A STUDY OF THE NEUROFIBRILS[1] IN THE GANGLION CELLS OF THE CEREBRAL CORTEX.

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Plate I.

As has often been pointed out, the use of the terms chromatic and achromatic to distinguish certain characteristics of the ganglion cell is misleading. The words are only applicable to describe the stained and unstained tracts in nerve cells which have been stained by the Nissl methylene-blue method. In order to avoid confusion in describing the morphological characteristics of the ganglion cells, it has seemed to the writer advisable to use terms which as far as possible admit of general application and do not have reference simply to the results given by one particular stain. For this reason Marinesco's classification of the constituents of the ganglion cell into (1) chromatic, (2) achromatic and (3) amorphous elements, is open to criticism. In the present state of our knowledge of the morphology of the ganglion cell it is not possible to give more than a tentative classification of the cellular constituents. The various elements of the nerve cell may be roughly grouped as follows: (1) cytoplasm, (2) Nissl bodies, (3) fibrils, (4) constituents whose morphological characteristics are not yet definitely known.

The achromatic tracts are made up of at least two constituents, the cytoplasm and a definite specific fibrillary substance which possesses morphological characteristics by which it can be easily differentiated from the surrounding protoplasm in which it is held. There is also another sense in which the use of the term achromatic is not exact. Under certain conditions the achromatic tracts exhibit marked chromophilic tendencies. This is often due to the fact that the fibrils

[1] In this paper the term neurofibril is used in a purely morphological sense to describe the fibrils which lie wholly or only partly within the ganglion cells of the cerebral cortex.

are capable of being stained by reagents for which under normal conditions they have little or no affinity. In view of the fact that the fibrils are now known to be an important and definite constituent of the ganglion cell and on account of the present confusion of terms in cytological nomenclature it has seemed best to make the foregoing suggestions in reference to the selection of terms.

Although it is often possible to stain the fibrils by various methods there are none that are satisfactory except the hæmatin or the gold stains of Apáthy and the as yet unpublished methods of Bethe. As Apáthy's methods are somewhat capricious when an attempt is made to stain the fibrils in the ganglion cells in the higher vertebrates, I have decided to publish a modified hæmatin method which often, but not always, gives excellent results. I hope others may be induced to try the method and be able to propose further modification in the technique which will make the method capable of more general application.

The sections represented in Plate I, Figs. 1 and 2, were both taken from the cerebral cortex of a fully developed pig. The tissue immediately after death was placed for 24 hours in a saturated solution of bichloride of mercury, to which 5% of glacial acetic acid had been previously added. Other methods of fixation can also be used. After the fixation the tissue is transferred to 95% alcohol. The alcohol must be changed twice during the first 24 hours and then every other day for a week. After this the fluid need not be changed oftener than once a week. In two weeks the sublimate is thoroughly removed from the tissues. I believe that better and more reliable results are obtained by not using the iodine solutions to remove the sublimate. This chemical has a very deleterious effect on the tissues. After being hardened in alcohol the tissue is ready for embedding. Either celloidin or paraffin may be used, preferably the latter, care being taken to use chloroform instead of xylol. The sections are fixed to the slide by the use of distilled water, and, after drying and the removal of the paraffin by chloroform, are put into 95% alcohol. The slide is then put for 1-2 hours into tinctura ferri Rademacheri as recommended by Weigert in his "mitosis stain." The sections are quickly rinsed in distilled water and are stained for 24 hours in the Apáthy[2] hæmatin solution. The

[2] *Mittheil. aus der zoolog. Station zu Neapel*,'1897, xii, Hft. 4.

whole section is then found to be over-stained. The preparations are then bleached under the microscope in an aniline oil and alcohol mixture (one part of the former to nine parts of 70% alcohol). The slides are finally well washed in tap-water, rinsed in distilled water, and after dehydrating and clearing are mounted in the usual way in Canada balsam dissolved in chloroform. The specimens when once fixed can be kept indefinitely.

