MEMOIRS.

Remarks *on the* Structure *of the* Gregarinæ. By Professor Edward van Beneden. (With Plate XI.)

In a work published in the 'Bulletins of the Royal Academy of Belgium' (vol. xxxi, No. 5, 1871), and 'Quart. Journ. Micro. Science' (vol. xi, new ser.), I have made known the successive conditions of the evolution of a new Gregarin found in the intestines of the lobster, and described on a former occasion under the name of *Gregarina gigantea* ('Report of the Royal Academy of Belgium,' vol. xxix, No. 11, 1869, and 'Quar. Journ. Micro. Science,' vol. ix, new ser.). I have established through my investigations that the psorosperms give birth to small protoplasmic globes, which differ from Amœbæ in that they are devoid of all cell-nucleus, and in that they never show any trace of vacuole. They represent, from a morphologic point of view, the Monera of Haeckel, and the Gregarinæ pass, during the course of their autogenic evolution, through the Monerian condition. At that time they are simple gymnocytods, and only become cells when a nucleus develops itself in their interior. On the surface of each cytod grow two protoplasmic prolongations. Simple buds in their beginning, these prolongations stretch out, absorbing at the same time the body of the cytod, and when they become free they move in the intestines of the lobster, like little nematod worms. From thence the name of Pseudofilaria, which I have given them. Not long after, they become shorter, and at the same time their movements become less active; they soon cease altogether; a voluminous nucleolus then appears inside the body, this at once becoming surrounded by a nuclear coating. From this time the cytod has become a cell; the separation of the chemical elements of the nucleolus and of the nucleus from the essential elements of the body of the cell has led to the differentiation of the original matter, which I have called "plasson," into three distinct layers—the nucleolus, the nucleus, and the protoplasma.[1]

[1] The beautiful observations that Eimer has recently published on the

The cell has only now to grow to become the beautiful Gregarine, of the $\frac{10}{1000}$th part of a mètre long, to which the lobster complaisantly gives home and nourishment; but at the time of its growth the cell undergoes in its protoplasmic body new phenomena of differentiation, and the complication which then appears in the cell-body allows one to affirm that certain monocellular organisms can present a real organization, and that they are composed of parts which must be distinguished, as much from a morphological point of view as from a physiological one. Before describing this complication of structure, and in particular those elements that constitute inside a monocellular being a real muscular system, I have thought it necessary to recall, in a few words, the results of my investigations on the evolution of the Gregarinæ, because they show that the Gregarina is but one single cell, and that it represents to us, without any doubt, a monocellular individuality.

The giant Gregarina's body is of a cylindroid shape; its diameter varies little, at the most a small progressive narrowing in its terminal parts, and a slight dilatation, the development of which is variable, near its anterior extremity. A cellular membrane, which I call cuticle by analogy with with the cuticle of Infusoria, limits the body externally, and the Gregarina is really only a long cylindrical sac, closed at each of its ends. This membrane shows no trace of mouth nor orifice, nor are any canalicular pores distinguishable; it appears perfectly homogeneous, and the nutritive liquids can only penetrate by endosmosis. The membrane is of the same thickness in every part. In individuals which have reached their complete development its limit is most exactly fixed on the inside as well as on the outside, and shows a strongly marked double contour. But this is not the same in young individuals; in them the cuticle is very difficult to find, because it is not completely isolated from the subjacent protoplasmic matter; there is an imperceptible passage between the contents of the cell and the external coating of the protoplasm, which gradually transforms itself into a cortical substance.

The contents of the cell, forming the parenchyma of the

psorosperms of higher animals confirm on all points my investigations on the evolution of the Gregarinæ of the lobster. The phases of psorosperms (figs. 53, 54, 56, and following in his work), of semilunar bodies, swelling at one end (fig. 34), of scythe-shaped bodies (fig. 36, and following), of semi-ovoid cells (fig. 47), and of nucleated Gregarinæ, correspond to the conditions which I have designated under the name of Monerian condition, of generative cytod, of pseudo-filarium, of young Gregarinæ, and of complete Gregarinæ.

body, may be divided, just as in the Infusoria, into "*a central column, or medullary parenchyma, a peripheric coating or cortical parenchyma, and a very thin subcuticular coating, which constitutes the muscular coating.*"

The medullary parenchyma appears in the greater part of the length of the cell, under the appearance of a dark band, occupying the axis of the body. It is composed of a very granular substance, and much more fluid than the cortical substance. The grains it contains are rather voluminous, and very refracting; they are seen to change their places, and move under the influence of the contractions of the Gregarinæ. The middle parenchyma really constitutes a massive column, which completely fills the hollow cylinder, circumscribed by the cortical parenchyma. The nucleus of the cell, of which the form is usually ellipsoidal, occupies the entire breadth of the cylinder. If the body of a Gregarine still alive is cut transversely, either above or below the nucleus, the central fluid matter runs off in a columnar shape, without carrying away the nucleus with it; and since the cortical matter remains in its place, there develops inside the body a cavity, circumscribed on the outside by the cortical parenchyma, at the top by the nucleus, at the cut end by the exuding medullary column (fig. 6). When the medullary matter spreads, it dilates, the granules separate one from another, and continue diverging, each animated by very intense Brownian movements, each oscillating by itself.

