[From the PROCEEDINGS OF THE ROYAL SOCIES, NO. 200, 1880.]

"On the Histology of *Hydra fusca.*" By T. JEFFERY PARKER, B.Sc., Lecturer on Biology in Bedford College, London, and Demonstrator in the Royal School of Mines. Communicated by Professor HUXLEY, Sec. R.S. Received December 11, 1879.

# (From the Biological Laboratory of the Royal School of Mines.)

### [PLATE 1.]

The few observations I have to offer on this much-discussed subject are partly confirmatory of, partly supplementary to, those of Kleinenberg,\* they present a certain agreement with those of F. E. Schulze,† while they are, in great measure, distinctly contradictory of the later researches of Korotneff.<sup>‡</sup>

1. The Ectoderm and the Muscular Layer.—The layer of longitudinal fibres between the ectoderm and the endoderm was discovered by Kölliker, who believed that each fibre was in direct connexion with an endoderm cell. Kleinenberg, in teased specimens, saw that the ectoderm cells tapered towards their inner ends, and that each was continued into a simple or branched process, of precisely the same character as the fibres seen in sections: from this observation the important conclusion was arrived at, that the fibres were in direct continuity with the ectoderm cells, thus forming a sort of nascent mesoderm.

Schulze figures the elements of the middle layer as fusiform fibres, with somewhat jagged edges. Korotneff, following Kleinenberg's directions as to methods of preparation, came to the conclusion that the ectoderm cells were expanded (*élargie*) at their inner ends, and that each carried a fusiform refringent fibre, attached by its middle to the enlarged base of the cell, and projecting beyond it in either direction, so that the cell appeared as a lateral appendage (*annexe*) of the fibre, rather than the fibre as a prolongation of the cell.

How M. Korotneff can have come to this conclusion as to the shape of the ectoderm cells, it is rather difficult to imagine; by any ordinary method of preparation it is perfectly easy to satisfy oneself that the cetoderm cells of the body are, as a rule, markedly distinguished from those of the endoderm by the tapering of their inner ends; and, in good specimens, that these ends are continued into longer or shorter filaments.

The question of the exact relations of the fibres is by no means so easy to decide. Anyone working at Hydra for a week or two,

\* " IIydra," 1872.

† "Ueber den Bau u. die Entwicklung von Cordylophora lucustris," 1871.

‡ "Histologie de l'Hydre et de la Lucernaire." "Arch. de Zool. exp.," t. v, (1876), p. 369.

and using various methods of preparation, might readily frame a dozen different theories on this point, all equally supported by appearances. But the matter seems to me to be entirely set at rest by thin longitudinal sections of specimens preserved in ammonic biehromate, which reagent usually has the effect of causing a certain amount of separation between the layers. In such sections (fig. 1) the eetoderm cells (ec.) are distinctly seen to taper off towards their inner ends; the fibres (m.p.) to pass from them, at a sharp angle, towards the endoderm, or, more correctly, towards the supporting lamella; and, in some cases (e.g., the fibre to which the line from m.p. points), the fibres can be distinctly traced into the attenuated extremities of the eells.

As to the true nature and functions of these structures, Dr. Kleinenberg calls the ectoderm cell with its filamentous process, a neuromuscle cell; M. Korotneff prefers to name it an epithelio-muscle cell; Prosessor Huxley\* considers that the fibres " are solely internuncial in function, and therefore the primary form of nerves." This last view is rendered, to say the least, decidedly improbable, by the great number and the regular disposition of the fibres. It seems, à priori, unlikely that an animal devoid of all muscular tissue should have a layer of elose-set longitudinal nerve-fibres throughout its whole body, while such an arrangement is perfectly intelligible in a set of specially contractile filaments, developed as a means of rapid retraction of the body.

The term "neuro-muscular" implies, as Kleinenberg explains, that the process only is contractile; the function of the cell itself being merely to receive and transmit impressions. But, as Professor Huxley points out, it is absolutely necessary to assume contractility in the cell proper, to account for the lengthening of the body. The fibres merely have a special degree of contractility assigned to them, in correspondence with the obvious advantage accruing to the animal from the power of instantaneous shortening, the general contractility of the cells serving for extension; this movement being, as observation of a living Hydra shows, a comparatively slow one. The fibres must also be of use in the characteristic "looping" movements of the animal.

The simplest and most reasonable way of looking at these structures is that adopted by Dr. Michael Foster, and illustrated in the diagram at the beginning of the third chapter of his "Text-book of Physiology." These show clearly enough that the ectoderm cell of Hydra, with its muscular process, is the equivalent of what, in the higher animals, becomes sensory cell, sensory nerve, nerve cell, motor nerve, and muscle cell. So that a fairly logical term might be made by

\* "Anat. of Invert. Animals," p. 64.

