Laboratory assessment of disorders of endocrinology

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Topic….

Case report

Part 1: Brief overview immuno assays

- **immunometric assay**: principle, compounds, technical evaluation interpretation

  problems

Part 2: Detailed endocrinology

- organization of endocrine system

- **laboratory evaluation of the endocrine axis**

Immunoassays

- **Physicochemical reaction :**
- X + Y ⇄ XY ex: Na + Cl ⇄ NaCl

- **Immunoassays or binding assays :**
- Ag + Ab ⟷ Ag-Ab

equilibrium

Antigen-Antibody complex

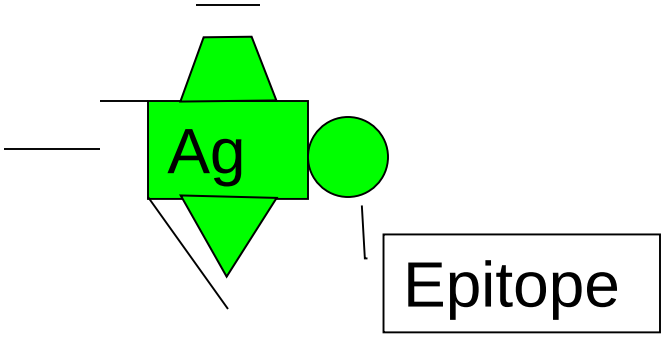
Immunoassays - Compounds

- **Antigen :** an antigen may be all kind of molecules :
  - protein
  - bacteria
  - virus…...

Immunoassays - Compounds

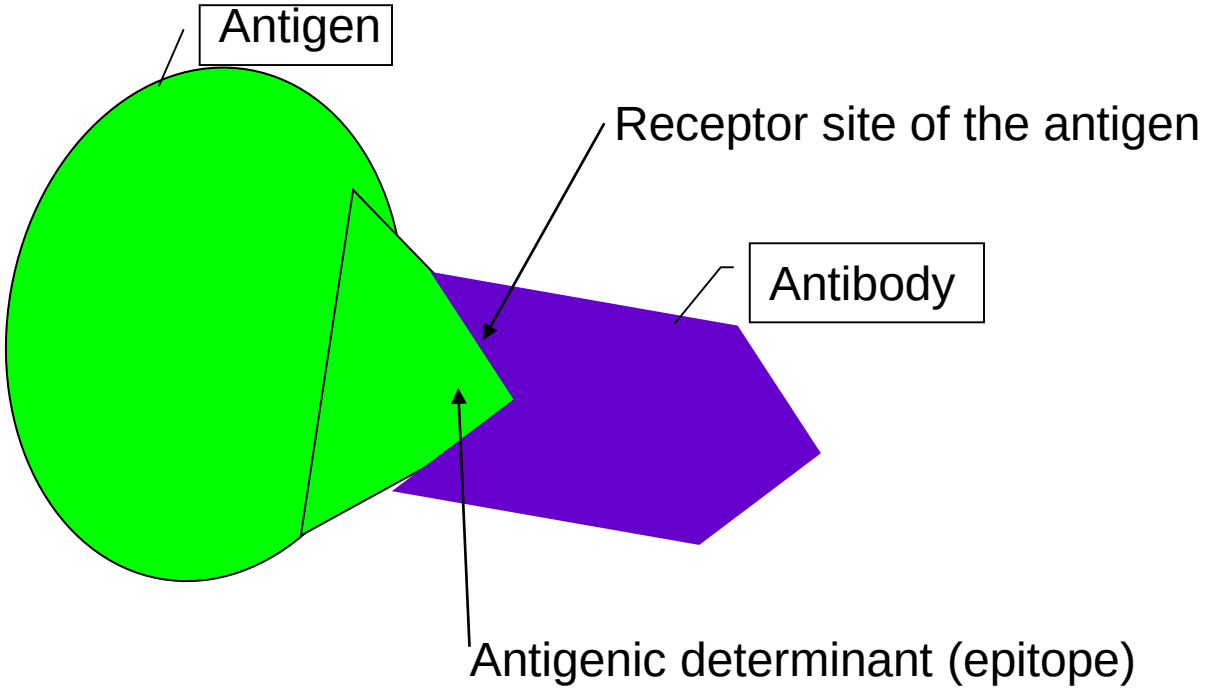
- **Antibody :** an antibody is a molecule which recognizes epitopes on antigens

  – An epitope is a sequence of amino-acids potentially existing on different types of molecules



Immunoassays - Compounds

- **RECOGNIZING STRUCTURE :** antigenic determinant (key) & receptor site (lock)



Immunoassays - Principles

- **Antigen - Antibody reaction**
  - The association between antigen / antibody is comparable with the image of the key-lock one
  - The immunossays are using a tracer which may be radioactive, enzyme, fluorescent, luminescent

Immunoassays - Principles

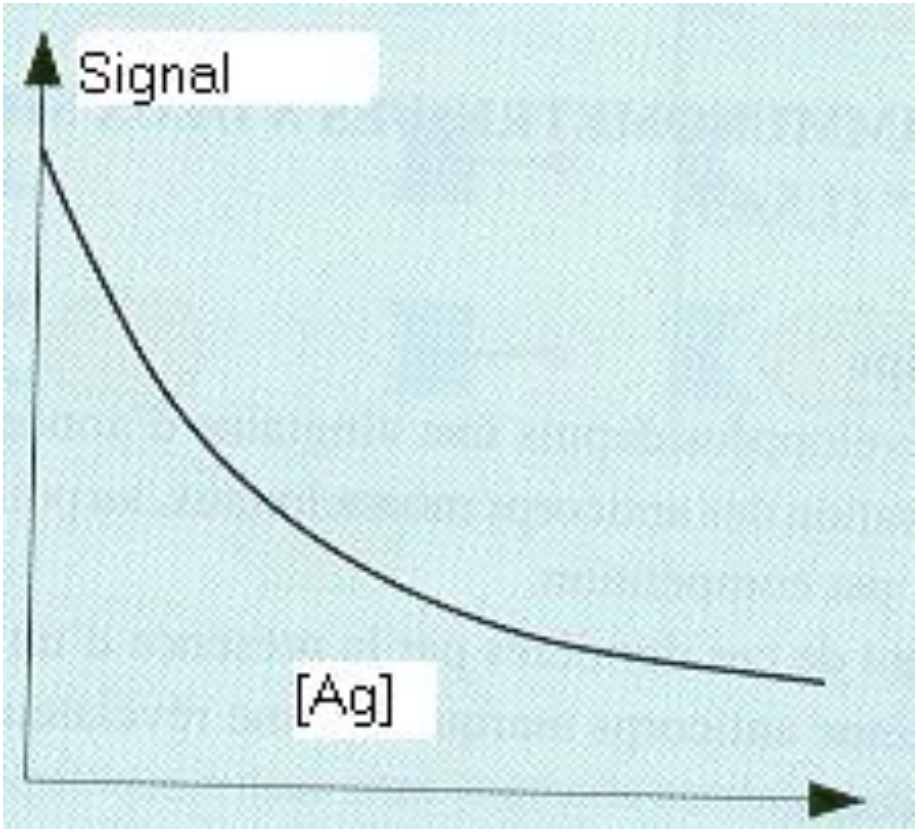
TRACER	METHODS
Radioactive isotope: ^{125}I, ^{3}H	RIA, IRMA
Enzyme : peroxydase, phosphatase	EIA, IEMA
Fluorescent compound : europium	TR-FIA, TRACE
Chemiluminescent compound : acridinium ester	CLIA

Immunoassays - Principles

- **Principles**
  - Competition
  - Immunometric (sandwich)

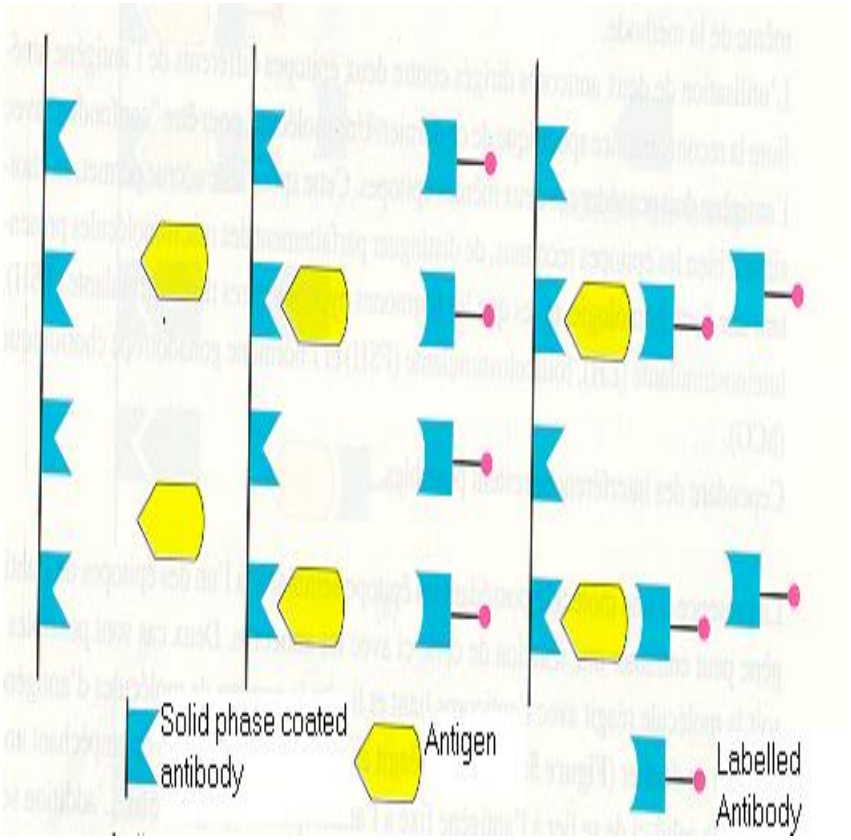
Immunoassays - Principles

- **Competition**
  - The more unlabelled antigen present, the less labelled antigen are binding to antibody and vice-versa
  - For small analytes (drug, thyroid, and steroid hormones) competitive immunoassay is the choice



