptor in human melanoma. *Methods:* The S-100B protein concentration was measured by luminescence immunoassay. Serum 5-S-CD concentration was determined by high performance liquid chromatography (Merck-Hitachi). LDH was determined at our Central Clinical Laboratory by optimised UV kinetic method. S-100B protein was detected in primary skin melanomas using monoclonal antibody to S-100B (DAKO). AMF receptor was detected with a rabbit anti-peptide antibody. *Results and new statements:* (1) Serum S-100B protein concentration in Stage II-III-IV is a reliable prognostic marker. It is significant independent prognostic marker in respect to disease specific survival. It is relevant marker for therapy monitoring and patient follow up. (2) During the comparison of immunohistochemical S-100B protein expressions and serum S-100B protein concentrations in tumour with normal concentrations focal and heterogeneous expression pattern and low intensity were found. (3) Due to the high number of false results during the evaluation of serum S-100B protein concentrations data suggest to determine the S-100B expression pattern and intensity of the primary tumour before therapy monitoring. In case of low S-100B expression (focal or heterogeneous pattern with low intensity) the clinical use of S-100B monitoring of disease progression may not be sensitive enough. (4) Serum concentration of 5-S-cysteinyldopa did not prove to be independent significant prognostic factor. However, the marker could be used for patient follow up. (5) S-100B protein is more specific and sensitive than 5-S-cysteinyldopa. It seems to be more relevant using them together to predict the prognosis, in spite of the relative low correlation. (6) In patients with metastatic disease S-100B protein proved to be the more specific and sensitive, however the LDH concentration was found to be significant prognostic factor. (7) In case of LDH 460 U/l is appropriate cut off level. (8) AMFR expression correlates with tumour thickness and vertical growing pattern of SSM, suggesting metastatic potential of the tumour.

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**MAGDOLNA DANK (2003)****Significance of predictive and prognostic factors in the treatment of breast carcinoma**

Supervisor: Dr. László Kopper

The clinical course of breast cancer could be very different. In some patients the course of disease is definitely indolent and local therapy can be applied, while others live many years after developing metastases and there are data on more than 10 year long survival without any medical treatment. The determination of the proper prognostic and prediction factors is needed for the selection of the appropriate personalized treatment strategy. The aim of this study was to examine the relationship between the prognostic and predictive factors and the treatment outcome of the disease among our pa-

tients. (1) The ten years disease-free survival of the breast cancer is determined by the tumour size (<3,5 cm), the number of the metastatic lymph nodes (< 3 affected axillary lymph node) and the lack of the mutant p53. (2) In the lymph node negative cases the early relapse could be predicted with 12,9% margin of statistical error according to the cluster analysis – based on tumour size, the histological grading, the NPI and the HER-2 state. The genetic differences can not conventionally identified by histologists using traditional methods. This could be compensated by the multiplier factor included in the NPI formula (tumour size x 0.2). (3) In the early stage of breast cancer the tumour localisation influences the disease outcome. The laterally localised breast cancers have poorer prognosis (because the clinical detection of the internal mammary nodes was not a routine process) compared to the medially localised disease. This observation supports the necessity of the exact determination of the clinical staging. (4) Among the high risk patients – more, than 3 affected axillary lymph node – there was a significant difference in the 3 years survival according to the number of affected lymph nodes ( $p < 0.01$ ) using the anthracycline treatment followed by sequential CMF therapy. The 3 years survival rate was 90% when the number of the affected lymph nodes was between 4 and 9, while this rate was reduced to 66% when the number of the affected lymph nodes was more, than 10. In the oestrogen receptor negative cases the prognosis was even more unfavourable. However, there was no correlation identified between the disease outcome and the progesterone receptor status. (5) The primary systematic treatment of the locally advanced breast cancer by doxorubicin/docetaxel combination resulted in 20% total histological remission, even in case of more extent tumour size (3.5–14.8 cm), N3 state and ECOG 2 values. (6) The metastatic breast cancer could be divided into three subgroups according to the disease free survival [the period of disease-free survival till the first relapse was less, than 12 months (1st group), between 12 and 36 months (2nd group), or more, than 36 months (3rd group)]. This classification determines the elapsed time until the start of the second line taxane treatment and the survival. The ratio of HER-2/neu overexpression and the mutant p53 is strongly correlated with the disease-free survival to the first relapse and the elapsed time until the start of the second line treatment.

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## BALÁZS DÖME (2004)

### The mechanism and significance of vascularisation in human malignant melanoma of the skin and in cerebral metastases

Supervisor: Dr. József Tímár

In the first part of the study, we determined the role and the fate of the peritumoral vascular plexus during the vascularization of human malignant melanoma (hMM) and in an appropriate murine melanoma model system. The prognostic significance of the vascularity of different tumor areas was also evaluated. Development of visceral metastases of hMM was exclusively correlated with the MVD of the tumor centre. 3D reconstruction of vessel networks of melanomas showed clearly that the peritumoral vascular plexus present at the tumor base is continuously being incorporated into the growing tumor mass. In contrast with the prevailing view, the pericyte coverage of endothelial tubes was complete in all of the investigated areas, in both human and murine melanomas. We have also found that the expression of the vascular adhesion molecule, VAP-1, is significantly decreased in intratumoral vessels compared to peritumoral ones, which observation also supports the idea that the phenotype of intratumoral blood vessels is important in the progression of malignant melanoma. Investigating the role of  $\beta 3$  integrins in melanoma growth, we found that transfection of the platelet integrin  $\alpha \text{IIb} \beta 3$  into human melanoma cells, promoted cell survival and in vivo growth. Upon orthotopic injection into SCID mice the  $\alpha \text{IIb} \beta 3$ -overexpressing clone (19H), grew significantly more

rapidly than the mock transfectant one (3.1P), due to a significantly increased microvessel density. Immunocytochemistry and flow cytometry indicated that the 3.1P cells did not express bFGF at protein level, unlike the transfected clone, characterized by a strong expression in the majority of the cells. Quantitative PCR studies indicated that the increased bFGF protein expression is due to transcriptional regulation. Previous studies indicated that bFGF expression in primary melanoma increases at the switch of radial to vertical growth phase: we propose here that this alteration could well be associated to the illegitimate expression of the  $\alpha 5\beta 1$  integrin in the constitutive  $\alpha 5\beta 3$  background demonstrated before. In the second part of our study our aim was to investigate the pathogenesis of glomeruloid bodies growing within cerebral metastases. Analyses of the cerebral vasculature showed clearly that the proliferating and migrating tumor cells pull the capillaries - and the adjacent capillary branching points - into the tumor cell nest. Initially, this process leads to the appearance of simple coiled vascular structures which later developed into chaotic and tortuous vascular aggregates with multiple narrowed afferent and efferent microvessels. Despite the absence of sprouting angiogenesis and the ruptures of the stretched and narrowed capillary segments observed frequently between the metastatic tumor nodules, necrosis was scarce in these lesions, implying that the blood supply from the multiple afferent microvessels and from the preexistent vascular bed sufficed to provide the tumor cells with oxygen and nutrients.

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## KÁROLY FAZEKAS (2004)

### The role of HGF-c-met system in tumor progression

*Supervisor: Dr. József Tímár*

It is known that c-met protein is overexpressed in several types of tumors including colorectal carcinomas. But it is not clear what is the role of this in the metastasis of colorectal cancers. Since HGF is one of the growth factors of the liver and the liver is the main target of metastasis of colorectal cancers it suggests that relation between HGF and c-met play role in this process. There are several possible mechanisms. The most obvious possibility is that the HGF - c-met relation helps the metastasis by mitogenic or motogenic or both way. Since it is known, that HGF, purified from blood plasma, induces angiogenesis secondly the role of HGF and c-met in angiogenesis of tumors was also studied. It seemed to be a real possibility that small basic presumably heparin binding peptides of HGF  $\beta$  chain and basic hexapeptides designed on the basis of HGF sequence could influence the behaviour of tumors because earlier studies has proven HGF is a heparin binding growth factor and basic heparin binding peptides have several biological activity. The effect of these peptides on cell proliferation, metastasis and angiogenesis was studied, too. We demonstrated that the expression of wild type c-met was detectable on metastatic cell lines, moreover the motility of metastatic human colorectal adenocarcinomas was increased by rhHGF treatment. On the other hand the expression of c-met was higher in Duke's C human colorectal cancer than in Duke's B tumors. These data suggest that the HGF - c-met system plays role in metastasis of colorectal cancers by its motogenic effects. We found that proliferation of HT25 human colorectal adenocarcinoma and M1/9 human melanoma was inhibited by HGP1 and HG2 HGF  $\beta$  chain peptides. Furthermore, the subcutaneous growth of HT25 human colorectal adenocarcinoma was inhibited by HGP1 HGF  $\beta$  chain peptide. Moreover the liver metastasis of HT25 human colorectal adenocarcinoma, M1/9 human melanoma and 3LL-HH rodent lung carcinoma was inhibited by HGP1 HGF  $\beta$  chain peptide. These results suggest that small HGF  $\beta$  chain peptides could have antitumoral effect. We showed that angiogenesis was inhibited in chicken choriallantois membrane model by HGP1 and HG2 HGF  $\beta$  chain peptides and the artificial BP4 basic hexapeptide. Moreover the proliferation of human cerebellar endothelial cells was inhibited by

HGP1 and HG2 HGF  $\beta$  chain peptides and the artificial BP4 basic hexapeptide. Furthermore the angiogenesis induced by subcutaneous HT25 human colorectal adenocarcinoma was influenced by HGP1 HGF  $\beta$  chain peptide. These findings suggest that small basic peptides of HGF  $\beta$  chain and small basic hexapeptides could have antiangiogenic effect. All of these data could mean that small basic peptides derived from growth factors could be developed as a new group of anticancer agents.

