PRELIMINARY REPORT ON THE CYTOLOGY OF MOLLUSCAN NERVE CELLS.

BY

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WITH SEVEN FIGURES IN THE TEXT.

The observations now reported are on the Opisthobranch, Haminea, the Lamellibranch, Venus, and the Pulmonates Planorbis and Limax. In addition to these forms Helix, Littorina, Melantho, Montagua gouldii, Aplysia and others have been worked during the past three years.

Interest in the structure of the cytoplasm not only of the nerve cells of both vertebrata and invertebrata but in gland, epithelial, egg and sperm cells has resulted in the accumulation of a very considerable literature during the past ten years and more especially the last five. (See HOLMGREN, '01; RHODE, '98, '03 a, '03 b, '04 a, '04 b; BERGEN, '04, and others.) Sufficient facts have been advanced by these writers to indicate that here are a large and varied number of conditions existing in the nucleus and especially in the cytoplasm of adult cells. My purpose is to refer but briefly at this time to these structures in nerve cells, reserving for the full paper a more extended survey of the literature.

The structures already found in the nerve cells of vertebrata and invertebrata are given a variety of names in part at least due to the theory of their origin and fate. These may be grouped under two headings for convenience in description, although in some instances at least the two classes are interrelated.

1. The *lymph spaces* which have no constancy of form or position in nerve cells, sometimes being found near the nucleus, or in the vicinity of the periphery of the cell, or again having a general distribution. Some writers regard them as artefacts while others maintain that they are normal and essential to the cell activity.

2. In this second class may be placed a large variety of bodies

which are differentiated by osmic acid and the basic stains. SCHNEIDER ('02) designates them in mollusca as NISSL bodies, thereby implying their similarity to the well known NISSL bodies of vertebrate nerve cells. In their distribution in the nerve cell, they are arranged usually in concentric rows around the nucleus, becoming fewer as the cell wall is approached. That such regularly disposed bodies exist in many invertebrate nerve cells there can be no doubt, but there is considerable doubt as to their distribution even in the same family; and as to their significance we possess no generally accepted conclusions.

Closely associated with these regular-shaped and regularly distributed NISSL bodies are some bodies to which the term mitochondrien and chondromiten (RHODE, '04 a) is given. RHODE would include here also the NISSL bodies. The term chondromiten is used when there is a considerable accumulation of a densely-staining substance in the cytoplasm. The collection of several bodies (mitochondrien) gives rise to a mass which is known as chondromiten and conversely the chondromiten may break up into a number of small bodies known as mitochondrien. RHODE makes a great deal of these various combinations in which he tries to establish a genetic continuity. The result is that he advances a hypothesis of elementary organisms for the cell which is a modification of ALTMANN's bioplastic hypothesis.

In general it may be said that HOLMGREN and his followers look on these structures as the expression of an activity in the cell and that under different physiological conditions the cytoplasm reveals different structural states.

After spending considerable time on the cytology of nerve cells in mollusca, I became convinced that, unless the cause of these remarkable structures could be resolved, not only was all theorizing futile, but also a correct interpretation of the facts as well. In the further work which is now well under way on this and kindred topics Dr. C. G. ROGERS will be associated with me and it is our purpose to follow careful physiological experiments by cytological observations.

Haminea solitaria.—Concerning the question as to whether the lymph spaces have a definite wall other than would be formed by the granules of cytoplasm, I do not wish at this time to make any statements for or against the contentions of HOLMGREN, RHODE and BERGEN.

There are located in the cerebral, pleural, pedal and visceral ganglia of most gastropods a few nerve cells much larger than those that make up the bulk of the ganglion. In Helix, Doris, Aplysia and others these are of enormous size. The small nerve cells are too small for a satisfactory study, so one is limited in the main to the few large cells in each ganglion.

All of the text figures were drawn with a camera lucida, the $\frac{1}{12}$ oil immersion and two-inch ocular, Bausch and Lomb.

a *b*

Fig. 1.