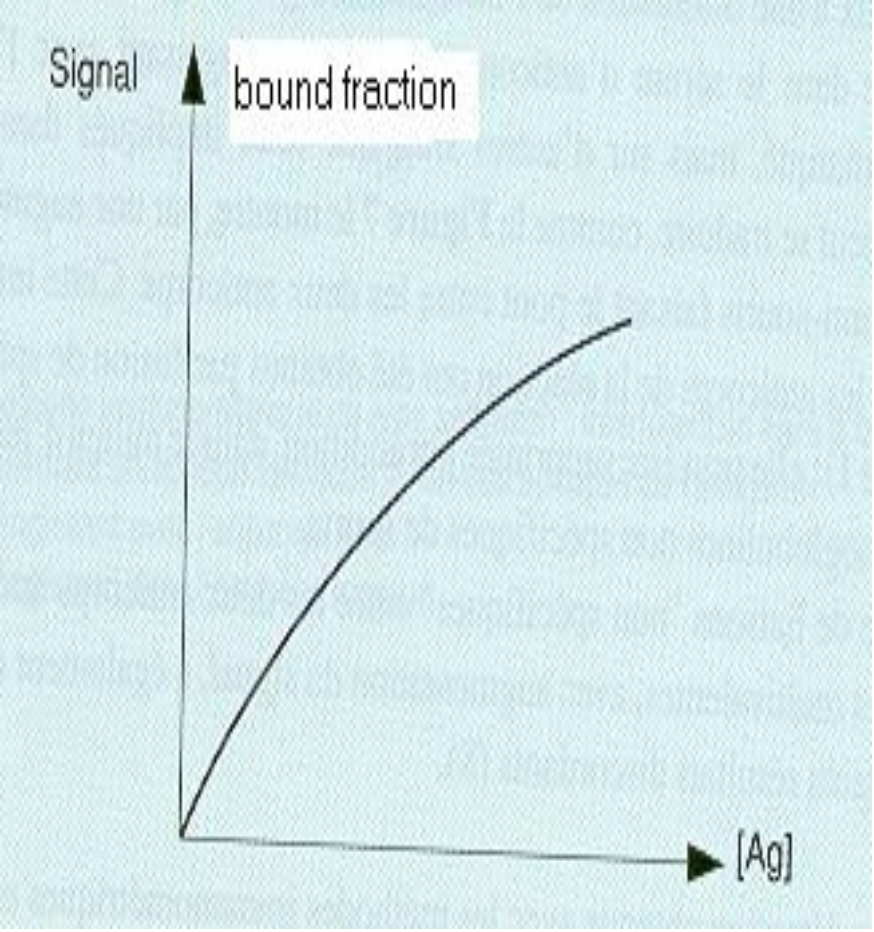
Immunoassays - Principles

- **Immunometric**
  - One antibody is bound to a solid phase , another one is labelled. The antigen will be captured between both antibodies in a kind of **sandwich**
  - The two kinds of antibodies are in excess



Immunoassays - Principles

- **Immunometric**
  - The more antigen is present, the more bound antigen and antibody complex is measured.
  - For large molecules (peptides, carbohydrates), having at least 2 epitopes, immunometric assay is the choice



In Vitro Diagnostic - Evaluation

- **Technical evaluation**
  - *Sensitivity:* *analytical* or detection limit
    functional
  - *Reproducibility* within run
    between run
  - Imprecision profile
  - recovery
  - dilution tests

In Vitro Diagnostic Technical evaluation

- **Analytical Sensitivity = detection limit = minimum detectable dose (MDD)**

  - MDD is calculated from the mean + 2 SD of 20 replicates of the zero calibrator performed within run.

  - It is usually calculated based on the response (radioactivity RIA or absorbance EIA) and read off the calibration curve to obtain the MDD

Detection limit of the determination of serum cortisol level

Method	Detection limit (SI) units
Fluorometric assay	1-10 mg/dl (276-524 nmol/l)
Enzime-linked immunoassay	1 mg/dl (27,6 nmol/l)
Fluorescent polarization liquid chromatography	80-500 ng/dl (2,2-13,8 nmol/l)
High pressure liquid chromatography (HPLC)	50-110 ng/dl (1,38-3 nmol/l)
Radioimmunoassay	3-200 ng/dl (0,08-5,52 nmol/l)
Enzyme-linked immunosorbent assay **(ELISA)**	1 ng/dl (27,6 pmol/l)
Isotope diluition gas chromatography-mass spectrometry	10-30 pg/dl (0,276-0,828 pmol/l
gas chromatography tandem mass spectrometry(LC-MS/MS)	15 pg/dl (0,414 pmol/l)

Sensitivity of the serum TSH measurements

Method	Sensitivity
I. generation immunoassay (IA)	0,6 -1,2 mU/l
II. generation IA	0,05 - 0,15 mU/l
III. generation IA	0,005 - 0,015 mU/l
IV. generation IA	< 0,005 mU/l

In Vitro Diagnostic: Technical evaluation Parameters

- **Reproducibility within run = within run imprecision**

  - using quality controls (QC)
  - ideally n = 20 replicates (often 10 !)
  - calculation of the mean $\bar{x}$
  - calculation of the Standard Deviation (SD)
  - coefficient of variation CV % = 100 x SD / $\bar{x}$

In Vitro Diagnostic: Technical evaluation Parameters

- **Reproducibility between run = between run imprecision**
  - using quality controls (QC)
  - daily QC results over a period of time preferentially over 20 days
  - calculation of the mean, standard deviation, coefficient of variation

In Vitro Diagnostic: Technical evaluation Parameters

- **Imprecision**
  - Generally between run imprecision tends to have higher % CV than the within run imprecision since variables are introduced from series to series and day to day
  - **Requirement:** The performance standard should be about 5% CV in the useful concentration ranges

In Vitro Diagnostic: Technical evaluation Parameters

- **Sources of imprecision**

A: **Technical or method dependent**

- **Reagent:** antibody capture, antibody conjugate, calibrator diluent, wash solution, substrate set, quality control
- **Antigen antibody reaction:** timing, temperature, separation, washing
- **Enzyme substrate reaction:** timing, temperature
- **Detection:** radioactive counter, spectrophotometer
- **Data reduction:** curve fit
- **Pipeting:** calibration, setting and reproducibility

- **Interference:** non specific, specific, carryover, high dose hook

B: Patient dependent

In Vitro Diagnostic: Technical evaluation Parameters

- **Interferences**
  - Human anti-mouse antibodies (H.A.M.A.)
  - Auto-antibodies
  - rheumatoid factors
  - lipemia, icterus, hemolysis
  - cross-reactivity

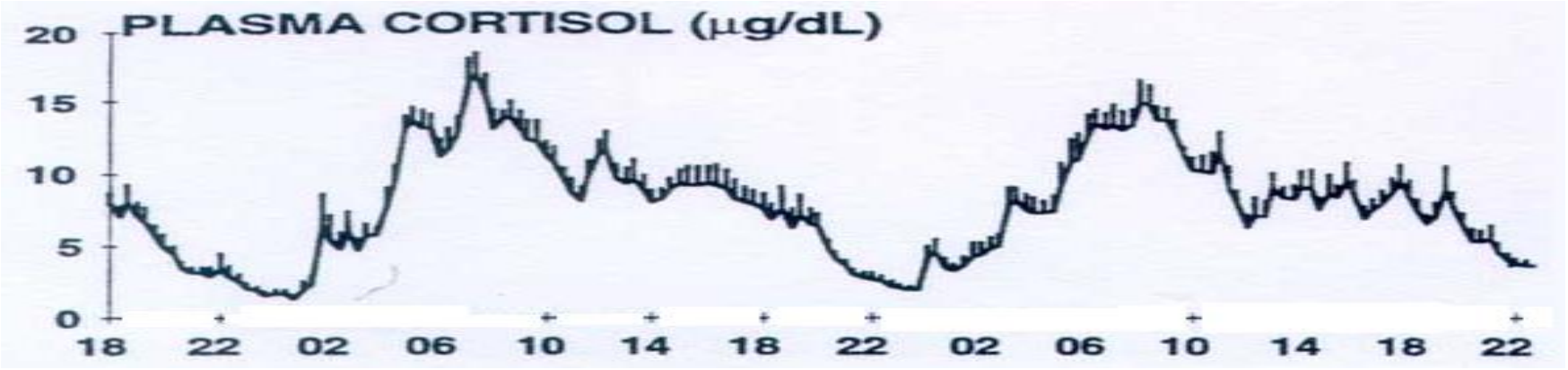
* NB: *be aware and cautious about discrepant results which may required further investigation*

Patient as a source of imprecision

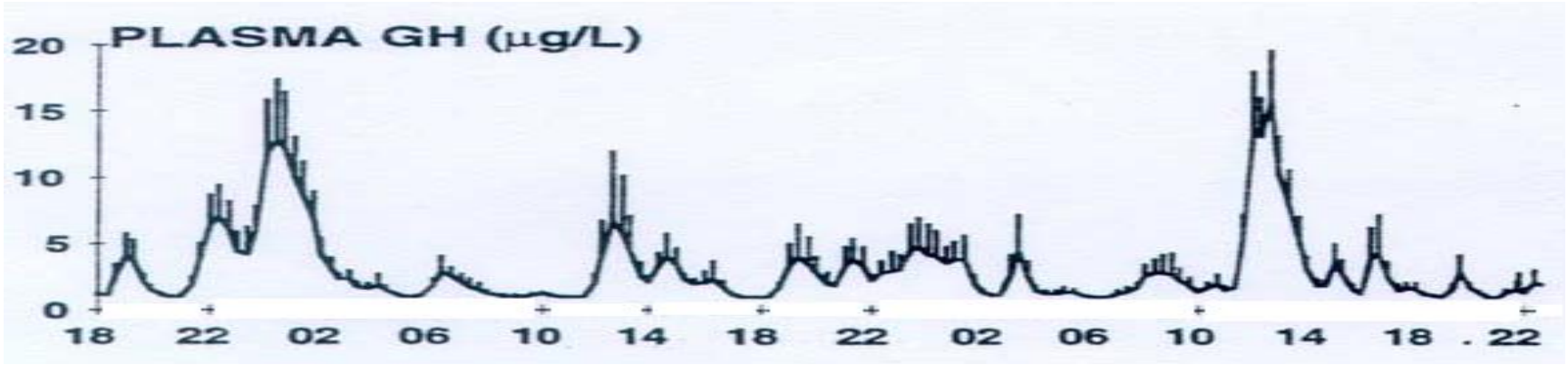
Physiological states which influence hormone assays

- age
- pregnancy
- menstruation cycle (LH, FSH, E2, P ….)
- nutrition
- daily rhythm of hormones (eg.cortisol, PRL…)
- stress (eg. renin activity, catecholamines)

Daily rythm of hormone secration



Maximum at 8.00 am, minimum level at midnight



GH maximum at midnight or during sleep.