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**GÁBOR GONDA (2003)**

## **Actual diagnostic problems of gastrointestinal tumors in the pathological practice**

*Supervisor: Dr. László Kopper*

The choice of subject, the examination of the tumors of the gastrointestinal tract is motivated because the morbidity and mortality is among the leading statistical malignancies. In the National Health Center a very large number of patients with different gastrointestinal tumors is treated in an interdisciplinary cooperation. In this work the different characteristics of the gastrointestinal tumors are examined, considering the possibilities of the diagnostic and prognostic conclusions. In cases of gastric carcinoma the overexpression of the p53 gene and the proliferation activity is evaluated in connection with the histological parameters of the cancers, and with the clinical and pathological stage of the diseases. The importance of rare metastases of gastrointestinal primary origin is discussed based on a series of cancer patients. Finally the detection of pituitary adenylate cyclase activating polypeptide is reported in the gastric mucosa. The overexpression of the p53 protein was observed in all but two cases. The number of p53 positive cells was significantly higher in carcinomas of the gastroesophageal junction than in carcinomas of the distal region of the stomach. Our findings correlate with the prognostic data published in literature. The proliferation rates were similar to that of p53 expression, but no significant difference was detected. The appearance of intramural metastases is of prognostic importance in cases of carcinomas of the cardiac region, as they must be considered as indirect signs of disseminated cancer disease. The significance of implantation metastases seems to be increasing, especially with the more and more widespread application of the endoscopic surgical procedures. Detection of PACAP in the parietal cells of the gastric mucosa resulted in a new experience, because as far as our knowledge, PACAP has not been demonstrated in this localization.

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RITA BEÁTA KOVÁCS (2005)

## Measuring telomerase expression, and study on galectin-3 in pathological samples

Supervisor: Dr. László Kopper

Telomerase (tel) is a special reverse transcriptase that is able to replace the ends of the chromosomes (telomer) by using an own inner template. This might compensate for the physiologically occurring telomeric loss, which – reaching a critical shortness – leads to the loss of the cell proliferation capacity and eventually to apoptosis. The tel expression makes the cellular immortality possible, which is a crucial step in oncogenesis, since the cell – during serial divisions, accumulating multiple mutations – can suffer a malignant transformation. Besides certain normal proliferating cells, in 85% of human malignant tumors, in 90% of carcinomas tel activity (TA) was detected, and it was also observed that in their majority the TA level correlated with the stage of the carcinogenesis. (An exception is represented e.g. by the clear cell renal cancer.) Consequently, tel is the best molecular tumor marker known so far, thus being an attractive target in the diagnosis, prognosis and therapy of tumors. Up to now no study examining TA in a routine material has been published in the literature. In the first part of our work we set the objective to study the tel reactivation in routine histological and cytological material. We also studied the correlation of the values received with the morphological factors. We divided the 118 (77 histologic and 41 cytologic) samples into five major groups: kidney tumors, soft tissue tumors, bladder tumors (urine), thyroid lesions and other lesions (of various localisations and origin). We measured the mRNA relative expression of hTERT (human telomerase reverse transcriptase), a catalytic subunit closely correlating with TA. It was determined with real-time RT-PCR method. Based on the results it can be established that measuring expression of hTERT yields valuable assistance in the preoperative diagnosis, particularly regarding the dignity of thyroid and breast lesions. The study of urine samples suggests that determination the expression of hTERT is well suitable for the early detection and monitoring of bladder cancer. The tel reactivation does not seem to play a key role in the development of soft tissue tumors although its study might be important for individualised treatment. Thyroid tumors show varying histopathological feature and clinical behaviour. Molecular markers, among them the telomerase studied in the first part of this work, are promising, however, they have currently limited availability for routine diagnostics. Nevertheless, the diagnostic immunohistochemical antibodies are getting more and more important in the differential diagnostics and grading of thyroid tumors. Out of them – based on literature data – the galectin-3 (gal3) seems to be highly specific and sensitive. In the second part of our work, in 91 thyroid lesions we retrospectively studied the gal3 immunohistochemical reaction and analysed its diagnostic value in the determination of the dignity of various lesions, with special regard to those of follicular origin. While gal3 was markedly and diffusely expressed in papillary carcinomas, it showed weaker, focal or varying positivity in other malignant lesions. In all the inflammations focal positivity was observed. The nodal goiter and normal thyroid tissue was negative. On the basis of our results the gal3 immunohistochemical reaction seems to be reliable in the histological diagnosis of papillary carcinomas. In the most difficult field of thyroid diagnostics, however, in the case of follicular lesions, our experience does support the observations described in most studies so far. In case of follicular lesions the method – although a useful supplementary examination – has no absolute value in itself, therefore fundamentally we must further rely on the classic morphologic criteria. Our most recent observations suggest that in the Hashimoto's thyroiditis the gal3 positivity occurring in papillary structures might indicate a malignant transformation already prior to the onset of the characteristic morphological criteria of papillary carcinomas.

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GÁBOR ISTVÁN LOTZ (2004)

## The pathogenesis of hepatitis C and a novel method for detection of HCV infection

Supervisor: Dr. Zsuzsa Schaff

The hepatitis C virus (HCV) in most cases causes the chronic inflammation of the liver, which often leads to the development of liver cirrhosis or liver cancer. The sensitivity threshold of the most sensitive nucleic acid detection methods used in the routine diagnostics of hepatitis C is not lower than approx. 100 virus copies/ml, thus there would be great need for a more sensitive HCV diagnostic technique. At the same time, the role of erythrocytes in carrying the HCV content of the blood is unknown. Accordingly, our aims were to study the pathomechanism of chronic hepatitis C (CHC) as well as the HCV-carrying ability of erythrocytes by RT in situ PCR technique, and to test this on a large number of HCV-infected patients, making comparisons with the solution phase RT-PCR based method used in routine diagnostics. The immunohistochemical detection of T- and B-cells, Fas/Apo1 receptor and Fas ligand (FasL), HCV core and NS4 protein, as well as the detection of apoptosis were accomplished in the liver biopsy specimens of patients suffering from CHC. HCV was detected using routine Amplicore RT-PCR method („serum-PCR”) in the blood samples of 105 chronic hepatitis C patients, 12 healthy individuals and 8 non-HCV liver disease cases, and by RT in situ PCR („erythrocyte-PCR”) in erythrocyte smears. The HCV-erythrocyte binding was studied by means of both in vitro inoculation and laser scanning confocal microscopy. Our studies revealed cytotoxic T-cell predominance in CHC, the close association of these lymphocytes and hepatocytes, furthermore enhanced Fas/FasL expression accompanied by apoptotic hepatocyte death. We were among the first to detect HCV in formalin fixed, paraffin embedded liver biopsy material. The core and NS4 proteins showed cytoplasmic localization. From the 125 individuals (105 HCV-infected patients, 20 controls) studied by erythrocyte-PCR, HCV RNA was detected in 99 HCV-infected patients' red blood cell smears, while only 79 cases were found positive using the serum-PCR technique. Both methods gave negative results for 5 earlier HCV-infected patients, as well as in the case of 20 controls. Using confocal microscopy, in the vertical slides of the erythrocytes, positivity was found at places in the cytoplasm, sometimes localized independently on the plasma membrane, proving the internalization capability of the virus. During the course of in vitro inoculation the HCV-erythrocyte binding was found to develop as early as after 30 seconds, referring to a receptor-mediated mechanism. Conclusions: (1) HCV causes immunopathomechanism based liver damage, a part of which is the enhanced Fas/FasL expression as well as the apoptosis of hepatocytes. (2) It is assumed that one of the main reservoirs of HCV in the blood is the erythrocyte-fraction. We have been the first to prove this on red blood cell smears, using the RT in situ PCR method. (3) The RT in situ PCR method used on erythrocytes is capable of detecting HCV more sensitively than the solution phase (serum-PCR) technique used in routine diagnostics, since erythrocyte-PCR gave positive results in 94 % of the studied HCV patients, in contrast to the result of 75% given by the Amplicor test. (4) HCV can be internalized into the red blood cells. e) Based on in vitro inoculation tests the HCV-erythrocyte binding may be specific (receptor mediated).