As said already, the method is not yet entirely satisfactory. In the human nervous system the results are only very rarely good; but I believe this is due to the fact that it is so difficult to obtain perfectly fresh material. The ganglion cells in the human cerebral cortex when stained by this method, as a rule, give pictures exactly similar to those obtained when sections have been made from the brains of animals which have not been fixed immediately after death. In both cases the fibrils are only very imperfectly stained. This fact has an important bearing if there are those who still contend that the fibrils are artefacts, or are only the results of post-mortem changes. Arnold [3] has said recently that he was unable to find any long fibrils in the ganglion cell, but only short ones together with small granular masses. His observations were made on the ganglion cells in the human nervous system. The continuity of the fibrils after death is soon broken and there follows a period of granular degeneration. As a rule, I believe, the fibrils are very susceptible to post-mortem changes, and these changes are first seen in the body of the cell. Apparently the fibrils in the processes, particularly in the apical process of the pyramidal cells, remain for a considerable period after death unchanged. This may account for v. Lenhossék's statement in regard to the fibrillary substance. He admits the presence of the fibrils in the apical processes, but does not believe that the fibrils exist in the cell body. It can be very easily shown that the post-mortem degeneration of the fibrils begins in the cell body, and in specimens which are imperfectly fixed the cell body often has a granular appearance in strong contrast to the apical processes where the fibrils can be plainly seen.

In both sections from which the drawings for Plate I were made, it is possible to follow fibrils which run through nearly the whole

[3] *Archiv f. mikr. Anat. u. Entwick.*, 1898, lii, p. 535.

length of the cell. Similar fibrils have been described by Bethe. In sections thicker than those represented in the plate, fibrils can often be seen running through the whole length of the cell. As already stated, Arnold does not believe that any long fibrils are found in the ganglion cell, but only "short fibrils and granular masses." I am convinced that the failure of Arnold to find longer fibrils in the ganglion cell is due to the technique which he has employed. The use of strong iodine solutions makes the staining of the fibrils difficult and often impossible.

In comparing Figs. 1 and 2 certain important differences are recognizable. In the section represented in Fig. 1 the fibrils run straight through the cell processes without being connected with each other, but in the cell body there are connections between the individual fibres, so that a very wide-meshed network is formed. This agrees with the description given by Apathy, and does not support Bethe's view. The latter failed to find any network formed within the cell body. In the apical process of the ganglion cell, shown in Fig. 2, it is easy to distinguish the fibrils, many of which can be followed through the whole length of the process and well into the cell body. In the preparation from which the drawing was made a bundle of fibrils can be followed from a point at about the outer fourth of the apical process through the remainder of the process into the cell body almost to its base, near where the axone is given off. Another bundle of fibrils can be followed which enter the ganglion cell from the ground substance on one side at about the level of the nucleus. This bundle passes inward and downward below the nucleus, and leaves the cell by one of the basal processes on the opposite side of the cell.

In the section from which the drawing for Fig. 2 was made it can easily be seen that the diffuse network of fibrils which is visible is not within, but is superimposed on, the cell and its processes. The fibrils here lie in a different and more superficial plane than that of the cell and its processes. I am convinced from my own observations that it is this extracellular network of fibrils which Held[4] has described as a pericellular network of terminal axones. The same is

[4] *Arch. f. Anat. u. Entwcklngsgesch.*, 1897, Suppl.-Bd., p. 273.

true also of the reticulum surrounding the nerve cell described by Golgi.[5] It is altogether impossible by means of the silver stains to differentiate between the fibrils and the processes of the cells, so that these methods are no longer of any service in studying the structure of the ganglion cells. By this modified hæmatin method it is possible to demonstrate the passage of the fibrils from the cell body, as well as from the processes, into the intercellular substance. The discussion of the relation of the neurofibrils to the intranuclear network as well as to the grey substance will not be considered in this paper.