Due to the pulsatile secration of GH one determination of the serum level of GH has a poor diagnostic value.

In Vitro Diagnostic: Technical evaluation Parameters

- **Autoantibodies**

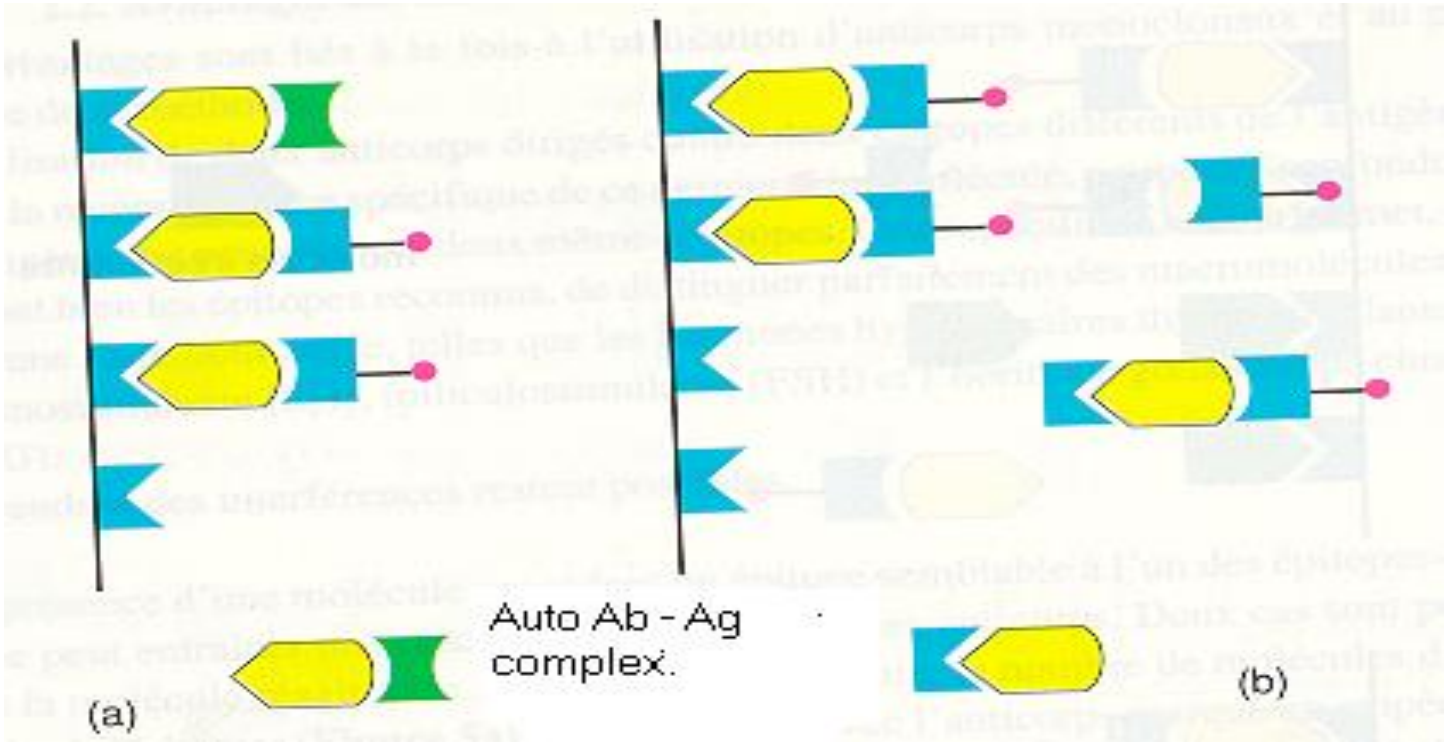
– In some cases, a patient can develop auto-antibodies directed against the antigen which is assayed ex: thyroglobulin autoantibodies

– These auto-antibodies can interfere on the antigen assay

– Detection and quantification of these auto-antibodies are done with different technique

In Vitro Diagnostic: Technical evaluation Parameters

- **Autoantibodies**



In Vitro Diagnostic: Technical evaluation Parameters

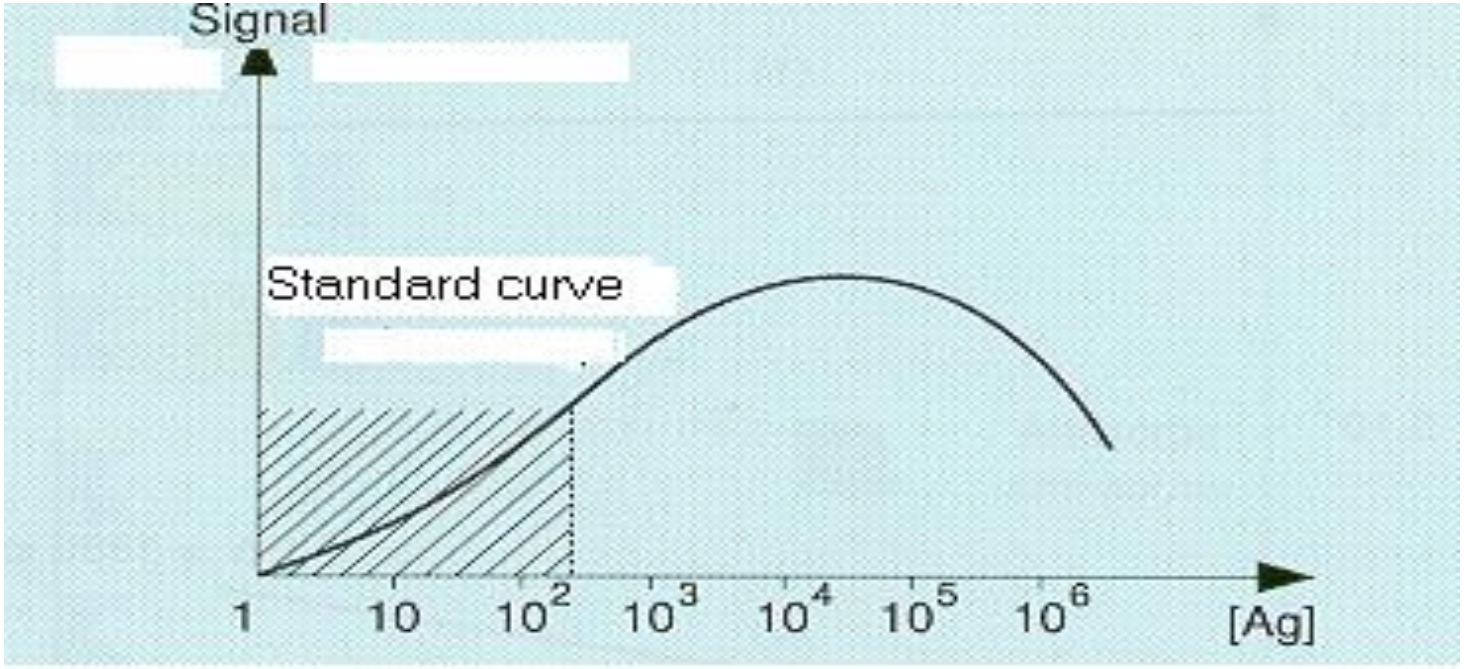
- **High dose hook effect**

– at the extreme high analyte concentrations, the antibody binding sites may be saturated with antigens making the antibodies unavailable to form a sandwich, that is, antibody-antigens complexes