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**EDINA MAGYAROSSY (2003)**

## **Clinicopathology of acute childhood lymphoid leukemia and non-Hodgkin's lymphoma**

*Supervisor: Dr. József Tímár*

Although pediatric cancers are rare, their significance is unquestionable since after accidents tumorous diseases are the second most common causes of death among children. The most frequent malignancy in childhood is leukemia (primarily the acute lymphoblastic form, ALL), while lymphomas are on the third place after brain tumors. Introduction of immunocytochemistry and histochemistry in the diagnostics of lymphomas was revolutionary in pathology. I have been involved in the development of the highly sensitive avidin-biotin immunoperoxidase technique, and also in the first evaluation of its impact on routine diagnostics. The significance of the methodology is well illustrated by the fact that immunohistochemical results changed the morphological diagnosis in more than half of the cases. In the last ten years particular attention was paid to ALL prognostic factors and for minimal residual disease. We have studied the expression of two metastasis-associated proteins (NM23 and CD44v6) in childhood ALL, and found that CD44v6 characterizes primarily the medium- and high-risk ALL cases, which suggests that this protein may be a marker for poor prognosis. Early detection of minimal residual disease (MRD) are now hot issues in the follow-up of childhood ALL. For the first time in Hungary, we have evaluated the potential of WT1 expression in the peripheral blood as a potential molecular marker for ALL. We have shown that monitoring WT1 in the peripheral blood of ALL patients can be efficiently used for the detection of relapse. Treatments of pediatric leukemias and lymphomas are based on similar principles. I have compared the efficiency of the two BFM protocols used in Hungary in the past decade for the treatment of pediatric ALL. I have been able to demonstrate that the more recently used BFM95 protocol produced better survival in the low-risk ALL group than the one used earlier. Within the BFM working group there is a subgroup for studying the side effects. In collaboration with this subgroup, I have developed a plan for follow-up, which gives the most advisable minimal criteria for the follow-up of patients with childhood ALL. Human tumor xenografts are valuable tools for preclinical studies of human cancer. Using several pediatric B-NHL cases, we have been able to establish and characterize two B-NHL xenografts. Furthermore, we have shown that the two NHL xenografts are characterized by different sensitivity to chemotherapeutic agents routinely used in the treatment of pediatric NHLs.

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**FARID PARVANEH (2004)**

## **Apoptotic and proliferative activity in neuroblastomas and PNET, tumors of the thyroid gland and parathyroid gland**

*Supervisor: Dr. Béla Szende*

The aim of our study was to examine apoptosis in neuroblastomas and few PNETs as they crossed our path with their rarity, to examine the Retinoic Acid Receptors (RAR) and find out about their relation. Our study on the tumors of the thyroid was also to determine apoptotic activity, percentage of p53, bcl-2 and bax. In studying the tumors of the parathyroid glands we examined the apoptotic and proliferative activities and percentage of p53, bcl-2 and bax. In neuroblastomas (NB) and PNET

apoptotic index and RAR index varied parallel to each other, i.e., when apoptosis was frequent, the RAR index was also elevated. As for NBs, not all were RAR positive in our study. The apoptotic index was low in general compared to other tumor types but it was elevated in cases with strong RAR positivity. In all cases of PNET, however, a relatively high apoptotic index as well as strong RAR positivity was found. To the best of our knowledge, immunohistochemical demonstration of RAR has not been reported yet. Our findings show that the RAR- $\beta$  Ab can be used successfully also for immunohistochemical purposes. The results suggest that spontaneous apoptotic activity in NB may be caused by endogenous retinoic acid and mediated by RAR. From the practical point of view the treatment of patients with RA was dependent on the result of our examination and in the case of RAR positivity the treatment was carried out in the clinic. Having examined p53, bcl-2 and bax in different thyroid tumors, a new dimension has been observed. Mutant p53 is very low in adenomas compared to its expression in papillary and follicular carcinomas, and mutant p53 suppresses cell death. Bcl-2 expression is high in adenomas and low in carcinomas according to our data, and bcl-2 secures cell survival. bax expression on the other hand, is relatively high in adenomas (but still lower than bcl-2) and low in carcinomas, and bax promotes cell death. The high expression of Bcl-2 explains the low ratio of apoptotic cells in the examined thyroid tumors, benign and malignant. The high expression of Bcl-2 in adenomas suggests the susceptibility to transformation to malignancy. Although few apoptotic tumor cells could be detected in our study in any of the benign or malignant tumors this may be relevant to the high expression of bcl-2. From the practical point of view, determination of P53, bax and bcl-2 ratio in thyroid tumors contributes to the differentiation between adenomas and especially follicular carcinomas. As to the parathyroid lesions the overwhelming majority of patients with primary lesions (PH) were female. Secondary hyperparathyroidism (SH) occurred nearly equally in females and males. The age of patients suffering from PH was on average higher than that of patients with SH. The majority of primary lesions were adenomas and only three carcinomas. In case of SH, hyperplasia was the most common finding. Apoptotic index was equally low in adenomas and hyperplasia and so was the mitotic ratio. The carcinomas showed slight elevation in mitotic and apoptotic activity. The results of our study on a relatively large number of parathyroid hyperplasias and adenomas clearly indicate that mitotic or apoptotic activity cannot differentiate between these two pathological entities. Bcl-2 and bax and in some cases p53 expression was found in hyperplasias as well as adenomas. The percent of cases considered as positive for p53 or bcl-2 was slightly higher in adenomas, but differential diagnosis can not be based on p53 or bcl-2 immunostaining. The same may be applied to bax expression (according to our knowledge expression of bax has not been studied yet in proliferative parathyroid lesions). The most interesting finding in our study was the co-expression of bcl-2 and bax in most of hyperplasias and adenomas. Spontaneous apoptosis is generally low in endocrine cells, but overall knowledge in this field reveals that hormones produced by different endocrine cells like PTH or LH induce apoptosis. This by itself probably can build up a protective mechanism in endocrine cells against apoptosis. The great emphasis in our study had been given to shed light over this subject and has called our attention to the co-expression of bax and bcl-2 and its relation to the protective mechanism against apoptosis in endocrine tumors.

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ZSUZSANNA PÁPAI (2003)

## Prognostic factors and new medication possibilities in malignant musculoskeletal tumors

*Supervisor: Dr. László Kopper*

Musculoskeletal tumors make out 2-3% of the malignant neoplasms. The past decades have brought essential changes in the diagnosis and treatment of sarcomas. Our aim is the analysis of prognostic factors known from the literature in our osteosarcoma samples and to search for methods, which could give further information on the prognosis helping to plan the treatment of these tumors. Apart from the conventional clinical, radiological and histological evaluation, we examined the p53 and mdm2 expression as well as the p53 and mdm2 expression in the biopsy material and the sample removed at the operation, using immunohistochemistry and molecular hybridization. Furthermore, the proliferative activity by Ki-67 MIB method, was measured. Results were evaluated by the middle- and long-term follow-up examination and analyzed by statistical methods. We did not find the age and sex of the patients of significant prognostic value, nor the pathological fracture or the radiographic appearance of the tumor. On the contrary, a tumor volume under 60 cm<sup>3</sup>, the wide or radical surgery, the highly distal location, a cartilaginous ground substance under 20% and a tumor necrosis above 90% after the preoperative chemotherapy are all positive prognostic factors. The last three factors proved to be significant in the multivariate statistical analysis and determinate survival. The increased expression of the p53 protein may also be a prognostic factor. The life expectancy of patients with advanced soft tissue sarcoma is rather bad in spite of the radical surgical methods and the radiotherapy. Successful medication is limited. In a retrospective study we examined the efficiency of a new drug combination, the VIP protocol (Vepesid, Ifosfamid, Cisplatin) at patients with advanced soft tissue sarcoma. Our results show that the efficiency of this newly introduced treatment exceeds the efficiency of the treatments applied so far in Hungary in this patient group. The side-effects of the treatment were acceptable. We consider the VIP combination a routinely applicable protocol in the treatment of patients with advanced soft tissue sarcoma.