1879.]

combining Kleiuenberg's and Korotneff's, and speaking of epithelioneuro-muscle eell; but, fortunately, it is unnecessary to employ any such cumbersome term, and quite sufficient to speak of ectoderm cell with contractile process.

The interstitial tissue, discovered by Kleinenberg, is quite readily made out in all parts of the body except the proximal end, where nematocysts are also absent. It is not mentioned by Korotueff, and, indeed, its existence would be impossible if the large ectoderm cells had the shape described by him.

I have found no interstitial cells in the tentacles (fig. 5); this would seem to show that the ordinary ectoderm cells may also be the mother cells of the nematocysts. The ectoderm cells of the tentacles also differ from those of the body from the fact that their nuclei are nonnucleolate, resembling indeed the nucleoli of the body cells, rather than their nuclei (fig. 5).

2. The Supporting Lamella. This structure is clearly distinguished by Schulze and by Korotneff, the latter of whom, however, figures it\* as almost equal in thickness to the diameter of an ectoderm cell! Kleinenberg states that the muscular processes are imbedded in a structureless cementing substance, and that this, continued beyond the muscular layer on the endoderm side, forms a layer—the "Stützlamelle" of Reichert—which can sometimes be obtained as a separate structure.

This description by no means expresses the distinctness of the supporting lamella. In specimens preserved in osmic acid, or ammonium bichromate, without subsequent treatment with alcohol, it is easy, by teasing with fine needles, to detach shreds of considerable extent, more or less free from attached muscular fibrils and from cells of the interstitial tissue (fig. 2).

3. The Endoderm.—The ciliation of the endoderm is a question about which there has been a good deal of discussion. Schulze figures a single flagellum to cach cell, as seen in optical section of the tentacle. Kleinenberg was unable to demonstrate the existence of flagella in the uninjured animal, or in preserved specimens, but in transverse sections of the living animal, he observed one or two cilia, in connexion with more or fewer of the cells, and noticed that they were not fixed structures, but were occasionally retracted, and then protruded again, the cells at the same time sending out pseudopodial processes.

It is quite easy to confirm this observation; the slow lashing movement of the flagelliform cilia, their continual disappearance and reappearance in fresh places can be made out without difficulty. But the best notion of the characters and relation of the cilia is obtained by teasing out, or still better, by cutting thin sections of osmic acid specimens. Such preparations quite lead one to think that the endoderm is ciliated throughout; in the sectious particularly, (fig. 3), cell after cell is seen bearing one, two, or three cilia. These latter are of great length, in fact nearly or quite as long as the cells to which they are attached; in some cases indeed, they are longer, as, for instance in the cell to the right in fig. 6. I have never seen anything like a "collar" at the base of auy of the cilia.

The amœboid character of the endoderm cells, as seen in sections or teased fragments of the living animal, is a well-known fact; but the extent and activity of the amœboid movements during life has not been sufficiently insisted on. In sections of pieric acid or ammonic bichromate specimens, large rounded pseudopodia are seen to be given off from the cells into the digestive cavity, sometimes to such an extent as completely to obliterate the latter. The length of the cells may, therefore, vary almost indefinitely; they may be but little longer than the ectoderm cells (fig. 3), or may be two or three times as long (fig. 1). This variation in the size of the endoderm cells, and the consequent variation in the diameter of the digestive cavity, is very marked in my series of sections, nearly all of which are taken from large specimens,\* killed in a state of half-extension. When the endoderm cells are fully extended, it is almost impossible to obtain them complete by teasing. They nearly always break across, and can only be obtained in a fragmentary condition.

A very noticeable point about the endoderm cells is the presence in their protoplasm, especially towards the far end, of dark-colonred irregular granules, of various sizes. It has been suggested that these are products of excretion; Kleinenberg makes the important observation that their number varies with the state of nutrition of the animal.

I am convinced that these bodies are food particles, taken into the protoplasm of the cells, from the partially disintegrated bodies of the *Entomostraca* in the digestive cavity. They are of quite the same nature as the contents of the alimentary caual in many of the common *Cladocera* and *Copepoda*; they occur chiefly in the free end of the cell, and in some cases they have all the appearance of being half in and half out of the protoplasm. (See fig. 5.) The particles of the more transparent parts of the body of the Crustaceans will naturally not be so evident in the cell protoplasm; even these, however, can be made out in a Hydra in full digestion, when the endoderm cells of the distal or gastric region are completely crammed with transparent spheroids.

The clearest case of ingestion of solid particles is that shown in fig. 1, d, when a diatom is seen to be completely imbedded in the protoplasm of a cell.

If this explanation of the dark grannles is the correct one, Hydra \* Supplied by Mr. Bolton, of Birmingham. will have been shown to exhibit a process of alimentation identical with that described by Metschinkoff, in the lower *Turbellaria* and in sponges.\* The Russian observer describes the complete obliteration, during digestion, of the digestive cavity in the Turbellarians, and of the canals in the sponges; and, in the former as well as the latter, he has undoubted evidence of the actual ingestion of solid particles by the endoderm cells.