– the end result is the severe underestimation of the analyte

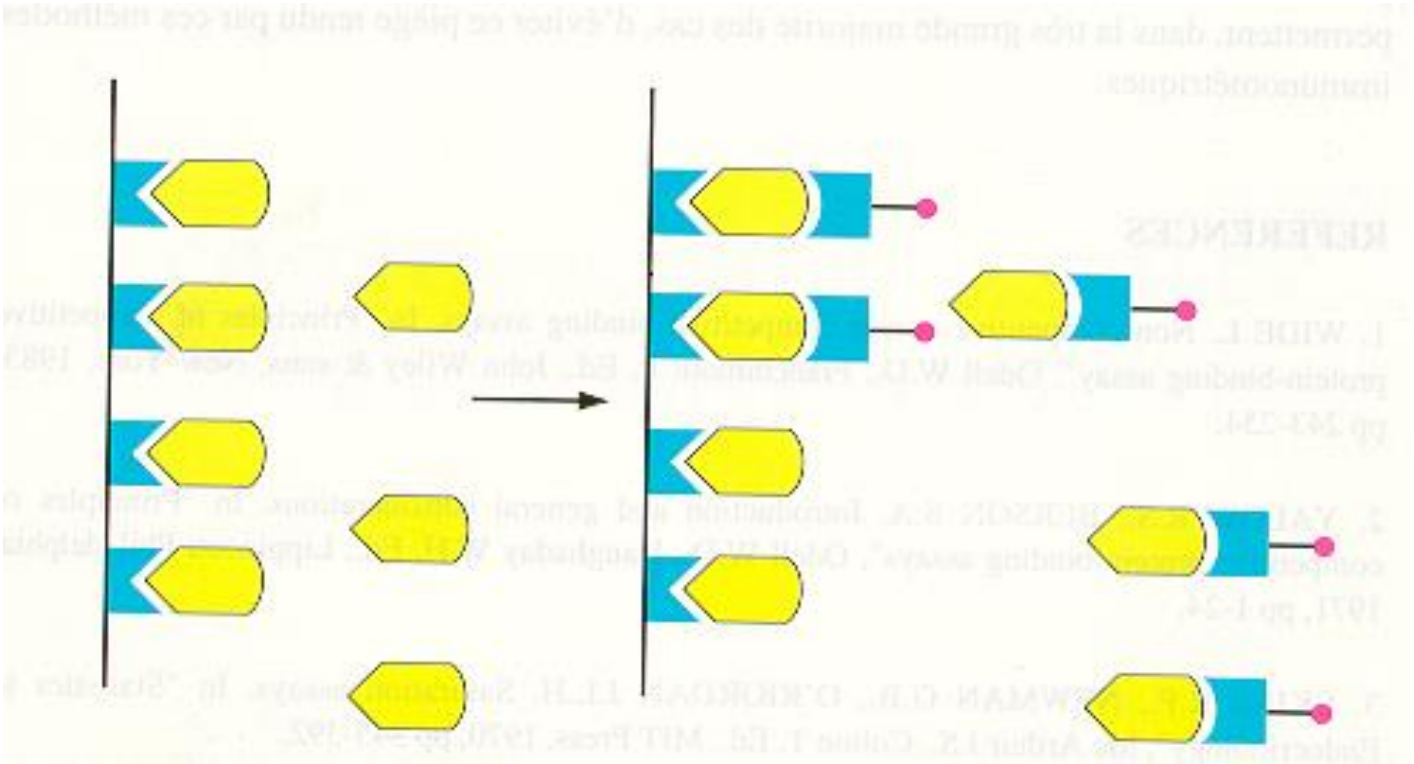
In Vitro Diagnostic: Technical evaluation Parameters

- **High dose hook effect**
  - This is a particular problem with analytes that could be present in wide concentration



In Vitro Diagnostic: Technical evaluation Parameters

- **High dose hook effect**



In Vitro Diagnostic: Technical evaluation Parameters

- **Samples :** serum, plasma

– **Serum** is obtained without anticoagulant

– **Plasma** is obtained with an anticoagulant : EDTA, Heparin, Citrate…

– *In some cases samples must imperatively be serum ☑ thoroughly follow the manufacturer's recommendations including about lipemia, hemolysis...*

In Vitro Diagnostic: Technical evaluation Parameters

- **Cross-reactivity**

– An antibody recognizes an epitope. If this epitope is common to other molecules, you will obtain a cross-reactivity

– The monoclonal antibody use has largely decreased this problem

In Vitro Diagnostic: Diagnostic evaluation

- **Clinical evaluation**
  - Reference value
  - Predictive value of a diagnostic test
  - Distribution of patient values
  - Disease management

In Vitro Diagnostic: Clinical evaluation

- **Reference values**

– Reference values should be established for a new technique using the population from the area concerned

– It requires a large healthy population n=120 or more

– Traditionally, reference values are defined as the central 95% interval of the healthy population [2.5% to 97.5%]

In Vitro Diagnostic: Clinical evaluation

- **Reference values (statistics)**
  - **Population**
    - **Gaussian** (normal) repartition: mean ± 2 SD
    - **Non-Gaussian** repartition: percentile method. The values at the 2.5th and the 97.5th or 95th percentiles become the reference value.

      Ex: n=120

      2.5 th percentile is 0.025 (120 + 1)=3

      and 97.5 th percentile is 0.975 (120 + 1)=118

      The reference values are the values at the rank 3 & 118

In Vitro Diagnostic Clinical evaluation

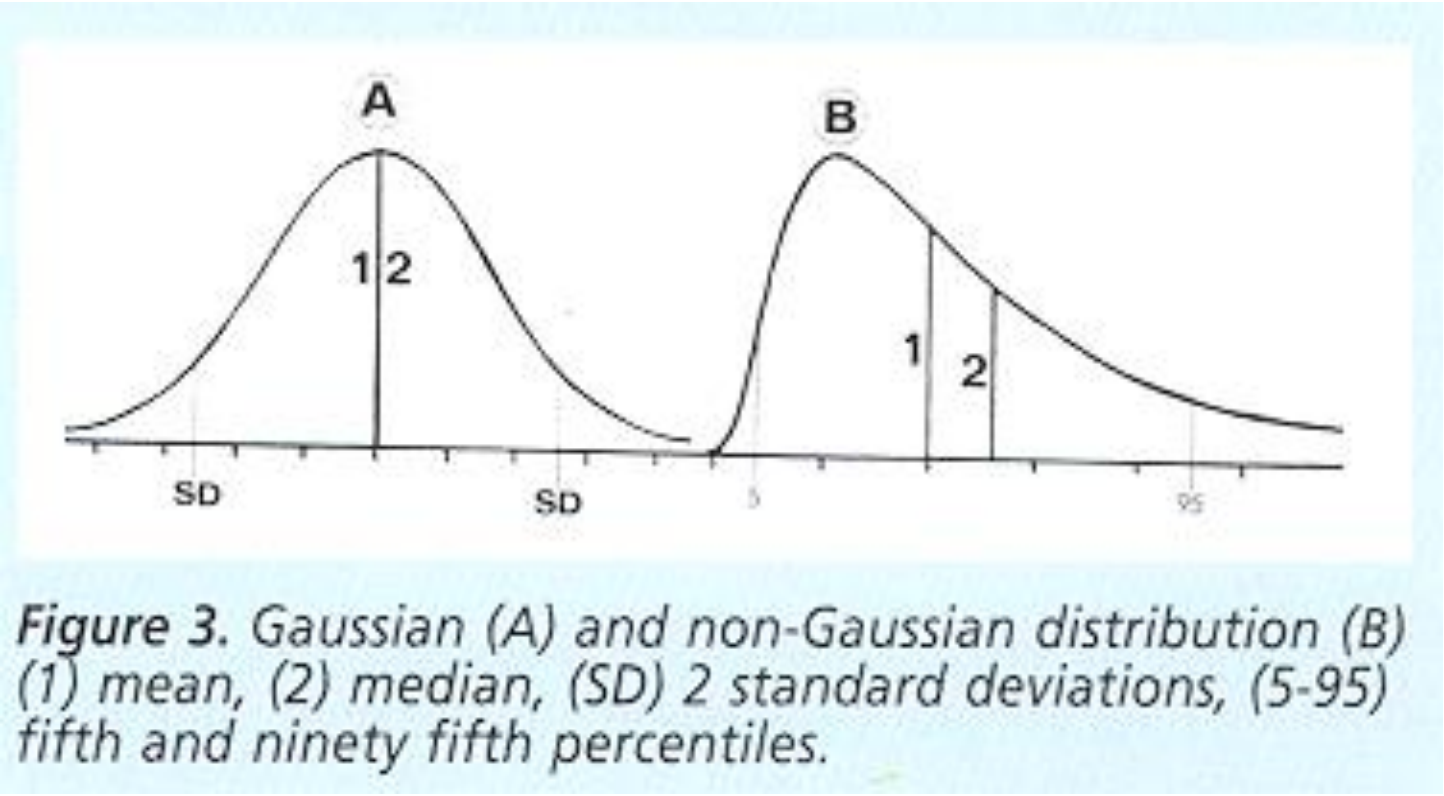


Figure 3. Gaussian (A) and non-Gaussian distribution (B) (1) mean, (2) median, (SD) 2 standard deviations, (5-95) fifth and ninety fifth percentiles.

In Vitro Diagnostic: Clinical evaluation

	Patients with disease	Patients without disease	Total
Patients with positive test result	TP	FP	TP + FP
Patients with negative test result	FN	TN	FN + TN
Total	TP + FN	FP + TN	TP + FP + TN + FN

→ PPV

→ NPV

Efficiency:

$$\frac{TP+TN}{Total}$$

Sensitivity

Specificity

TP: true positive, FP: fals positive, TN: true negative, FN: fals negative

Present and future in diagnosis of endocrine disorders

Demand for new methods

- Need for **increased turn-around time** (need for faster, precise analysis, determination of multiple analytes from the same sample, decreasing the sample volume)

- Developing **new molecular biological methods** resulted in substantial increase in genetic data

- Demand for molecular biological methods in routine genetic testing of genes involved in endocrine disorders