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ZOLTÁN TAKÁCSI-NAGY (2005)

## Analysis of application and results of high dose rate brachytherapy in the treatment of base of tongue cancer

*Supervisor: Dr. György Németh*

Approximately 30% of tumours of the oropharynx are base of tongue cancers. In 2002, 228 new cases were registered and 251 patients died of this disease in Hungary. In the non-surgical treatment of these tumours, the „organ preserving” modalities have become more and more important, because they provide – beside the practically complete retaining of speech and swallow functions as well as good cosmetic results – a high locoregional tumour control. At present radiotherapy is the most important means of this kind of treatment. Local dose escalation, an important factor in increasing local tumour control with irradiation and brachytherapy (BT), is a possible choice for this purpose. Low dose rate (LDR) BT has been applied for a long time in the treatment of base of tongue tumours, but no detailed analysis can be found in the literature about the application and efficacy of high dose rate (HDR) BT. The purpose of my work has been to investigate HDR brachytherapy and to draw conclu-

sions from its application. Between 1992 and 2000, 77 patients with biopsy proven carcinoma of the base of tongue (T1-4N0-3) were treated at the Department of Radiotherapy of the National Institute of Oncology. Forty patients received exclusively external beam irradiation (60-72 Gy), thirty patients were treated with boost BT (12-30 Gy) following teletherapy. In seven patients tumour excision and postoperative BT (24-30 Gy) of the tumour bed and additional neck dissection or percutaneous irradiation were performed. The results of exclusively external radiotherapy, the combination of percutaneous irradiation and boost BT, and the LDR series known from the literature were compared. The role of postoperative BT was studied. First rigid needles, later flexible plastic tubes were used for the interstitial treatment. Traditional and CT based interstitial radiation planning, and the radiophysical characteristics of percutaneous boost irradiation and BT were analysed by means of modelling. Local (LTC), locoregional tumour control (LRTC), overall survival (OS) and prognostic factors were studied. HDR boost BT significantly increased complete remission (80 % vs. 55 %;  $p = 0.0257$ ). The 5-year probability of LTC, LRTC and OS for all stages were 60 %, 52 % and 46 % or 36 %, 34 % and 26 %, respectively, in favour of the boost group. Brachytherapy increased LTC by 24 % ( $p = 0.0188$ ), LRTC by 18 % ( $p = 0.0753$ ) and OS by 20 % ( $p = 0.0545$ ). The rate of local tumour control in our T2-T4 patients was 100 %, 83 % and 50 % (there was no T1 tumor), while in the boost LDR studies it was on average 80 %, 83 % and 73 %, respectively. Our results were comparable to these latter results. Combination of postoperative tumour bed BT and external irradiation gave good results in the postoperative treatment of selected early base of tongue cancers, but no conclusions could be drawn because of the low number of cases. Incidence of serious mucositis with or without boost occurred in 10 % and 5 %, respectively. The occurrence of late side-effects was negligible. On the other hand, in the LDR studies the rate of soft-tissue necrosis and osteoradionecrosis varied between 2.5 and 27 %, and 0 to 6 %, respectively. The use of flexible applicators is more advantageous from the point of view of both radiation physics and biology. With CT-based BT planning – compared to traditional planning – the coverage of the target volume with the reference dose was 9 % better (87 % vs. 78 %). The radiation exposure of the critical organs was lower with boost BT than with teletherapy. In univariate analysis boost BT for LTC ( $p = 0.0449$ ), tumour size for LTC ( $p = 0.0443$ ), LRTC ( $p = 0.0125$ ) and OS ( $p = 0.0025$ ), nodal status for OS ( $p = 0.0098$ ) were significant prognostic factors. Summarizing our results, HDR BT combined with external irradiation seems to be suitable for the exclusive radiotherapy of base of the tongue cancer, because it improves local tumour control significantly without considerably increasing the risk of side-effects.

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MÁRTA UJPÁL (2004)

## Correlations of tumors of the oral cavity and diabetes mellitus

Supervisor: Dr. Zsuzsanna Suba

The oral signs of diabetes mellitus are well known: gingivitis, parodontosis, and lesions of the oral mucosa (glossitis and candidiasis). However, the literature to date does not detail any connection between diabetes mellitus and tumors in the oral cavity. The author has carried out epidemiological, clinical and histopathological studies on this topic. The stomato-oncological screening of diabetics revealed that subjects with diabetes (and particularly that of type 2.) display an elevated incidence of inflammatory lesions, benign tissue growth and precancerous states. Diabetics who smoke are at high risk of the development of precancerous processes in the oral cavity. A retrospective investigation of malignant tumors in the oral cavity demonstrated an enhanced frequency of diabetics (mainly of type 2.) among those with oral carcinoma. The most frequent malignomas among diabetics are tu-

mors of the gingiva and the lip. It emerged from the clinical and histopathological studies that the progression of oral carcinomas is accelerated by diabetes: the tumors then form metastases more quickly, and death occurs earlier. A new hypothesis is proposed as regards the correlation of tumors in the oral cavity and diabetes mellitus, study of which demands further detailed research work.

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## 8/2. PROGRAM

### Alterations of Cells, Fibres and Extracellular MATRIX and Diagnostic Pathomorphological Studies in the Course of Heart and Vascular Diseases and in Certain Tumours. Experimental and Diagnostic Pathomorphological Studies

#### *Coordinator*

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Two of leading causes of morbidity and mortality in Hungary are the cardiovascular diseases, especially atherosclerosis and malignant tumors, e. g. breast cancer in females. The Program offers a multi faceted analysis of the above diseases, completed with the development of liver diseases including liver tumors. The research studies apply various pathohistological approaches with the extension of clinical retrospective and prospective studies. The project is dealing mostly with human materials, though experimental models are also induced.

#### *Sub-programs*

Locoregional staging and prognostic factors

Molecular biology and immunological importance of adhesion proteins

Macro-and microstructure of blood vessels

Alterations of extracellular matrix in atherosclerosis and in various tumors

Experimental prearteriosclerotic vascular lesions

Prognostic detections in breast carcinomas

Screen detected in situ breast carcinomas

Prognostic significance of adhesion molecules and extracellular matrix components in breast carcinomas

Breast screening: frontiers of radiology and pathology

Factors in the development of liver diseases

Extracellular matrix in chronic liver diseases

Growth factors in the progression in chronic liver diseases

The role of cell adhesion molecules in the development of liver cirrhosis and in hepatic tumors

#### *Supervisors*

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András FALUS

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**Ph.D. candidates**

Tibor Glasz*	na
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Anna Kádár
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**Ph.D. graduates**

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Anna Polgár	pt
Péter Sótonyi	pt
Zoltán Wiener	ft

**Supervisors**

Anna Kádár
András Falus
Anna Kádár
András Falus

a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

**ESZTER HORTOVÁNYI (2004)****The pathogenesis of atherosclerosis: small steps of the thousand miles journey**

*Supervisor: Dr. Anna Kádár*

The purpose of the first study was to determine the effects of ovariectomy and long-term combined sexual hormone replacement on the gap junctional protein of aortic smooth muscle cells in rats. Rats were ovariectomized, and received estrogen and/or progesterone. Neither the ovariectomy nor the hormone replacement had any effect on the gap junctional protein expression of aortic smooth muscle cells as compared to control animals. These results indicate that sexual steroids do not influence the gap junctional protein of the medial layer of aorta. Chlamydia pneumoniae has emerged as the most likely pathogen to have a causative role in the development and/or progression of atherosclerosis. In an effort to better understand the significance of finding Chlamydia pneumoniae in atheroma, we examined different artery segments – such as coronary arteries of young adults, carotis endarterectomies, dissected aortic specimens, failed coronary grafts and also aortic valves by immunohistochemistry. Chlamydia pneumoniae was not found in the intimal and medial layer of normal-appearing coronary arteries. The rate of Chlamydia pneumoniae positivity increased with the severity of lesions. In non-atheromatous segments of coronary arteries a sequence of preatherosclerotic changes was identified which consisted of medial thickening followed by intimal thickening. In the third study the intimal and medial thickness of the left descending coronary artery of young adults were measured, and were correlated with the presence of Chlamydia pneumoniae antigens. In the proximal segments, atherosclerosis was associated with the widening of both the intima and the media of lesion free sites. In the distal segments the proportion of the intimal thickening had a significant association with atherosclerosis. Compared to non-infected arteries, Chlamydia pneumoniae infection was associated with higher hypertrophy index in the intima as well as in the media. Chlamydia pneumoniae may favour arterial wall hypertrophy and plays a role in lesion progression. The presence of C. pneumoniae correlates with the severity of atherosclerosis, thus may initiate atherosclerotic injury or facilitate its progression, or both.

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## ANNA POLGÁR (2003)

### Immune recognition of cartilage matrix components and soluble interleukin-6 receptor in rheumatoid arthritis

Supervisor: Dr. András Falus

Autoantigen of rheumatoid arthritis (RA) is still debatable, there are several questions to clear in its pathomechanism as well. Cartilage matrix components, especially small, leucin rich proteoglycans were tested as potential rheumatoid arthritis autoantigens. Role and production of soluble receptor for interleukin-6, which is a key cytokine responsible for autoantibody production, were also investigated. Serum and synovial fluid biglycan, decorin, aggrecan, type II. collagen and fibronectin specific IgG and IgM autoantibody levels of RA, psoriatic arthritis (PsA), other seronegative spondylarthritidis (SNSA) and osteoarthritis (OA) patients were determined by ELISA. To all of the five cartilage matrix components humoral recognition was detectable, especially in synovial fluids. Isotypes of collagen specific autoantibodies were mainly IgG, while biglycan and decorin specific ones IgM. The highest relative frequency of elevated antibody levels were decorin specific synovial IgM found at RA and SNSA patients. RA and systemic lupus erythematosus (SLE) patients' and healthy controls' sIL-6R levels were determined in sera and 48 hours lymphocyte culture supernatants without and after incubation with dexamethasone by ELISA. sIL-6R mRNA was also detected by RT PCR. Serum sIL-6R levels didn't correlate with disease activity, but sIL-6R serum concentrations of patients with inactive RA were significantly lower than that of patients with active RA, SLE and healthy control group. Supernatants' sIL-6R levels showed similar pattern to sera, which suggest that lymphocytes have important role in the production of circulating sIL-6R. In vitro glucocorticoid treatment inhibited RA lymphocytes' sIL-6R production in concentration dependent way.