The cortical layer (Leidy's muscular layer) is composed of a slimy protoplasmic matter, much less fluid, much less granular, and, therefore, clearer than the medullary substance. The granules of the cortical parenchyma are not only less numerous, but much more slender and refracting, than those of the central column. Just as in Infusoria, there is to be found no strong line of demarcation between the two layers; there is an imperceptible passage from one to the other. Near the posterior extremity of the body these two substances are with difficulty distinguished. The surface of contact between the medullary parenchyma and the cortical parenchyma is not always a simple cylindroid surface; the medullary coat presents sometimes on the external surface channelings approximated more or less one to the other, into which the cortical substance moulds itself. The ridges of the medullary column, and the corresponding sides of the cortical substance, are more or less numerous, and more or less near to each other. As they are always parallel to the axis of the cylindrical body of the Gregarine, they communicate to it a longitudinal striation, the sides of the cortical

column producing the effect of lighter longitudinal striations. The channelings and the longitudinal striations that are their consequence appear and disappear, and it is impossible for one to tell the signification of this arrangement.

Many naturalists have described the longitudinal striation of the body of certain Gregarinæ. Lieberkühn [1] recognised such a striation at the posterior extremity of the body of Gregarinæ that are found in the testicles of Lumbrici ('Monocystis et Zygocystis' of Stein). Just as Claparède,[2] who observed a double system of striations on the surface of the body of a Gregarina, from a Phyllodoce, so Lieberkühn has neither inquired the cause nor the signification of these striations. Leidy[3] first described a layer distinctly characterised by longitudinal striation, and called it the muscular coating (it corresponds to our cortical coating). Leuckart [4] confirms the observations of Leidy, but he emits the opinion that the striation depends on the momentary folding of the subcuticular cortical membrane. This perfectly correct interpretation has been recently adopted by Ray Lankester.[5] According to him, also, the longitudinal striations are only the result of a state of momentary contraction of the would-be muscular tunic of Leidy. When I published my first work on the Gregarinæ of the lobster, I then also recognised the true value of the longitudinal striations, attributing them, not to an organic permanent disposition, but to a passing state of the cortical tunic of Leidy.[6] Nothing, however, proves the muscular nature of this coating; the longitudinal striations are not muscular longitudinal fibrils, but the result of a thickening, following a longitudinal direction, of the cortical substance. This is probably susceptible of local contractions; it is likely that it allows the Gregarine to roughly bend, in elbow form, and also determines the movements of translation of the granules of the medullary fluid layer; but it consists only of protoplasm, not transformed into muscular substance.

A third very thin coating, which has completely escaped the observation of naturalists who have studied Gregarinæ,

[1] Lieberkühn, 'Evolutions of the Gregarines,' p. 24, pl. i, fig. 1.

[2] Claparède, 'Anatomical Investigations among the Hebrides,' p. 43, pl. v.

[3] 'Transactions Amer. Phil. Soc. at Philadelphia,' 1855, vol. x.

[4] Leuckart, 'Bericht über die Leistungen in der Naturgeschichte der niederen Thiere wahrend der Jahre,' 1841—1853, p. 108.

[5] Ray Lankester, 'Transactions Micro. Soc.,' 1, VI, pp. 23-28, Tab. V.

[6] Edouard van Beneden, "Sur une nouvelle espèce de Gregarines designée sous de nom de Gregarines," 'Bull. de l'Acad. Roy. de Belg.,' 2 série, t. xxviii, p. 447. 'Quart. Journ. Micro. Science,' vol. ix, new ser.

is found between the cuticle and the cortical parenchyma. It is about as thick as the cuticle; it enlarges slightly at the anterior extremity of the body, and it is this that is inflected to constitute the transverse partition which separates the anterior from the posterior chamber. This layer is developed on the whole surface of the posterior chamber; but it stops a little in front of the partition of separation between the two chambers, so that the cephalic chamber is only covered on its posterior face, and on a small part of its lateral face by the layer of which we are speaking.