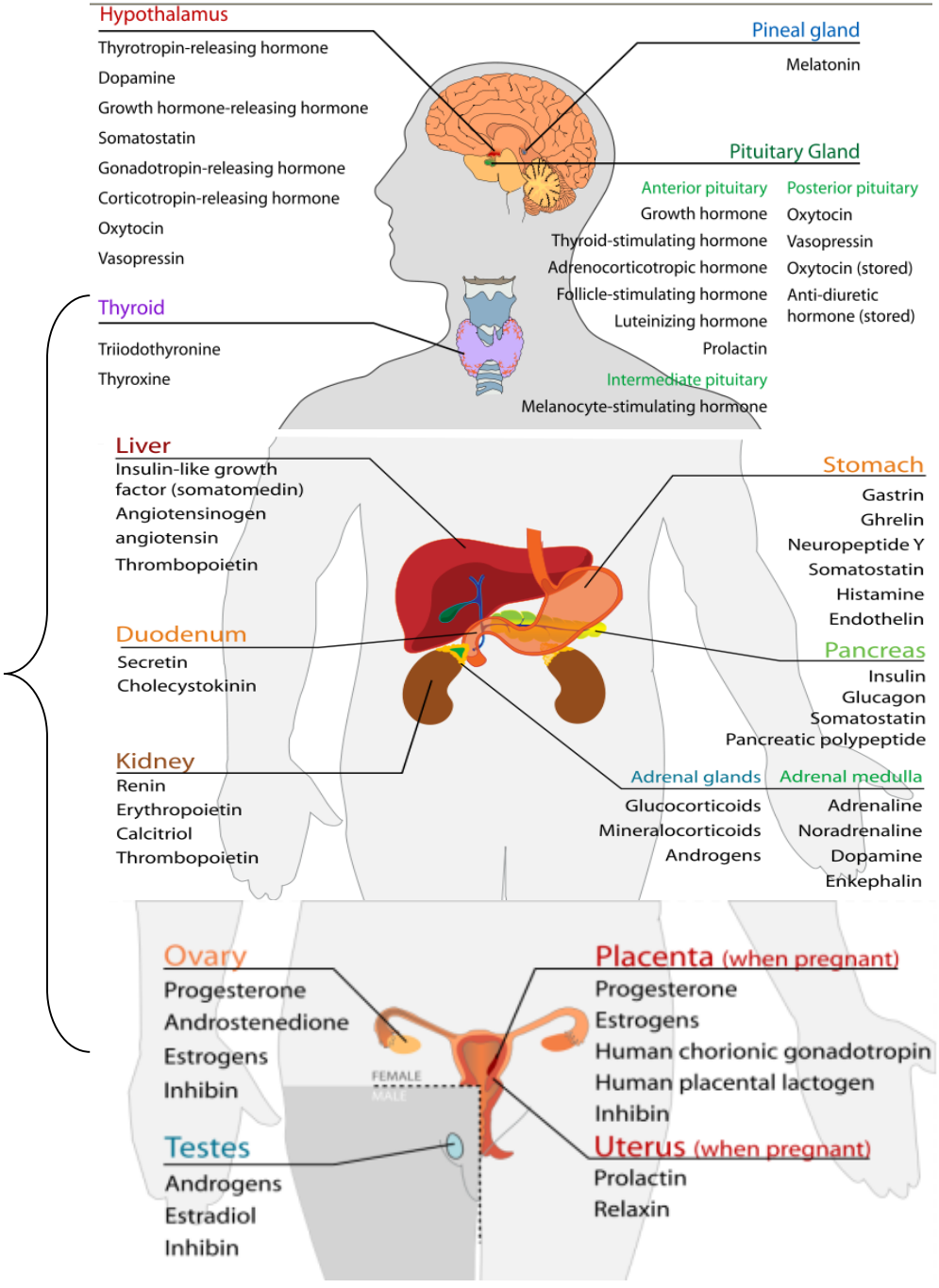
New methods:

-Routine hormone analysis: GC, HPLC, mass spectrometry, microarray

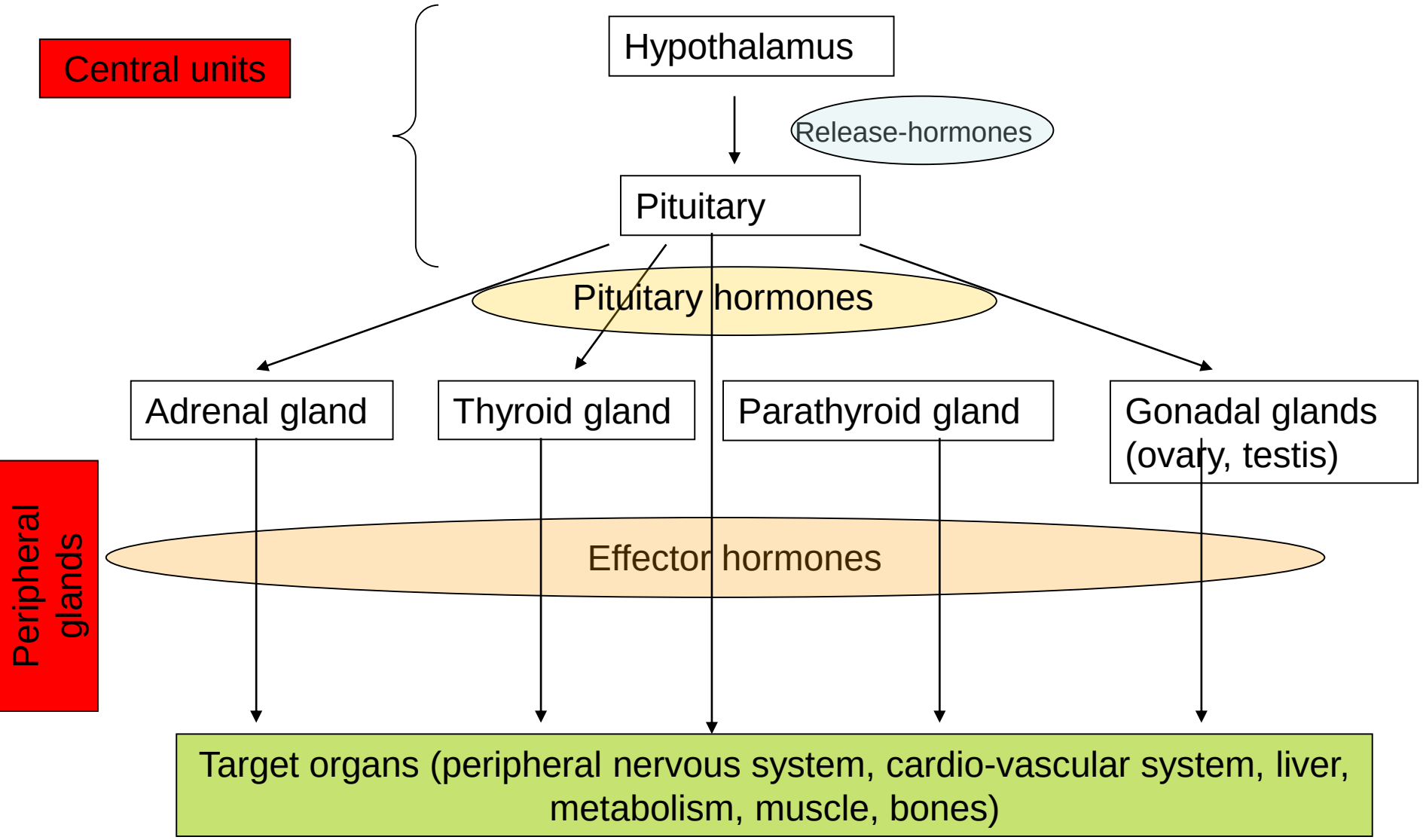
-**Molecular biological methods**: PCR, DNA sequencing, gene expression array, protein detection (western blot, immunohistochemistry, FISH)

Central, endocrine organs and secreted hormones

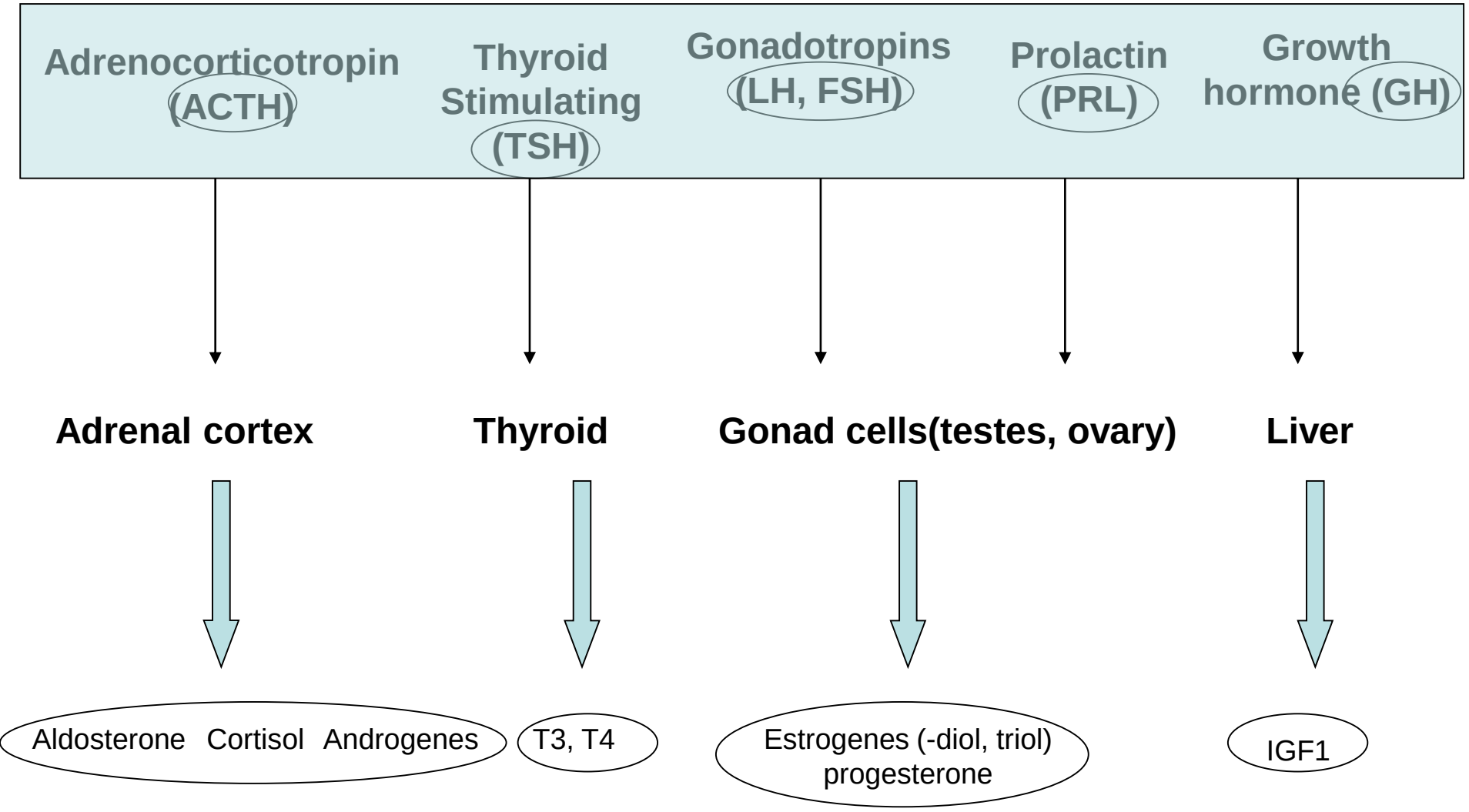
Peripherial endocrine organs and secreted hormones



Organization of the endocrine system



Regulation of endocrine system is made through hormone axis



Laboratory evaluation of pituitary function

Syndromes with Hormone overproduction (hormone-secreting tumors)

- GH-producing pituitary tumors
  Acromegaly, gigantism (GH ↑, IGF-I ↑)
- Prolactin-producing pituitary tumors (prolactinoma)
  syndrome called galactorrhea-amenorrhea (PRL ↑, LH, FSH ↓)
- ACTH-producing pituitary tumors
  Cushing disease (ACTH ↑, cortisol ↑)
- TSH-producing pituitary tumors
- hyperthyrodism (TSH ↑, fT4 ↑, fT3 ↑)

Hormone deficiency syndromes

- Panhypopituitarism (all are missing)

- Isolated hormone deficiency

Case 1.