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## PÉTER SÓTONYI (2004)

### Cardiovascular effects of Tinuvin 770 in animal model

Supervisor: Dr. Anna Kádár

Tinuvin 770 bis(2,2,6,6-tetramethyl-4-piperidinyl)sebacate pharmacologically active agent used world wide as a light stabiliser for plastic materials. In vitro studies show that it is an L-type Ca<sup>2+</sup>-channel and neuronal nicotinic acetylcholine receptor blocker. Tinuvin 770 is a widely applied component of plastic materials used in the medical field and also in the food industry. Hypotension, veg-

etative dysfunction and neurological symptoms are frequently observed during a haemodialysis treatment. During the haemodialysis the plastic materials can come to direct contact with the circulation on huge surface and long exposure time. The release of Tinuvin 770 from plastic materials applied in haemodialysis may play a part in the development of clinical symptoms. The present studies investigate: (1) the Tinuvin 770 content and release of four different commonly used haemodialysis membranes, (2) in vitro effect of Tinuvin 770 on isolated myocardial cells, (3) acute haemodynamic changes in Tinuvin 770 treated dogs and 4. the chronic cardiotoxicity of Tinuvin-770 in vivo in rat model. Results: (1) Tinuvin 770 release was detected from all examined membranes. (2) The Tinuvin 770 causes irreversible cell damage of isolated myocytes with hypercontraction and decreased level of high energy phosphates. (3) Intravenous administration of Tinuvin 770 results expressed cardiodepression and vasodilatation leading to cardio-vascular insufficiency. (4) In chronic model the morphological results correspond to catecholamine induced myocardial damage. Current literature as well as our research indicates that more detailed toxicological analysis of Tinuvin-770 should be required and current regulations in medical and food industries should adopt the new results.

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## ZOLTÁN WIENER (2004)

# The role of histamine in the differentiation and functions of mast cells in vitro

Supervisor: Dr. András Falus

One of the key steps in revealing the effects of histamine was the production of histidine decarboxylase (HDC) deficient mice which are unable to express the only enzyme involved in histamine synthesis. These mice are histamine-free and show unexpected phenotypes, for example less mast cells than the wild type ones. The lower number of mast cells in HDC<sup>-/-</sup> mice may be the consequence of their disturbed differentiation processes. To test this hypothesis we established in vitro bone marrow-derived cell cultures. We observed a lower proportion of mast cells and promastocytes in HDC<sup>-/-</sup> cultures. However, when adding histamine to HDC<sup>-/-</sup> cultures or the inhibitor of HDC enzyme,  $\alpha$ -fluoromethyl-histidine to wild type cells, we could detect no difference compared to the untreated controls. Based on these experiments and on semisolid colony assays performed with bone marrow-derived cells we concluded that the progenitor cell content in the bone marrow of HDC<sup>-/-</sup> mice is modified. The effect of histamine on mast cell development was also confirmed by testing embryonic stem cell-derived differentiation processes. HDC<sup>-/-</sup> tissue mast cells were described to contain granules with abnormal morphology which raises the interesting question whether these cells function in a modified way compared to wild type ones. Our attention turned towards the effect of IL-9, the level of which is elevated during intestinal infections and results in mastocytosis. The overexpression of IL-9 causes asthmatic syndromes in mice and it has been implicated in human asthma, too. First we tested its effect on the cytokine expression profile of wild type mast cells. According to our results IL-9 is a potent inducing agent only in the presence of ionomycin or Ige/antigen and elevates the mRNA level of IL-1 $\beta$ , IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13 and MIF. It seems to act through the production of IL-1 $\beta$ . In HDC<sup>-/-</sup> mast cells the IL-9-induced IL-9 synthesis is inhibited. IL-1 $\beta$  and ionomycin also results in a lower IL-9 mRNA level in histamine-free mast cells. These data show that HDC<sup>-/-</sup> mast cells are able to produce less IL-9 upon different stimulations compared to wild type controls. Based on our experimental results we conclude that the lack of histamine modifies mast cell differentiation by influencing the size of the progenitor/stem cell population, and the different cytokine expression profile of HDC<sup>-/-</sup> mast cells may explain the reduced asthmatic symptoms observed in histamine-free mice.

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## 8/3. PROGRAM

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### MICROBIOLOGY

#### Coordinator

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Infections caused by bacteria, viruses, parasites and fungi have shown significant leading roles in morbidity, mortality and health economy all over the world including Hungary. This Program has offered a variety of studying the causative agents, pathomechanisms, pathogenesis, transmission, epidemiology, control, rapid diagnosis and prevention of most frequent infections in Hungary from molecular to host level. Outstanding parts of the Program have been the *in vitro* and *in vivo* pathomechanism and pathogenesis of natural and nosocomial infections including the pheno- and genotypical features of infectious agents with special regard to the presence and expression of genes responsible for toxin production, cell-surface properties, and resistance to different antimicrobial groups. It has been extended to investigate the structure and function of adenovirus epitopes, the interaction between virus and host cell, the origin and spread of hepatitis viruses, the regulation of viral oncogen expression. Most recently the effects of infections on the quality of life as well as on health care economy have been involved in the program.

#### Sub-programs

Biological effects of immunomodulators

Antibiotic resistance, serotype and genotype of *Streptococcus pneumoniae*

Identification/characterization of bacterium species by molecular genetic techniques

Application of molecular microbiological methods in rapid microbiology diagnostics

Molecular biological examination of resistance mechanisms in Gram-negative bacteria

Investigation of the interactions between virus infected cells and host cells at molecular level

Studies on antigenic structure and biological function of adenovirus hexon

Molecular examination of viruses (HBV, HCV, HGV and TTV) from hepatitis virus carriers aiming to reveal their origin and spread

Molecular studies and diagnostics of RNA viruses

Studies on host dependent methylation patterns of latent Epstein-Barr virus genome using automated fluorescent genomic sequencing

#### Supervisors

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György BERENCSEI

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János MINÁROVICS

Regulation of expression of virus oncogens in cells carrying latent Epstein-Barr virus genome	János MINÁROVICS
Role of pathogens in development of atherosclerosis: molecular mechanisms	Éva GÖNCZÖL
Molecular study on the role of human retroviruses (HIV, HTLV) in immunopathological diseases	Károly NAGY
Role of human herpesvirus 8 (HHV-8) in the development of Kaposi sarcoma	Károly NAGY
Epidemiology, pathogenesis and laboratory diagnosis of parasitoses	Zsuzsanna SZÉNÁSI
ustrighInfection control and clinical epidemiology	László GULÁCSI

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a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

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**ORSOLYA DOBAY (2004)**

## Epidemiology of *Streptococcus pneumoniae*: Molecular characterisation, antibiotic sensitivity and serotyping of Hungarian isolates

*Supervisor: Dr. Ferenc Rozgonyi*

*Streptococcus pneumoniae* is one of the most important pathogens world-wide, causing mainly upper and lower respiratory tract diseases, but also capable of causing invasive diseases such as meningitis, with high morbidity and mortality rates. During the 1990s, extremely high resistance rates were reported from the country, however, the pneumococcal situation was not investigated in the last few years in Hungary. Therefore we wanted to perform a large-scale epidemiological study, based on genotypic and phenotypic methods. We had a collection of 304 pneumococcal isolates (confirmed by PCR-based identification) from five different centres in three subsequent seasons, and we deter-

mined their antibiotic sensitivity, serotypes and genetic relatedness by pulsed-field gel electrophoresis (PFGE). This kind of survey has never been performed in Hungary before. We found a contradictory low penicillin resistance proportion compared to the previous reports, however, we found very high macrolide resistance levels. Although the fluoroquinolones showed very good efficacy, there was a gradient increase in the MICs through the three seasons of the study. We focused our attention on the penicillin- and macrolide non-susceptible (PNS and MNS) isolates. Instead of the predominance of serotype 19A (as also reported earlier), a big diversity of serotypes was found among them. The serotypes and antibiotic sensitivity showed good correlation with one another and the clinical data, especially the age of the patients. The presence of fewer, but larger genetic clusters was characteristic to the PNS isolates, while rather many smaller groups were identified among the MNS ones. We confirmed the presence of at least five internationally wide-spread resistant clones in Hungary based on PFGE comparison. As serotypes and genotypes did not always show clear correlation, we suggested that epidemiological studies should always be based primarily on genotypic methods. The molecular examination of the MNS isolates showed the predominance of *erm(B)* determinant in Hungary, but we also found a few strains with *mef* genes, including 3 isolates with unusually high macrolide resistance. We identified interesting novel mutations in the ribosomal RNA and proteins of these strains, as well as in others, by sequencing. We have also found the *erm(TR)* gene in one of the strains, which is very rare in pneumococci, emphasising the importance of testing the isolates in the presence of 5% CO<sub>2</sub> when we are looking for resistance determinants.