In Fig. 1, *a* there are two conspicuous lymph spaces and four smaller ones. These are not connected with one another or with the surrounding neuroglia cells. The cytoplasm is quite uniformly granular. The nucleus is sharply defined by a membrane and the larger size of its granules. On the side toward the axone, the nuclear membrane is sharply bent in toward the center of the nucleus and the larger granules assume a radial and beadlike arrangement as if there were some marked physiological activity taking place. In Fig. 1, *b* the lymph spaces are smaller and more evenly distributed. The cytoplasm differs from the conditions in Fig. 1, *a* in that there are several rodlike and granular bodies present. These bodies take the same kind of a stain that the large granules in the nucleus of each cell takes. In one instance the granules of the cytoplasm were arranged radially around one of the rodlike bodies having very much the appearance of a centrosome and sphere. MCCLURE ('97) describes similar conditions in gastropod nerve cells and explains them on the ground of the persistence of the centrosome and sphere.

It is further to be noted that the granules in the nucleus have the same radial, beadlike arrangement around the infolded nuclear membrane. These granules are very noticeable because of their intense reaction to basic stains. The nucleolus is solid and large; a single one is figured in this section, but as many as seven have been counted in some nerve cells of Haminea. These two nerve cells were taken from the same animal which was fixed with picroacetic (BOVERI) under normal conditions.

Venus.—A large number of preparations of the visceral ganglion of the common market clam were made in the hope that the conditions existing in these cells could be harmonized with other mollusca. Clams just taken from the sea and those that had been transported to Syracuse, N. Y., were used, but no constant difference was found to exist. The tissue was fixed in picric acid-sublimate (HOLMGREN), as well as sublimate and osmic acid.

In Fig. 2 the cytoplasm is loosely granular, showing many fibers present in the region where the axone arises. The nucleus is limited by a conspicuously staining membrane; the chromatin is pretty generally distributed throughout the nucleus. On one side of the nucleus there is a cone-shaped mass of deeply-staining granules which are much like the usually described NISSL bodies for molluscan nerve cells, but it should be noted that these bodies do not have the general concentric distribution so characteristic of NISSL bodies. In close connection with each other and the nuclear membrane, there are various shaped bodies and granules which occupy the remainder of the space around the nucleus. The smaller of these bodies are indistinguishable from the cytoplasmic granules, while the two larger are more like nucleoli than anything else. On the side of the nucleus opposite to the cone-shaped mass of NISSL granules here is an interesting condition. A number of the cytoplasmic granules have increased slightly in size and form a rather complete boundary to what is evidently a lymph space. In this space there is a solid staining body similar to the two already mentioned. It would seem as if we had an early stage in the formation of the limiting wall.

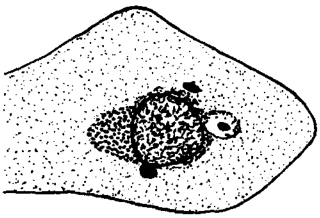
Fig. 2.

Figure 3 reveals one or two additional characteristics. The chromatin shows a tendency to mass around the periphery of the nucleus sim lar to the condition already described for Haminea. There seems to be an entire absence of NISSL bodies as such. There are two conspicuous lymph spaces sharply limited, apparently empty. Associated with these two are several smaller ones, some likewise empty of stainable substance, others containing one or more bodies. Surrounding these lymph

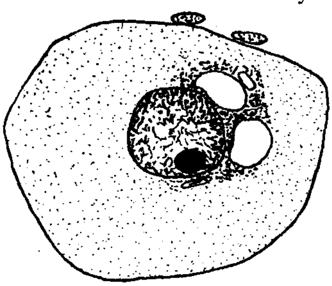
Fig. 3.

spaces there is a considerable amount of densely staining subtance similar to that described in Fig. 2.

Figure 4 again shows the NISSL bodies rather numerous and completely filling one end of the cell. The nucleus is evidently undergoing division as indicated by the state of the chromatin lying free in the cytoplasm. There are a few lymph spaces near one edge of the cell, close to the cell membrane.

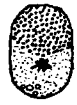

Fig. 4.

Planorbis.—The fresh nerve collar of Planorbis was placed in a 3 per cent. solution of osmic acid for eight days. In hundreds of cells obtained by this method, the conditions were much as shown in Fig. 5. Here there seems to be a nearly typical distribution of NISSL bodies. The appearance of these bodies is more like the usual conditions for the NISSL flakes. When other stains are used a fine granular cytoplasm is then evident. There are scattered in the cytoplasm a number of lymph spaces with no apparent regularity of form or position. Most of the nucleoli contained a central vacuole. Apart from the lymph spaces represented in the drawing this nerve cell would be typical of many of the nerve cells reported by RHODE.