48 yr old woman

Hands torpidness

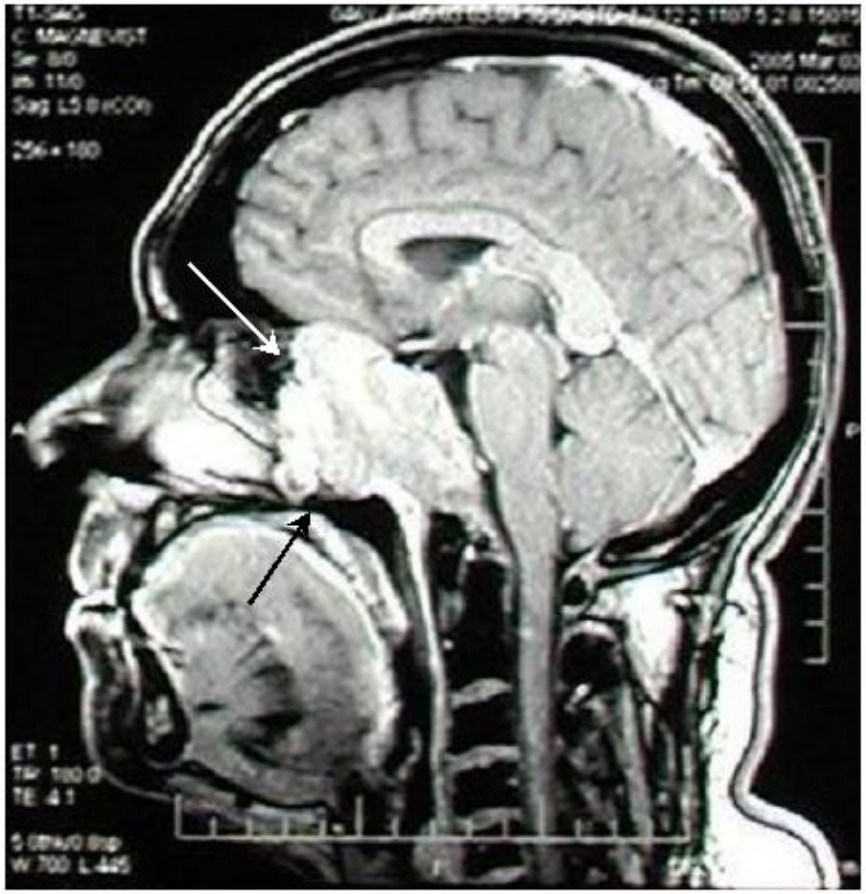
- **Neurology:** carpal tunnel syndrome
- **Surgery:** before surgery routine laboratory evaluation
- **Endocrinology:** thyroid struma with normal function

Three month later rhinorrhea

- **Dentistry:** allergy, rhinitis, sinusitis, polypus sinus maxill.

Case

- History
  - Size of shoe increased from size 39 to size 42
  - Can not wear rings
  - Sweating
  - No menstruation since 1 year
- Hormone levels
  - **Serum GH: 36 ng/ml** (norm<5), no suppression after OGT
  - **Serum IGF-1: 885 ng/ml** (norm: < 280)
  - **Serum prolactin: 4866 ng/ml** (norm: < 15)
  - Normal, thyroid and adrenal function



Clinical signs of pituitary tumors

- **Due to compression**
  - headache
  - chiasma opticum lesion
  - ophthalmoplegia
  - liquorrhoea
  - hypopituitarism (with or without diabetes insipidus)

Acromegaly

Cause: overproduction of growth *hormone* (GH)
(before ossification –gigantism)

Incidence: 3-4/1 million/year

Prevalence: 55-69/1 million

Man/woman: no difference

Age: any time, but is common in 40-50 years
(rapidly increasing tumors are typically in young adults)

Time between diagnosis and first signs : 7-12 years

Etiology:

98% GH-producing pituitary tumors

2% GHRH-producing tumors (hamartoma of hypothalamus, pancreas and bronchoid carcinoma, medullary thyroid cc.)

Acromegaly


aged 14
aged 16
aged 18
aged 19
aged 20
aged 21
aged 23
aged 24
aged 27

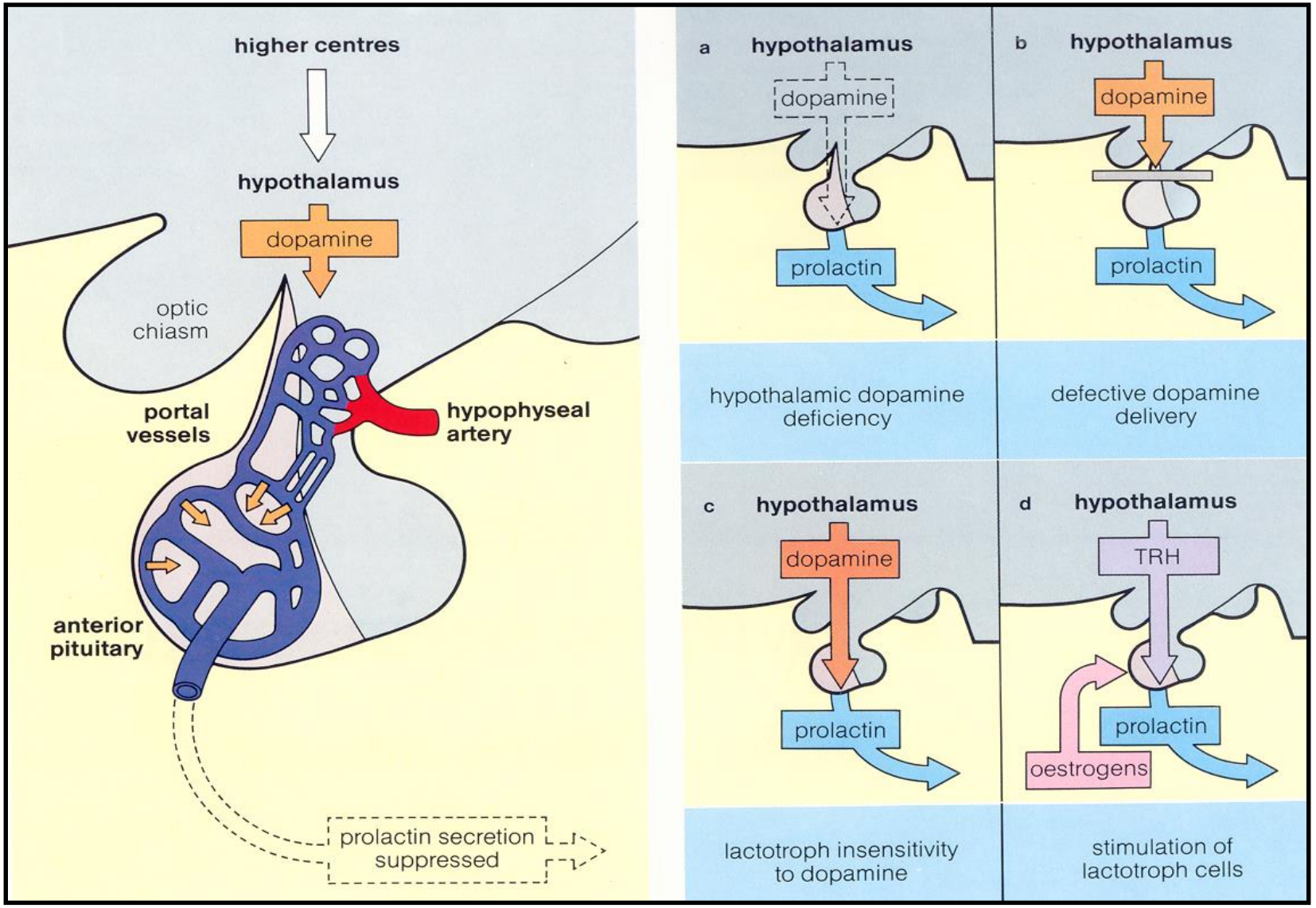
Acromegaly

- Why the diagnosis is important?
  - The expected lifetime is reduced by 10 years
  - Mortality is increased compared to the general population
  - After successful therapy no difference in mortality compared to the general population
- <u>Diagnostics</u>
  - random **GH** useless (the secretion is pulzatily)
  - **Dynamic test:** oral glucose tolerance test (75 g glucoes p os and measure blood glucose and GH)
  - **IGF-I** (for screening, and for therapy monitoring)
  - GH profile (7-9x serum GH)
  - Pituitary MRI

Case 2.