It would seem, therefore, that Hydra adds another instance to the two already brought forward by Metschnikoff, of a Metazoon exhibiting what is usually considered to be a distinctively Protozoan mode of digestion. It is quite possible that a preliminary disintegration of the animals taken in is performed by juices secreted by the endoderm cells, but the final digestion seems to take place in the actual protoplasm of the cells, into which the food articles are taken in the solid form.

The endoderm cells of the tentacles resemble those of the proximal and of the body in possessing larger vacuoles (fig. 5). Their nuclei are in some instances, although not constantly, simple and non-nucleolate like those of the ectoderm cells for the same region (fig. 5).

Finally, I have been able fully to confirm Professor Huxley's statement<sup>+</sup> as to the presence of nematocysts in the endoderm (fig. 1, n), a statement which, as far as I am aware, has not been made, with regard to Hydra, by any other writer on the subject. This fact is, like the absence of interstitial tissue in the tentacles, an argument against Kleinenberg's view that the tissue is the sole source of the nematocysts.

4. Methods.—For sections, the Hydræ were either killed with hot water, and placed in Kleinenberg's pieric acid for two hours, or were placed alive in ammonic bichromate, 1 per cent.—which always kills them in the half extended condition—and kept in it for two or three days. In either case they were afterwards transferred to 50 per cent. alcohol, and then placed successively in 75 per cent., 90 per cent., and absolute alcohol. The specimens were stained either with carmine or pierocarmine, and imbedded in cacao butter, after soaking for a short time first in oil of cloves and then in melted cacao butter. By this means they became so thoroughly permeated with the imbedding material that they could be cut without the loss of a single section; even longitudinal sections of the tentacles could be made with case.

For teasing I employed ammonic bichromate, acetic acid (0.5 per cent.), or osmic acid (1 per cent.), and for this purpose the specimeus were not transferred to alcohol, but to weak glycerine (equal parts of glycerine and water), in which they were teased out.

\* "Zool. Anzeiger," Bd. I (1878), p. 387, and "Zeitsch. f. wiss. Zool." Bd. xxxii (1879). It need hardly be said that the above view of the physiology of digestion in *Hydra* was suggested by these papers of Metschinkoff's.

+ Huxley and Martin, " Elementary Biology," p. 100.

## On the Histology of Hydra tusca.

For sections showing the cilia of the endoderm, the Hydræ were kept for twenty-four hours in 1 per cent. osmie acid, then washed, and preserved in weak glycerine until required for eutting. The sections were eut by Dr. Pritchard's very convenient freezing microtome, the specimens being placed in gum water before freezing.

### DESCRIPTION OF PLATE.

#### Reference letters.

ec., ectoderm.	en., endoderm.
m., middle layer.	f., food particles.
m. p., muscular processes.	d., diatom included in an endoderm
s. l., supporting lamella.	cell.
i. t., interstitial tissue.	c., cilia.
ne nematoarsts	

- Figure 1. Longitudinal section of the body, distal end, (ammonic bichromatealcohol-cosin). The specimen was killed in full digestion, the endoderm cells being gorged with food particles, amongst which is a diatom frustule. The ectoderm and endoderm are unnaturally separated by the action of the bichromate, and the supporting lamella is not shown. The nuclei are not well shown, the specimen having been unfortunately stained with cosin instead of carmine.
- Figure 2. Fragment of the supporting lamella, obtained by teasing, (osmic acidweak glycerine). The wavy character of many of the adhering muscular processes is well seen.
- Figure 3. Longitudinal section of the body, distal end, (osmic acid—weak glycerine), showing particularly the flagella of the endoderm cells. The boundaries of the ectoderm cells are not seen.
- Figure 4. Transverse section of the body, proximal end, (pieric acid—alcohol carmine), showing the pseudopodia of the endoderm cells and the included food particles, as also the absence of interstitial tissue and of nematocysts in this region.
- Figure 5. Longitudinal section of a tentacle, (pieric acid—alcohol—carmine). In this section, selected as showing the food particles in the protoplasm of the endoderm cells, no nematocysts, nor palpoeils are to be seen.
- Figure 6. Longitudinal section of the body, distal end, to show the typical structure of the body wall.
- Figures 1—5 are accurately drawn, each from a single specimen, except for the fact that the diatom d, in fig. 1, has been inserted from a similar, but otherwise less favourable specimen. Fig. 6 is a combination of several preparations, being the correct proportions retained.

All the figures were drawn with a Gundlach's  $\frac{1}{16}$ th immersion (for the use of which I am indebted to Professor Huxley) on Hartnack's small stand, and with his No. 3 eye-piece.

HARRISON AND SONS, PRINTERS IN ORDINARY TO HER MAJESTY, ST. MARTIN'S LANE.



Fig.3.





Fig.6.





Fig.4



Fig. 5.



Fig. 2.