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- Dobay O, Rozgonyi F, Amyes SGB (2004) Virulence factors, antibiotic resistance mechanisms and the prevalence of resistance worldwide in *Streptococcus pneumoniae*. *Med Microbiol* 15:27-39.
- Dobay O, Rozgonyi F, Hajdú E, Nagy E, Knausz M, Amyes SGB (2005) The relationship between serotypes and PFGE genotypes in isolates of *Streptococcus pneumoniae* from Hungary. *Clin Microbiol Infect* 11:673-676.

## 8/4. PROGRAM

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### PUBLIC HEALTH AND HEALTH SCIENCES

#### *Coordinator*

**Péter SÓTONYI**

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The program includes issues and joint areas of public health, forensic medicine, hygiene and dietetics. The various subprograms sum up knowledge in the fields of organ damages caused by environmental injuries, endocrinological diseases and virology. A special sub-program deals with topics of health education and nursing. The various sub-programs have a common basis and following the branching-off there is an opportunity to select a special subjects.

#### *Sub-programs*

Epidemiology, physiology and pathophysiology of alimentation  
 Epidemiological, diagnostic and clinicopathological aspects of the relationship between iodine- supply and the endocrine system  
 Researches in project-planning, methodology and effect-analysis in health education and nursing  
 Radiobiology and radiohygiene  
 Pathomechanisms of health damaging effect of environmental chemicals

#### *Supervisors*

Magda ANTAL  
 István SZABOLCS  
 Judit MÉSZÁROS  
 Györgyi RONTÓ  
 Péter SÓTONYI

*Further topics*

Support of tutors in the health education of 3-18 yr children  
 Molecular diagnostics of respiratory tract viruses  
 Medical insurance  
 Health education for women leaders  
 Personality in the social contacts of nurses  
 Patient's guiding  
 Preparation of students in higher education for health education  
 Nursing structures  
 Death caused by drugs (especially heroin abusers)  
 Study on combined (physical, chemical) environmental factors with  
 cytogenetics and cell biology  
 Role of anti-oxidants in the prevention  
 Epidemiology of tumor diseases  
 Developments of epidemiological surveillance  
 Hygiene in hospitals  
 Life style and health conditions  
 Life style problems among secondary school students  
 Risky behaviour of hungarian secondary school students  
 Effect of lipid composition on atherogenesis  
 Unemployment and environment  
 Effect of environmental chemicals on health  
 Effect of metals on maternal haemodynamic, transplacental transport

**Ph.D. students**

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a: absolutorium, ft: full-time, pt: part-time, it: international, na: non-affiliated

**PÉTER HERMANN (2004)****Oropharyngeal candidosis in immunocompromised patients***Supervisor: Dr. Péter Sótónyi*

80 persons with haematological malignancies receiving stem cell transplantation (SCT) were examined over a 24 months period. Altogether 28 samples were collected using transport media from each of the 80 patients, except one, who died in Candida sepsis before the collection of the last +7.day samples. Therefore the total number of microbiological specimens was 2233. In three samples of one patient was found *C. inconspicua*, which was first identified as *Candida norvegensis* by the ID32C-system. On the basis of the RFLP patterns and also by RAPD analysis our clinical isolates proved to be *C. inconspicua*. The fluconazole sensitivities and the activities of the main extracellular enzymatic virulence factor aspartic protease and phospholipase of these *C. inconspicua* isolates was determined and compared them with those values of oropharyngeal isolates of *C. albicans* and *C. krusei* from SCT patients. Clinical isolates of *C. inconspicua* differed from the type strain by 20%, but resulted in completely identical patterns. This confirms that isolates belong to *C. inconspicua* and also indicates that they belong to the same strain/clone. By using the E-test we determined the fluconazole sensitivity of the *C. inconspicua* isolates. These MICfluconazole values are in good ac-

cordance with the earlier findings that fluconazole resistance is an inherent trait of *C. inconspicua* and they can explain the failure of the fluconazole pretreatment in this particular case. The aim was to investigate the effect of alkaline earth metals (NaCl, KCl and LiCl) on the growth and on some known virulence factors of this yeast. The results support the observations in that, in case of the *C. albicans* strains tested, due to the effect of alkaline earth metals, the ability to adhere to acrylic sheets and hydrophobicity has changed in an analogous way, i.e. both have decreased. Antifungal treatment was successful during the SCT treatment periods, since the colonisation decreased, but more attention should be paid not only to the oral hygiene of these patients, but to the restoration of the salivary flow rate even prior to the SCT treatment.

- Hermann P, Berek Z, Nagy G, Kamotsay K, Rozgonyi F (2001) Pathogenesis, microbiological and clinical aspects of oral candidiasis (candidosis) *Acta Microbiol Immunol Hun* 48:479-495.
- Dombi C, Vörös-Balog T, Hermann P, Czeglédy Á, Vincze N, Bánóczy J (2001) Risk group of oral precancer attached to X-ray lung-screening examination. *Community Dent Oral Epidemiol* 29:9-13.
- Hermann P, Forgács K, Gál E, Lenkey B, Nagy G, Rozgonyi F (2003) Effects of alkali metal ions on some virulence traits of *Candida albicans*. *Folia Microbiol* 48:173-176.

## CSABA KISS (2004)

# Epidemiology, metabolism and clinical aspects of diffuse idiopathic skeletal hyperostosis

Supervisor: Dr. Magda Antal

Diffuse idiopathic skeletal hyperostosis (DISH) is a chronic degenerative disease of the musculoskeletal system. It is characterized by increased inclination of tendons (enthesis) to ossification. Its primary symptom is the ligamentous ossification of the anterolateral ligament of the spine. The importance of DISH, which is a frequent problem in rheumatology and general practice, lies in its prevalence, complex relationship to metabolic disorders in the etiology, clinical symptoms affecting quality of life, as well as its rare complications, which may be life threatening. Our aim was to determine DISH prevalence and incidence in Hungary together with obesity prevalence, which is often associated with DISH. We evaluated the relationship of DISH to obesity and parameters of metabolism in comparison with a control group of patients with spondylosis in a case-control study. Furthermore, the relationship of DISH to obesity was studied in obese and non-obese patient groups. Quantity and quality of bone in patients with DISH were measured in a case-control study in comparison with a healthy control group. Quality of life in DISH patients was compared with that of spondylosis patients and the effectiveness of physiotherapy in DISH was evaluated in a non-control study. DISH prevalence (27.3% in men and 12.8% in women), incidence (37/1,000 years in men and 17/1,000 years in women) and obesity prevalence (18.1% in men and 15.4% in women) were determined in both sexes. In comparison with international data, DISH prevalence in Hungary proved to be in the upper section of the expected region both in men and women. Of metabolic parameters, obesity and higher levels of serum uric acid were significantly more frequently observed in the DISH group than in the spondylosis control group. Correlation between DISH and obesity was the strongest. Complex metabolic disorder (a metabolic syndrome) is likely to be the underlying cause of the disease. Densitometry detected higher lumbal density in males in the region of entheses and lower bone quality in comparison with the healthy control group outside of the entheses. According to our study hyperostosis is not only a generalized disease of the entheses because it affects bone quality as well. Data of quality of life questionnaires indicate that DISH is accompanied by life quality which is similar to that of spondylosis, however, it involves less pain, but significantly greater disability, and occasionally severe complications. According to the non-controlled study, quality of life can be successfully improved, pain and disability can be significantly reduced with physiotherapy.

- Kiss C, Szilágyi M, Paksy A, Poór G (2002) Risk factors for DISH: A case-control study. *Rheumatol* 41:27-30
- Kiss C, O'Neill T, Mituszova M, Szilágyi M, Poór G (2002) The prevalence of diffuse idiopathic skeletal hyperostosis in a population based study in Hungary. *Scand J Rheumatol* 31:226-229.
- Kiss C, Poór G, Donáth J, Gergely P, Paksy A, Zajkás G, Antal M (2003) Prevalence of obesity in an elderly Hungarian population. *Eur J Epidemiol* 18:653-657.