- Woman , 28 years old, 15 month after labour
  - Complains: **galactorrhea , no menstrual cycle**
  - Weariness, weigth gain (10 kg)
  - Vision problems
- Examinations (gynecology)
  - No alteration
  - **plasma prolactin: 108 ng/ml** (reference: <15);
  - plasma LH, FSH and estradiol: low
  - ophthalmology: V: 5/5, 5/5, Fundus: decolorált papillák,
  - T: 16, 14 **Visual field defect on the left temporal, and on the right lower and upper temporal side**
  - sella MRI

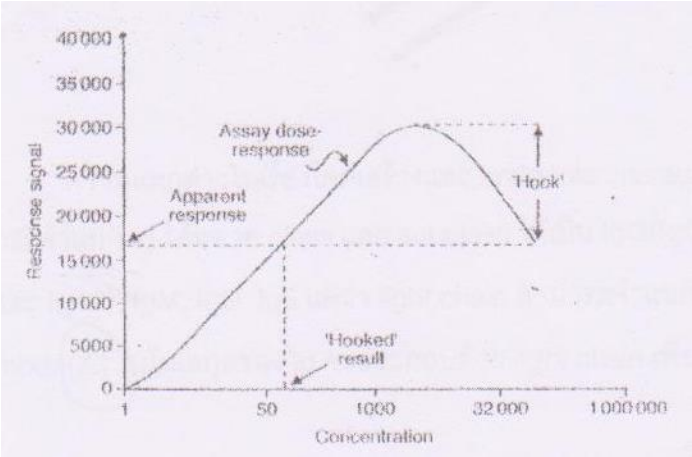
Hyperprolactinaemia

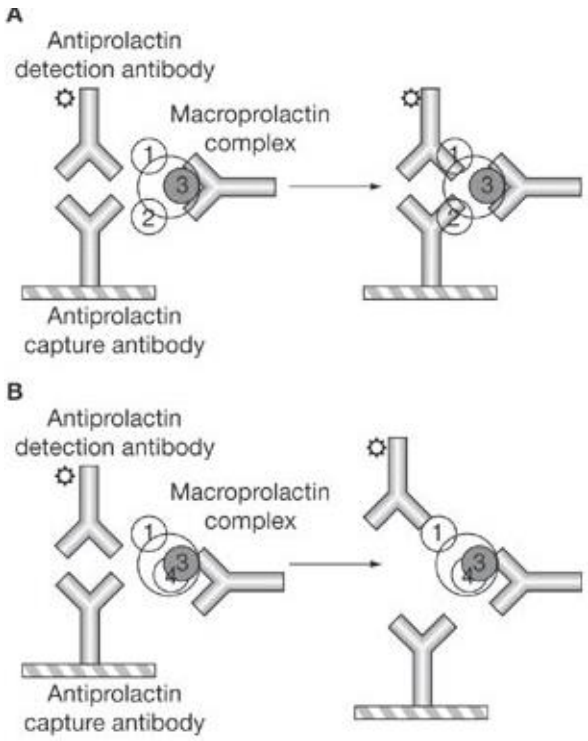


Causes of hyperprolactinemia

- **Hormon-producing pituitary tumors**
  - Prolactinoma
  - Acromegaly
- **Diseases of hypothalamus and pituitary stalk lesions**
  - Tumors and cysts (craniopharyngeoma,cyst of Rathke-pouch, meningeoma, stb.)
  - Empty sella
  - Radiotherapy
  - Trauma
  - surgery
- **Ectopic prolactin-producing tumors (**bronchus carcinoma)
- **Other endocrine and systemic diseases or drugs**
  - Primary hypothyreosis
  - Polycystic ovary syndrome
  - Renal insufficiency
  - drugs

Problems with prolactin measurement





- Hook-effect
  - ”sandwich” method
  - The sample should be diluted
- Macroprolactin
  - ”big-big” prolactin
  - The sample should be precipitated

Clinical Manifestations of Hyperprolactinaemia

women

galactorrhoea
30 – 80%

menstrual
irregularity

infertility

men

galactorrhoea
< 30%

visual field
abnormalities

headache

impotence

E.O.M. paralysis

anterior pituitary
malfunction

Cushing disease

- **Cause**:
  - ACTH-producing pituitary adenoma (often microadenoma)
  - Adrenal tumor producing cortisol
  - Drug induced (iatrogenic Cushing disease)

- **Prevalence**:
  - 2,4/million/year
  - More frequent in women (3-8x than in man
  - Any age but more prevalent between 25-45 years

Clinical signs

Slow progression

- weight gain, especially in the face, supraclavicular region, upper back, and torso.
- Skin: purple stretch marks, easy bruising, skin thinning.
- progressive proximal muscle weakness, patients may have difficulty climbing stairs,
- Menstrual irregularities, amenorrhea, infertility, and decreased libido
- Psychological problems depression, cognitive dysfunction, and emotional lability
- hypertension and diabetes mellitus,
- difficulty with wound healing, increased infections,
- osteopenia, and osteoporotic fractures

Laboratory diagnosis of Cushing disease

Hormone measurements

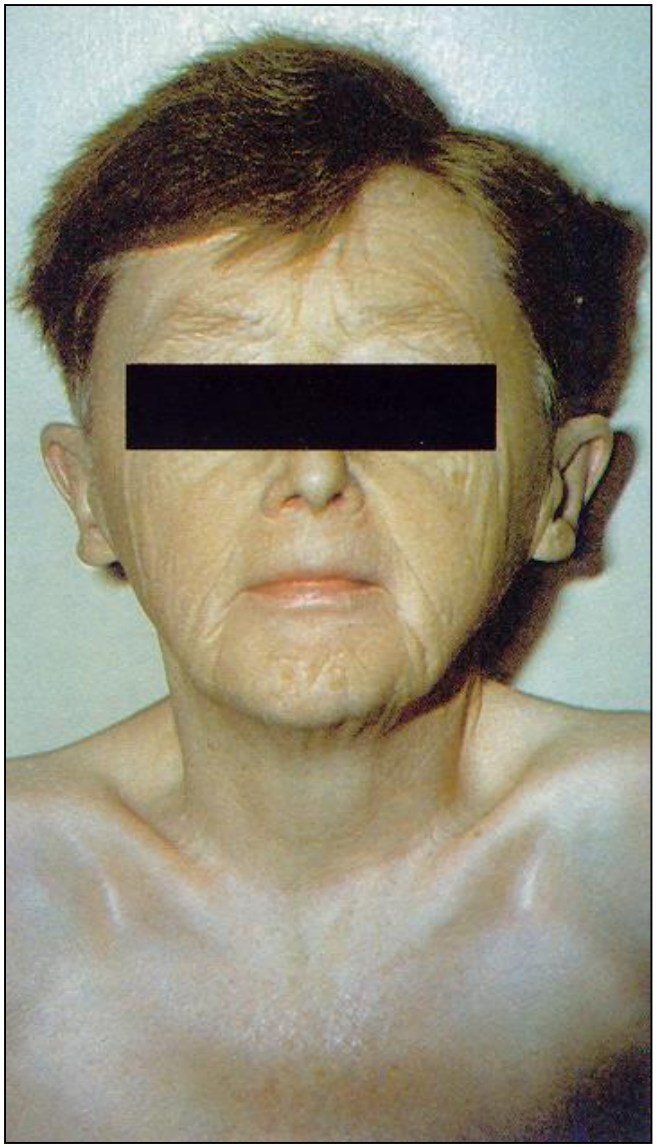
1. Demonstration of **Hypercortisolism (screening tests)**
   - cortisol in urine (collected 24 h)
   - in blood after low dose dexamethsone suppression
   - daily rhythm
   - cortisol in saliva

2. To separated the origin of **Cushing syndrome**

   (the three most common causes: ACTH-producing pituitary tumor, cortisol-producing adrenal tumor and ectopic ACTH-production

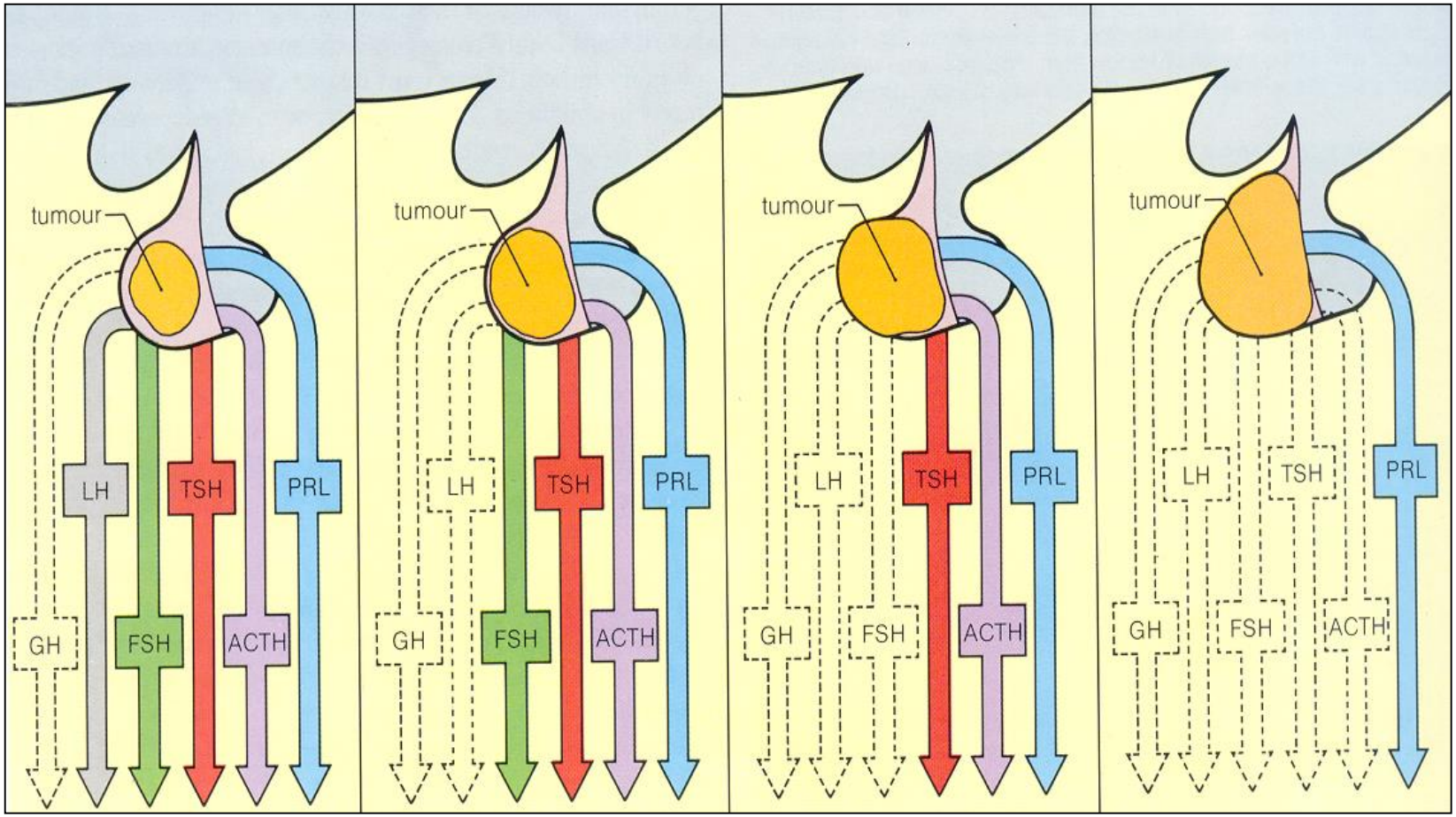
   - plazma ACTH
   - high dose dexamethason test
   - CRH test (vasopressin, desmopressin, metopyron teszt)
   - blood sampling from sinus petrosus inferior
   - Imaging (sella MRI, adrenal CT,