It consists of a colourless substance, homogeneous and transparent, formed by transverse fibrils, composed of a very refracting substance; these present all the appearance of the muscular fibrils of Infusoria. These fibrils form either circular rings or a continual spiral developed on the whole surface of the Gregarina; but they are wanting in the transverse partition, which is exclusively composed of a transparent colourless substance.

If we examine the *surface* of the body of a Gregarina with a high power (obj. 9 or 10 immersion of Hartnack) in the intestinal liquid of the lobster, or in the serum of its blood, we distinguish a transverse striation (very evident) in the sub-cuticular layer (fig. 1). These dark striations are very near each other; they are placed with perfect regularity always equidistant, and they are nearly as evident as the transverse striation of the muscular fibres of an Arthropod or a Vertebrate. They become still more distinct under the influence of acetic acid, chlorhydric acid, or a weak solution of osmic acid.

These striations are not the result of a momentary plication of the sub-cuticular membrane; *they depend on real preformed organs*, on transverse fibrils, situated in the sub-cuticular layer; for if, instead of placing the microscope so as to observe the surface of the Gregarina, it is placed so as to observe its optical section, one distinguishes very clearly on the edges immediately under the cuticle refracting corpuscles of circular shape, situated at equal distances from each other, the diameter of which is just the same as that of the transparent layer in which they are found (fig. 2). By changing progressively the focus of the microscope, it is seen that these corpuscles are only really optical sections of the small transverse bands seen on the surface, and that consequently *these striations are produced by real transverse circular fibrils*. These fibrils, composed of a very refracting substance, alternate with light clear striations, formed by the fundamental substance of the muscular layer. The

clear substance must be considered as forming the foundation of this muscular layer, since where it thickens near the anterior extremity of the body, on a level with the transverse partition, the fibres no longer occupy the entire thickness of the layer; there the transverse fibrils are really held in suspension in the transparent substance, which in itself alone constitutes the entire partition. The fibrils are not always found, at this level, near the cuticle (figs. 2 and 3); sometimes the first fibrils envelope like so many rings the posterior part of the anterior chamber (fig. 1).

If, after having in some places torn the cuticle, the body of the Gregarine is slightly compressed, the contents flow away, carrying here and there the muscular coating with the fibrils it contains. These are then seen isolated, and it can be perfectly recognised that these fibrils are composed of small refracting corpuscles, elongated transversely, and very close to each other (fig. 5). After having by this proceeding recognised the structure of the fibrils, I was able to see the constituent corpuscles of these elements, in the living Gregarine. For that, it is only necessary to slightly compress it, and to examine the fibrils on a level with the nucleus of the cell with a high power. At this point the granular matter of the medullary column is replaced by a homogeneous and transparent nucleus, and it is far easier, aided by this greater transparency, to distinguish the details on the surface.

If it were still possible to admit Dr. Bowman's ideas on the structure of the striated muscular fibres of higher animals,[1] I would believe it possible to compare the muscular coating of the Gregarina to a muscular fibre in its developing stage, whilst it still shows in its central part protoplasm not modified, and whilst the peripheric part alone has been transformed into a muscular substance. For at this time the transverse discs formed by the juxtaposition of sarcous elements are only simple rings, which may be compared to a circular fibre of the Gregarinæ. The clear and little refracting fundamental substance of the muscular coating of the Gregarina might be compared to the clear and monorefracting layer of substance, separating, in a striated muscular fibre, the discs formed by "*sarcous elements.*"

We should suppose, in fact, that in a single cell the peripheric coating of the protoplasm can become transformed into muscular substance, as well as in a nucleated protoplasmic mass,

[1] Bowman, 'On the Minute Structure and Movements of Voluntary Muscles,' London, 1840.

really formed by the fusion of a certain number of cells. But the last works of Krause,[1] Hensen,[2] Flögel,[3] and Merkel,[4] on the structure of striated muscular fibre have so much modified our ideas on the organization of these elements, they have shown to us so complicated a structure in these fibrils, that all assimilation of the muscular fibres of the Arthrod and the Vertebrata to the muscular mechanism of the Gregarine, appears to me now impossible. It is only by comparing the muscular fibrils of the Gregarina with the fibres of Infusoria that the meaning which I have given to these elements appears to me justifiable.

To finish the description of the Gregarina it is necessary to say something more as to the contents of the anterior or cephalic chamber. These contents are always very granular and very opaque, at least in the central part of the chamber. The refracting granules that this part of the body contains are remarkable for their pretty considerable size, and by the ease with which, under the influence of an increasing pressure, they fuse one into another, and form thus irregular piles of a highly refracting substance.