**RÓBERT LANGER (2003)**

## Selectin inhibition mitigates both ischemia/reperfusion injury and allograft rejection by blocking production of the cytokine/chemokine response

*Supervisor: Dr. Péter Sótonyi*

Presented work describes for the first time the effects of the selectin inhibitor BIMO on the survival of kidney allografts and on ischemic/reperfusion injury. The results documented that BIMO alone inhibits both allograft rejection and ischemic/reperfusion injury. Furthermore, BIMO in combination with other immunosuppressive drugs (cyclosporine, sirolimus or FTY720) acted synergistically to block allograft rejection. Finally, the inhibitory effect of BIMO on allograft rejection correlated with reduction of cytokines and chemokines mRNA expression. These results suggest that selective inhibition of selectins produces a very complex pattern of changes in the immune response. We postulate that the selectin inhibitor may be an excellent choice to produce a non-toxic immunosuppressant that may complement standard drugs, such as cyclosporine. Concerning the action of mechanism of the above mentioned characteristics the ribonuclease protection assay (RPA) was applied to examine the mRNA expression of different cytokines, chemokines and chemokine receptors. It was proven that BIMO blocks allograft rejection by reduction of intra-graft production of cytokines and chemokines resulting in decreased activation of graft infiltrating cells. The action of mechanism for preventing the ischemia-reperfusion injury can not be explained by the present investigation because the tested cytokines, chemokines and chemokine receptor gave us no information. The same was true for any involvement with the apoptosis cascade and any information concerning the spleen as a potential site for immune capacity bearing cells of the same animals.

- Langer RM, Kahan BD (2002) *Incidence, therapy, and consequences of lymphocele after sirolimus-cyclosporine-prednisone immunosuppression in renal transplant recipients. Transplantation* 74:804-808.
- Langer RM, Hong DM, Van Buren CT (2002) *Basiliximab-sirolimus-prednisone induction regimen followed by delayed low-dose cyclosporine in renal transplant recipients of living donors. Transplant Proc* 34:3162-3164.
- Langer RM, Kahan BD (2002) *Alexis Carrel's legacy: visionary of vascular surgery and organ transplantation. Transplant Proc* 34:1061-1066.

**LÁSZLÓ MANGEL (2003)**

## Radiation dose escalation and combined therapies in postoperative treatment of malignant gliomas. Experimental and clinical results

*Supervisor: Dr. Egon Hidvégi*

The technical evolution of neuroradiology and radiotherapy brought with it new theoretical and practical advances in the irradiation of malignant gliomas. The great number of local relapses encourages radiotherapists to apply focal radiotherapy techniques which prevent the normal tissues from being damaged, and create the possibility for dose escalation, and the simultaneous application of systemic modalities, such as chemotherapy and immunotherapy. The aim of our experimental and clinical investigation was to elaborate the optimal dose escalation strategy and to prove the efficacy of combined modality treatments with the increased control of therapeutic side-effects. We examined under laboratory conditions the Hungarian-developed dibromodulcitol, the widely-used carmustine, as well as procarbazine which is the active metabolite of temozolamid keeping the most efficacious cytotoxic medicine against glioma. The combination of these drugs with X-ray irradiation

resulted in advantageous co-operation and increased life span of brain tumourous animals. The combination of X-ray irradiation and auto-vaccination with cytokine producing glioma cells also proved efficacious. Based upon radiobiological calculations, we succeeded in proving that the daily fractions of irradiation can be elevated without the increased probability of normal tissue damage. This way we elaborated a novel biological dose escalation strategy. The analysis of our retrospective clinical observations shows that the HDR AL boost increases the median survival time for T1 glioblastomas, and the novel intensified hypofractionated external beam radiotherapy can be the alternative for conventional irradiation in case of advanced diseases. The use of adjuvant or salvage chemotherapy, combined with our focal dose escalation methods, results in increased survival of glioblastoma patients. We developed a novel CT-densitometry based brain edema observation system to prevent the early neurotoxicity of irradiation. Using CT-densitometry, we succeeded in completing radiotherapy courses without any difficulties in most cases. We were able to avoid the routine usage of steroids as well, which is a relevant aspect when systemic therapies are applied. Our results support the theory that in the future the radiotherapy of malignant gliomas could be based on focal dose escalation and combined systemic modalities.

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- Mangel L, Vönöczky K, Hanzély Z, Kiss T, Ágoston P, Somogyi A, Németh G (2002) *CT densitometry of the brain: a novel method for early detection and assessment of irradiation induced brain edema. Neoplasma 49:237-242.*
- Mangel L, Skriba Z, Major T, Polgár C, Fodor J, Somogyi A, Németh G (2002) *Modelling normal tissue isoeffect distribution in conformal radiotherapy of glioblastoma provides an alternative dose escalation pattern through hypofractionation without reducing the total dose. Acta Oncol 41:162-168.*

**ERIKA MEDVE CZKY (2003)**

## **Conductive education: a special educational method to promote development**

*Supervisor: Dr. Judit Mészáros*

The objective of our research was to demonstrate the role of learning in the conductive education process.

Our intention was to prove the existence of the learning process by studying voluntary urination in 112 children with spina bifida (age range: 2-7 years). The condition of the urinary tract is a factor that limits the lives of spina bifida children. In order to protect the children, the status of the urinary tract was regularly checked by urologists in our multidisciplinary team. In compliance with urological protocol, we examined the children for vesicoureteral reflux (VUR) and urinary tract infections. The amount of residual urine in the bladder was measured prior to the start of the conditioning process for voluntary urination and then continuously during the conductive education period while the condition of the urinary tract was followed as well. The extent of VUR diminished in 10% and did not change in 32% of the cases while urination conditioning was applied. After the first 4 months the incidence of urinary tract infections significantly ( $p < 0,0001$ ) decreased by half, primarily as a result of less residuum and significantly more efficient ( $p < 0,0001$ ) emptying of the bladder. By the end of conductive education period 67% of kindergarten aged spina bifida children have learned independently voluntary urination. Children whose bladder had been bacteria-free did not become infected and the number of those on permanent antibiotics and disinfection therapy decreased. The former routine of catheterisation could be avoided. The number of socially continent children increased: ( $p = 0,02$ ) became able to hold urine for more than 2 hours in between voluntary urination sessions.

With careful multidisciplinary prevention, our tests demonstrated the existence of the learning process in cases of neurogenic bladder due to organic neurological damage (spina bifida). We advise against applying the method (voluntary urination) as a routine procedure or in combination with some other method.

- Czeizel EA, Medveczky E (2003) *Periconceptional multivitamin supplementary and multimalformed offspring. Obstet Gynecol 102:1255-1261.*
- Medveczky E, Puhó E, Czeizel EA (2004) *The use of drugs in mothers of offspring with neural-tube defects. Pharmacoepidemiol Drug Saf 13:443-455.*
- Medveczky E, Puhó E, Czeizel EA (2004) *An evaluation of maternal illnesses in the origin of neural-tube defects. Arch Gynecol Obstet 270:244-251.*

**ANDREA RADÁCSI (2004)**

## **Thyroid disorders in different life periods and in areas of different iodine intake (experimental, epidemiological and clinical studies)**

*Supervisor: Dr. István Szabolcs*

We investigated the interactions of different environmental factors (old age, pregnancy, iodine supply, oxidative DNA damage, humin substances in drinking water) on the presence of thyroid disorders. Old age, iodine supply: We screened the thyroid function in chronically ill geriatric patients (N=124) at admission and hospital stay in a moderate iodine deficient area. A measurable TSH at hospital admission practically excludes hyperthyroidism in the follow up. Suppressed TSH levels remain suppressed but subnormal levels should be controlled because their normalization frequently occur in the follow up. Screening upon hospital admission is sensitive enough to detect cases of thyroid dysfunction and justified by their high prevalence. We investigated the thyroid functions and the mortality rate in chronically ill geriatric patients (N=93) from a moderate iodine deficient area in a 2-year follow up. We concluded that subclinical hyperthyroidism is associated to higher mortality rate, justifying the screening for thyroid dysfunction and treatment of subclinical hyperthyroidism. A subnormal but measurable TSH is not indicative for the future development of hyperthyroidism. Antibody positivity in the euthyroid case is not predictive for the future development of hypothyroidism. We investigated the diagnostic value of serum Tg and Tg/TSH ratio for goitre screening in elderly nursing home residents from areas of different iodine intake (group A: N=75, g/g creat., µg/g creat., group B: N=53, MIE: 101µmedian iodine excretion (MIE):62 g/g creat.). We showed that goitre screening by Tg in old group C: N=71, MIE:496 the calculation of the Tg/TSH ratio has no advantage over; age is not justified in the non-iodine deficient elderly, the addition of Tg to TSH screening; Tg would have some benefit in exclusion of goitre. Pregnancy, iodine supply: We performed a cross-sectional thyroid screening study in pregnant women from areas of different iodine intake in Hungary. However, the iodine excretion was not different between the iodine deficient and iodine sufficient region. Thus, iodine prophylaxis during pregnancy is recommended in all Hungary. Screening for thyroid dysfunctions in the I. trimester is justified by their high prevalence. Screening for antiTPO level in pregnancy is also justified because of the high prevalence of antiTPO positivity and the increased prevalence of subclinical hypothyroidism in the third trimester in the antiTPO positive cases. Oxidative DNA damage: Oxidative DNA damage (DNA-adduct) was detectable by immunohistochemical analysis in medullary and papillary thyroid cancer, but the method was not sensitive enough for quantitative measurements. Therefore, in further studies primary cell cultures from cancer tissue should be used. Humin substances in drinking water: The content of humin substances in the drinking water was examined in 30 different Hungarian communities. There was no correlation between the content of humin substances and the prevalence of goiter.