Panhypopituitarism



- **Etiology:** heterogeneous
  - Pituitary tumor, inflammation, infiltration, trauma, irradiation, surgery
- **Clinical signs**
  - Secondary adrenocortical insufficiency, hypothyroidism, hypogonadism, GH-deficiency, diabetes insipidus
- **Diagnosis**
  - Evaluation of pituitary and peripheral hormones
- **Treatment**
  - Hormone substitution (thyroid, adrenal and gonadal hormones)
  - Dezmopressin
    - Hyponatraemia occurs when overdosed

Hormone deficiencies due to pituitary tumors



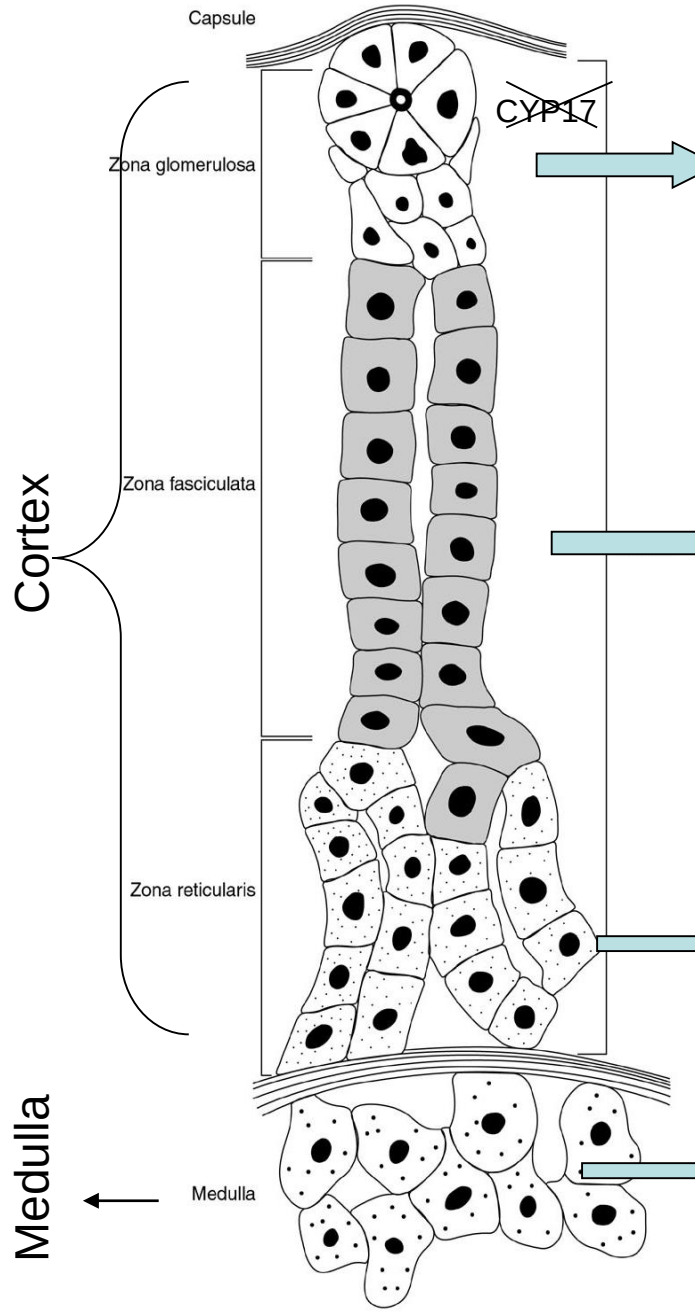
GH-deficiency

Hypogonadism

Adrenocortical insufficiency, Addison disease

Adrenal disorders

Organisation of adrenal cortex and origin of adrenal steroids



Main hormones

Enzymes

Aldosterone, NO cortisol

No CYP17, but CYP11B2 only here

Cortisol

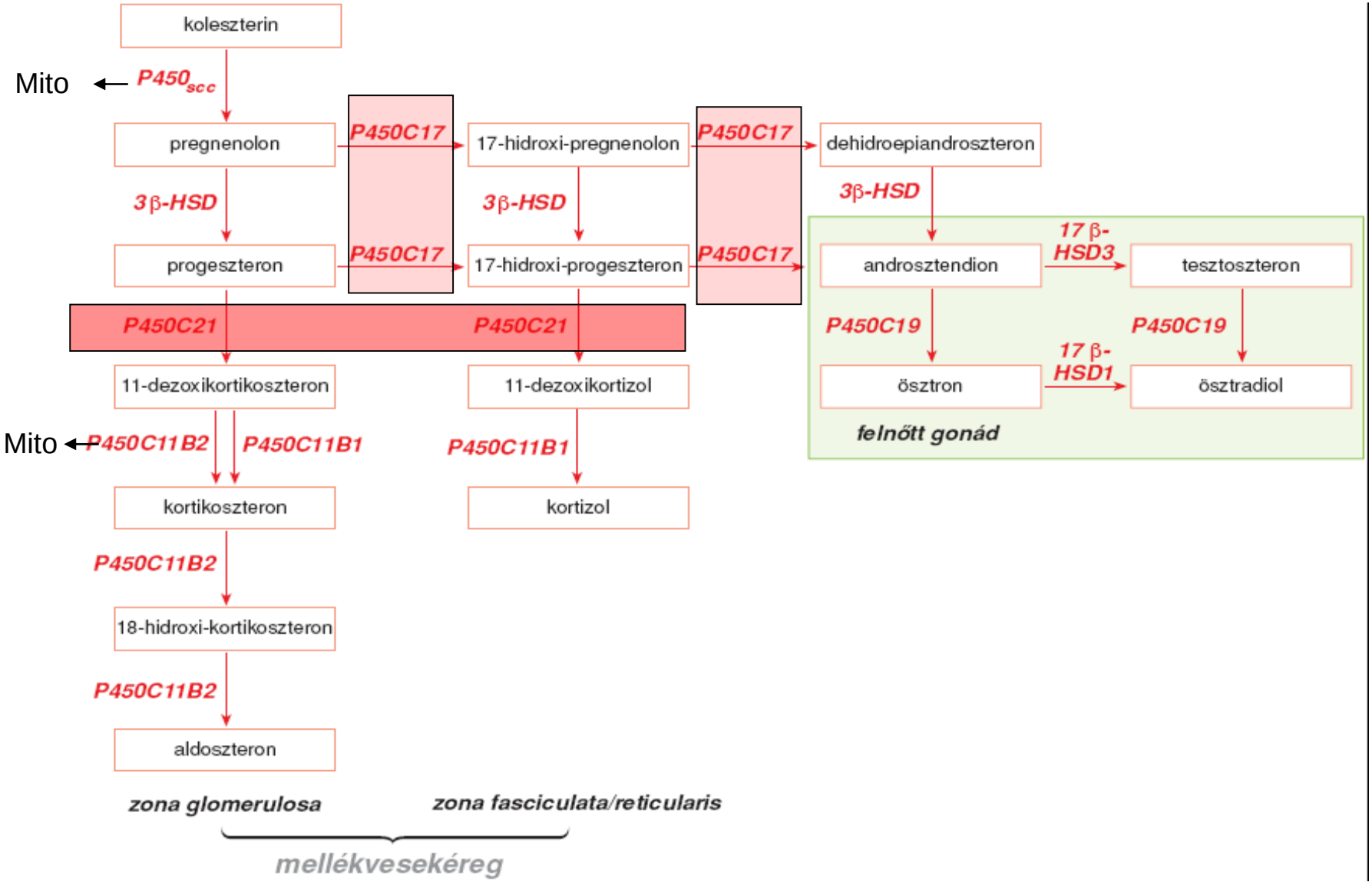
No CYP11B2, but CYP11B1 yes

Androgens

DHEA-DHEAS only here

Catecholamines, CRH

Steroid biosynthesis



Adrenocortical diseases

- **Overactivation:**
  - tumors
    - hormone producing-functioning tumors
    - non-functioining tumors
- **insufficiency**
  - impaired function
  - *congenital deficiency*

    Important

    lack of end point hormones and increased level of precursors (the highest concentration of the hormone before the block occurs)

Clinical signs of primary adrenocortical insufficiency

• Weakness, fatigue	100%
• Weight lost	100%
• Gastrointestinal signs	95%
• Vomiting	85%
• Nausea	80%
• Abdominal pain	30%
• Obstipation	30%
• Diarrhea	25%
• **Hyperpigmentation**	95%
• Hypotonic or orthostaticus hypotonic	90%
• Hypoglycemia	50%
• Anemia	40%
• Vitiligo	15%
• Muscle pain	15%

Hyperpigmentation in Addison disease