After a careful study of the development of the ganglion cell[6] I have been brought to the conclusion that the presence or absence of "gemmules" is dependent upon the existence or non-existence of the fibrils. Hill[7] concluded that these lateral buds or gemmules were nothing more or less than "the cell end of an unstainable nerve filament." This is, I believe, only partly true. A gemmule is formed by a silver deposit at the point where the nerve filament or fibril enters the cell process, and it is also found if the fibril is simply lying upon the process as is the case with most of the fibrils in Fig. 2. The gemmules are found either at the point of entrance, or at the point of simple contact of a fibril with the protoplasm of the cell process. It can be easily shown that the appearance of the gemmules in the cortical cells of embryos is synchronous with the appearance of the fibrils. In the adult as well, the gemmules depend upon the existence of the fibrils and any pathological process which destroys the latter causes a corresponding diminution or absence of the former.

DESCRIPTION OF PLATE I.

Both sections are from the cerebral cortex of a fully developed pig. Thickness of sections 2μ. Zeiss, Apochromat. No. 6, Ocular, 2 mm.

FIG. 1. The neurofibrils can be seen running through the cell-body and apical process. In the section fibrils can be seen entering the ground-substance from the cell-body as well as from the processes.

FIG. 2. Most of the fibrils, which look as if they were within the cell-body, are really extracellular, and form part of the fibre-network of the ground-substances.

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[5] *Arch. ital. de biol.*, 1898, xxx, p. 71.

[6] The histogenesis of the cellular elements of the cerebral cortex. Contributions to the Science of Medicine dedicated by his Pupils to William Henry Welch on the Twenty-fifth Anniversary of his Doctorate, p. 709. Baltimore, 1900.

[7] *Brain*, London, 1897, xx, p. 131.

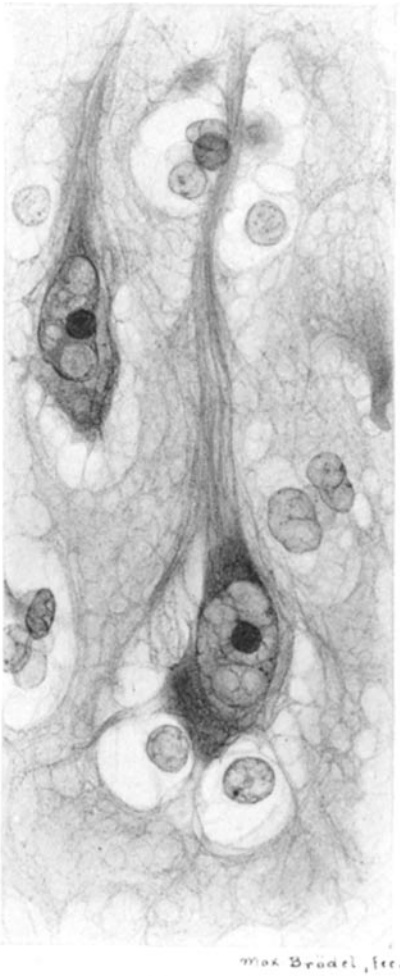

FIG. 1.

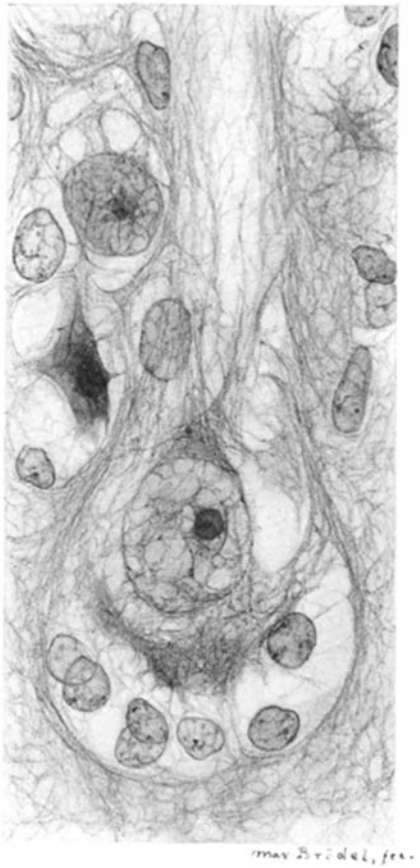

FIG. 2.