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- Takáts IK, Péter F, Rimanóczy É, Dohán O, Földes J, Vadász J, Feldkamp J, Szilágyi G, Góth M, Kovács L, Radácsi A, Szabolcs I (2000) *The blood spot thyrotropin method is not adequate to screen for hypothyroidism in the elderly living in abundant-iodine intake areas: comparison to sensitive thyrotropin measurements. Thyroid 10:79-85.*
- Radácsi A, Dohán O, Kovács G, Bernard W, Feldkamp J, Horster FA, Szabolcs I (2003) *Mortality rate of chronically ill geriatric patients with subnormal serum thyrotropin concentration. Endocrin 21:133-136.*

## IMRE RURIK (2003)

**Primary care evaluations of nutrition and health of elderly***Supervisor: Dr. Magda Antal*

The number and ratio of elderly has been increasing worldwide, also in Hungary. Medical treatment provided for the elderly means the greatest part of health-budgeting and workload in hospitals and primary care offices. The quality of life also of old age is influenced by nutritional factors. Nutritional habits can change during life, even those which are already fixed. This means an important task for physicians. Evaluations were planned for assessment of nutritional habits of elderly people in a primary care office, in Budapest. The largest possible proportion of the target population was planned to involve in this study. We intended to evaluate the nutritional habits, nutritional status, biomarkers and their possible relations to morbidity. Special attention was turned toward obesity, diabetes type 2, hypertension and osteoporosis which are of great importance from the point of view of Public Health. A cross-sectional study was planned including some retrospective elements like body weight and height in the former life decades. During the 3 stages of evaluations the 264 people involved gave information on their state of health, nutritional habits (including FFQ). Their anthropometric data, serum biomarkers, bone mineral density, laboratory parameters and analysis of nutritional records were also measured. People involved randomly in the study represented 75% of the local elderly population, whose level of education and age distribution was similar to those of the national data. Smoking and drinking habits proved to be similar to those detected in former evaluations. Triglyceride level was in the highest part of the reference range and the mean serum total cholesterol level was beyond it. Almost all registered anthropometric parameters were higher than in a former national-wide survey 15 years ago. The mean BMI was in the overweight category, which means a great cardiovascular risk. On average, 16-17.000 HUF were spent each month by every person on foods and 10% of this amount on alcoholic beverages. With age, the number of daily meals is increasing. It was more visible by men, many of whose had breakfast again. Women ate frequently. In the food frequency questionnaire a higher consumption of meat, eggs, and cold-cuts, fresh-green was detected by men and that of diary products, fruit, bread and coffee by women. The total energy intake was higher by men. A higher proportion of fats, cholesterol, sodium and smaller amounts of carbohydrates, iron, copper and calcium were consumed. A higher ratio of obesity was detected within the families of obese subjects and less among people educated at college or university. People who are recently heavier had a higher body weight also in their youth. The increase of body weight by women was higher than by men. By people within the obese or overweight group a higher ratio of increase in body weight could be observed using a retrospective body weight analysis. The higher daily meal frequency was accompanied by lower BMI. The ratio of diabetics was higher in the higher BMI groups. The body weight of diabetic men was lower in the youth. A rapid increase of body weight occurred about 60 ys by men and about 40 ys by women. In the study population the prevalence of hypertension was high (67%). Men with hypertension use more added salt to meals. By obese men a higher ratio of hypertension was observed. The entire population is threatened by osteoporosis and diabetes. The real prevalence of osteoporosis is much higher in the entire population than reported by health statistics. The retrospective body weight analysis is a useful method to register the age-related body weight increase. With the use of the questionnaire the complaints of patients could be better evaluated. The ways available for the primary care physician for the interventions are the education for health improvement, life style counseling, and diet advice for the prevention of obesity.

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**ESZTER SARKADI NAGY (2003)**

## **Effects of long chain polyunsaturated fatty acid supplementation on essential fatty acid metabolism in term and preterm non-human primates**

*Supervisor: Dr. Lajos Szollár*

Arachidonic (ARA) acid and docosahexaenoic acid (DHA) are important for normal brain and retina development. We studied the effect of ARA/DHA supplementation and prematurity on tissue total fatty acid composition and on ARA/DHA synthesis in a non-human primates model. Baboons were randomized to one of four groups: Term breastfed (B); Term formula-fed (T-); Preterm (155 of 182 days gestation) formula-fed (P-); and Preterm DHA/ARA-supplemented formula-fed (P+). The P+ contained 0.61±0.03% DHA and 1.21±0.09% ARA, and breast milk had 0.68±0.22% and 0.62±0.12% as DHA and ARA, respectively. At 14 days adjusted age, neonates received a combined oral dose of [U-13C]-linolenic acid and [U-13C]-linoleic acid, and tissues were analyzed 14 days post-dose. After analyzing the total fatty acid composition of tissues and the correlation between tissue vs. plasma and RBC fatty acid levels, we conclude that (1) DHA drops precipitously in term and preterm primates consuming formula without long chain polyunsaturates, while 22:5n-6 concentration rises; (2) tissue ARA levels are insensitive to dietary LCP supplementation or prematurity, (3) plasma and RBC levels of ARA are uncorrelated with total ARA levels in tissues; (4) DHA levels are correlated with group effects and are uncorrelated within groups. Analyzing the tracer data we found that brain accretion of LNA-derived-DHA was about 3-fold greater for the formula groups than the breastfed group, and dietary DHA partially reduced excess DHA synthesis among preterms. Brain LA-derived-ARA accretion was significantly greater in the unsupplemented term group but not in the preterm groups compared to breastfed. These data show that formula potentiates biosynthesis/accretion of DHA/ARA in term and preterm neonates compared to breastfeeding, and that inclusion of DHA/ARA in preterm formula partially restores DHA/ARA biosynthesis to lower, breastfed levels. Current formula DHA concentrations are inadequate to normalize conversion to that of breastfed levels.

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**ATTILA PÉTER VÉGH (2005)**

## **Membrane protein association processes in a bacterial model system and in the human epidermal growth factor receptors (HER/ErbB)**

*Supervisor: Dr. Judit Fidy*

In this work we studied the association processes of membrane proteins in a bacterial model system and in the Human Epidermal Growth Factor Receptors. First, with a systematic approach to investigate the structural and functional aspects of integral membrane proteins we analyzed the stepwise assembly of the antenna protein complexes from purple bacteria. Studies of the polypeptide

oligomerization and the characterization of a newly isolated tetrameric subunit form (B851) in the process of the formation of the native protein allowed a more detailed description of the mechanism of the association. Our studies of the intra-membrane interactions through the construction of mutant transmembrane polypeptide sequences showed that intramembrane H-bonding is a key interaction motif in the assembly. The identified H-bonding clearly enhances the structural stability of the protein complexes in a context dependent manner. By using rescue model proteins the impact of specific interaction motif(s) were systematically examined, in particular that of single mutations at the membrane-embedded protein interfaces. Critical motifs could thus be detected within the context of the destabilized model system, which may pass unnoticed in the wild-type sequence context due to multiple, compensating native interactions. Through extending on the insights gained on the studied model system, we further investigated membrane protein association processes of pathological significance in the Human Epidermal Growth Factor Receptors (HER). Activation of these receptors through dimerization is essential in the development of a large number of cancer, including breast, ovary, lung, prostate, colon or head and neck carcinomas. By determining both homo- and heterodimerization constants between the transmembrane domains of all four members of HER (HERtm), we show that dimerization occurs on a wide range of affinity from relatively highly efficient dimerization for HER1tm and HER2tm to marginal dimer formation for HER4tm. It was revealed that the hierarchy in the dimerization of the transmembrane domains correlates with the clustering preference of the whole receptors. Making estimations of the apparent free energy of the homo- and heterodimerization processes yielded comparable energy levels involved in HERtm dimerization as in that of the extracellular domains. By mapping the putative interaction sites of the HERtms we found that the number of the interacting motifs in heterodimers shows some correlation with the order of the dimerizing potential of HERtms. Modeling the conformational structures of the receptors demonstrated that HERtm interactions are compatible with the proposed extracellular domain structures. Our findings support an active role of the HERtms in the dimerization and activation of the receptors, which explains some of the differences in the carcinogenic potential of the various dimers. These results confirm the utility of the transmembrane domains as a novel drug target to treat HER overexpressing cancer.

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