41. Cerebellum (immunohistochemical reaction to GFAP, rat)

*(The knowledge of *-marked details is not necessary for the Ist year students.)*

The specimen represent three histotechnical 'novelty': it was sectioned without embeddeding, by a vibration microtome, then an immunohistochemical reaction was performed, but still before mounting, in the free-floating sections. The immunohistochemical reaction detected the GFAP ('*glial fibrillary acidic protein*' – the characteristic intermediate filament protein of astroglia). Polyclonal antibodies were applied, labeled with peroxidase enzyme, and the product of immunoreaction was visualized by diaminobenzidine reaction. The reaction stained the main astroglial processes rich in intermediate filaments in dark brown. The background is to be whitish, but even it is frequently yellow or faint brown in the grey matter rich in peroxidases (e.g. cytochrome oxydase) which also catalyze the oydation of diaminobenzidine. White matter is devoid of this effect and the background is really white.

The system of folia* is conspicuous. Already low power objective but even more the medium one help to distinguish grey and white matters. In this latter one two characteristic patterns are formed by the dark brown glial processes. Below the pial surface, they are perpendicular to the surface, and almost parallel to each other. It is the **Bergmann-glia*** (modified astrocytes), characteristic of the molecular layer*. In the granular layer true astrocytes form a dense, irregular system. Under high power objective, on the meningeal surface maybe the end-feet of the Bergmann-glia are visible, which contiguing each other (to visualize this properly, electron microscope is requested) form the *glia limitans**. High power objective also help to recognize individual true astrocytes with their stellate-like or spider-like process system, from which their name was taken. The process system is easier to study in the white matter where the cells are less densely packed. Astrocytic perikarya are usually devoid of immunoreactivity. But if it does, it becomes obvious that the processes are not distributed evenly around the body but emerge from one point. The higher the quality of the immunoreaction, the more numerous and finer branches are visible, which can delineate the positions of the large neurons (here: that of Purkinje-cells).

Immunohistochemical reaction against GFAP is not capable of labeling every astrocytes, but only those which are relatively rich in it, first of all the **fibrous astrocytes**, the perivascular glia, and the submeningeal astrocytes. The too intense fixation can destroy epitops, the molecular structures responsible for the immunoreactions. Beside the GFAP, several other astroglial markers are in use: glutamine synthetase, S100β-calcium-binding protein, glutaminic acid transporter-protein (GLAST), and the Cx43, the major protein of connexons, which interconnects the astrocytes into a functional syncytium.

The finest glial processes fill all the spaces between the neurons. These processes are visible, however, only under electron microscope. That eosinophilic 'ground substance' in the hematoxylin-eosin stained sections, into which the neural perikarya are apparently embedded consists of plexus of neural and glial processes. The name of this substance in the electron microscopic terminology is 'neuropil'.

The immunohistochemical reaction can also be performed in paraffin-embedded or frozen materials (after proper sectioning), and it is also adapted to electron microscopy. Two, or even three or four substances may be detected, if the different antibodies are labeled with different dyes (double, etc. labeling). The result of the immunochemical reaction can be counterstained, e.g. according to Nissl, or it can be combined with classical histochemical reactions (.e.g. enzyme-histochemical reactions), as well as the in situ hybridization, which detect the localization of mRNAs specific for different biochemical substances. Nowaday the immunohistochemistry is a fundamental procedure of the basic and applied researches aswell as that of pathohistologic diagnistics. In the case of the GFAP, e.g. if a brain tumor is rich in it, probably its cells are mature and less malignant.

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m, g: molecular and granular layers, arrow, arrowhead: asztrocytes, broken arrow: position of Purkinje-cell.