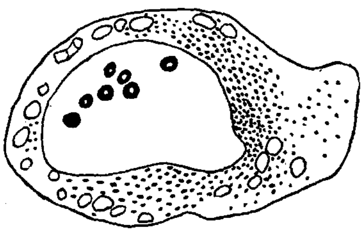

Fig. 5.

Limax.—Collections of Limax were made October 13, 1903, from a brick walk on our campus. A part of the specimens were killed at once in several of the best fixing reagents and the remainder were placed in a dark, moist chamber having plenty of grass. The conditions in Fig. 6 are such as are common in the freshly collected animals, while in Fig. 7, the nerve cells exhibit many unusual structures after being kept in the dark for seventy-two hours with plenty of grass.

In Fig. 6 the lymph spaces are very large and distributed irregularly. The cytoplasm seems to be free from NISSL bodies or other densely-staining granules. In the nucleus there are two large nucleoli. The chromatin is evenly and loosely

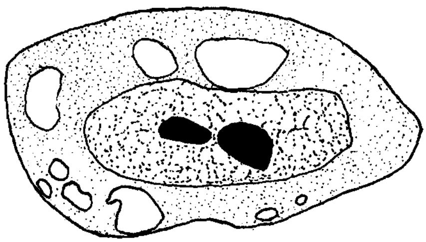

Fig. 6.

distributed. The nuclear membrane is distinct. One would hardly imagine the nerve cells shown in Fig. 7 was from the same species as that represented in Fig. 6. In the axone there are four large lymph spaces containing densely-staining bodies of irregular shape. There are also nine roundish lymph spaces each containing a rodlike or roundish body as well as many small spaces rather evenly distributed. The remaining bodies illustrate very well RHODE's ('04 a) idea of chondromiten and mitochondrien as well as sphere substance. I am inclined to doubt very much the value of his distinctions in regard to the several structures of the cytoplasm. In some instances in the figure these irregular masses lie free in the cytoplasm; while in others, the granules of the cytoplasm are arranging themselves to form a wall which will result in a lymph space. Some of the bodies are quite large and regular shaped, while others are equally irregular and very small.

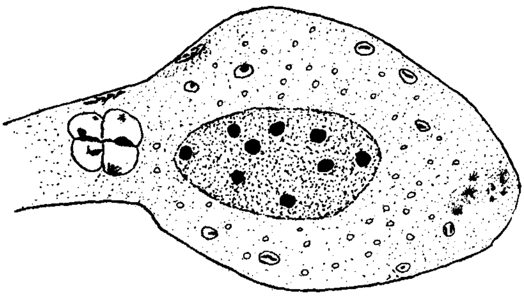

Fig. 7.

The conditions of the cytoplasm and nucleus described above represent several different states. A later paper will attempt to correlate them.

Syracuse University, Department of Zoölogy, May 10, 1905.

LITERATURE.

BERGEN, FREDERICK VON. '04.—Zur Kenntnis gewisser Struktur-bilder (Netzapparate, Saftkanlchen, Tropospongien) im Protoplasma versshiedener zellmarten. *Archiv f. mikr. Anat.*, Vol. 64.

HOLMGREN, EMIL. '01.—*Anatomische Hefte*, Vol. 11.

MCCLURE, C. F. W. '97.— The finer Structure of the Nerve Cells of Invertebrates. Gastropoda. *Zool. Jahrb., Abt. Morph.*, Bd. 11.

RHODE, E. '96.—Ganglion Zellkern und Neuroglia. *Archiv f. mikr. Anat.*, Vol. 47.

'98.—Die Ganglion Zelle. *Zeit. f. w. Zool.*, Vol. 64.

'03 a.—Untersuchungen über den Bau der Zelle. I Kern und Kernkurper. *Ibid.*, Vol. 73.

'03 b.—II.—Ueber eigenarte aus der Zelle wandernde "Spharen" und "Centrosomen," ihre entstehung und ihren Zerfall. *Ibid.*, Vol. 75.

'04 a.—III. Die entstehung von Mitochondrien und Chondromiten aus eigenartigen intra- und extra-zellularen Spharen (Idiozome). *Ibid.*, Vol. 76.

'04 b.—IV. Zum histologischen wert der Zelle. *Ibid.*, Vol. 78.

SCHNEIDER. '02.—Vergleichende Histologie der Tiere.