When the Gregarina reaches its complete development, notwithstanding its monocellular nature, it appears to us then to be a being with a rather complex structure. As in pluricellular organization, the division of physiologic work brings about the differentiation of the cells and the progressive complication of the organization, so, in the same way, this principle of the division of work brings about in certain monocellular beings a local differentiation of the protoplasm, and causes the formation of distinct organs.

In the Gregarine such are the cuticle, the muscular layer, the cortical substance, the medullary column, the transverse partition, and the cephalic chamber. All these parts are but the result of the slow transformation of the protoplasmic body of the young Gregarina; progressively the different layers show themselves during the course of the autogenic evolution; it is also at a relatively advanced epoch of development that a transparent partition appears between the anterior extremity of the body, characterised from the beginning by an accumulation of refracting globules, and the posterior chamber. All these modifications are produced

[1] Krause, 'Zeitschrift für Rationnelle Medizin,' iii Beitsc., 33 Bd., p. 265.
[2] Hensen, 'Arbeiten des Kieler Physiol. Institut,' 1868, p. 1.
[3] Flögel, 'Archiv für Microsk. Anat.,' Bd. 8, 1 Lief.
[4] Merkel, 'Archiv fur Microsk. Anat.,' Bd. 8, 2de Lief, p. 244.

in the cell by the transformation of the protoplasm into cuticular, muscular, cortical, and medullary substance.

An important question of which, before concluding, I wish to speak, is the question of the affinity between Gregarinæ and Infusoria, or rather between Infusoria and the cell. The opinion that Infusoria should be considered as monocellular beings was generally given up the day the complex structure of these organisms became known. That complication appeared to contradict the monocellular nature, for the cell appeared to be the final expression of organic simplicity. Nevertheless it has been impossible until now that statements have been based on the anatomical study of these organisms, or that what one knows of their development, can have been taken into consideration in the attempt to show their pluricellularity.

All that we have said and made known on the structure of Gregarinæ shows, first, that, contrary to the generally received opinion, a monocellular organism can attain a high degree of complication; second, that there is a great analogy between the tunics of which our Gregarine is composed and those recognised in Infusoria. We have no reason therefore, at any rate in consequence of their rather high organization, to sustain *à priori* that microscopic animals are pluricellular beings; and it is a question if the muscular layer, the cortical parenchyma, and the medullary substance of Infusoria are not homologous with these same elements in the Gregarinæ; the solution of this question in the affirmative would demonstrate the unicellularity of Infusoria. Without wishing to maintain that these organisms are of a monocellular nature, I think we may ask ourselves the question, for our present knowledge of Infusoria has not solved it as yet. The exact knowledge of the autogenic development of these organisms could alone decide the question of the homology of their layers with those of our Gregarina, and throw light on the genealogic affinity which binds Infusoria to the most simple monocellular organisms.

EXPLANATION OF PLATE XI,

Illustrating Professor Van Beneden's paper on Researches on the Structure of the Gregarinæ.

Fig. 1.—Shows the anterior portion of the body of an adult Gregarina, seen on the surface, and showing the transverse striations, the different tunics or layers, and the anterior chamber, with the partition of separation between the two chambers. (Obj. 9 à immersion with ocular 3 of Hartnack.)

Fig. 2.—Anterior part of the body of another individual as it presents itself in optical section. (Obj. 8 oc. 3 of Hartnack.) Remark that the optical sections of the fibrils on a level with the partition of separation between the two chambers are very numerous.

Fig. 3.—Same part of the body of another individual remarkable for a greater development of the anterior chamber, a greater thickness in the partition, and a different arrangement of the transverse fibrils near the partition. The longitudinal striation is also to be seen appearing on the surface of the medullary column; it depends on a particular state of contraction of the cortical layer. The transversal fibrils have been represented in optical sections. (Obj. 10 à immersion and oc. 1 of Hartnack.)

Fig. 4.—Optical section of the body, to show the different layers and the distinct character of the granules of each of them. Under the cuticle is seen the muscular tunic composed of a fundamental homogeneous transparent substance and of transverse fibrils seen in this plate in optical section. (Obj. 10 à immersion and ocular 3 of Hartnack.)

Fig. 5.—Three isolated transverse fibrils. It is seen that they are composed of refracting corpuscles quite close to one another. (Obj. 10 à immersion of Hartnack.)

Fig. 6.—Part of the body near the nucleus. The body having been torn, the medullary column has partly run off, and a cylindroid cavity has been developed between the nucleus and the receding medullary column. The cortical substance remains in its place.

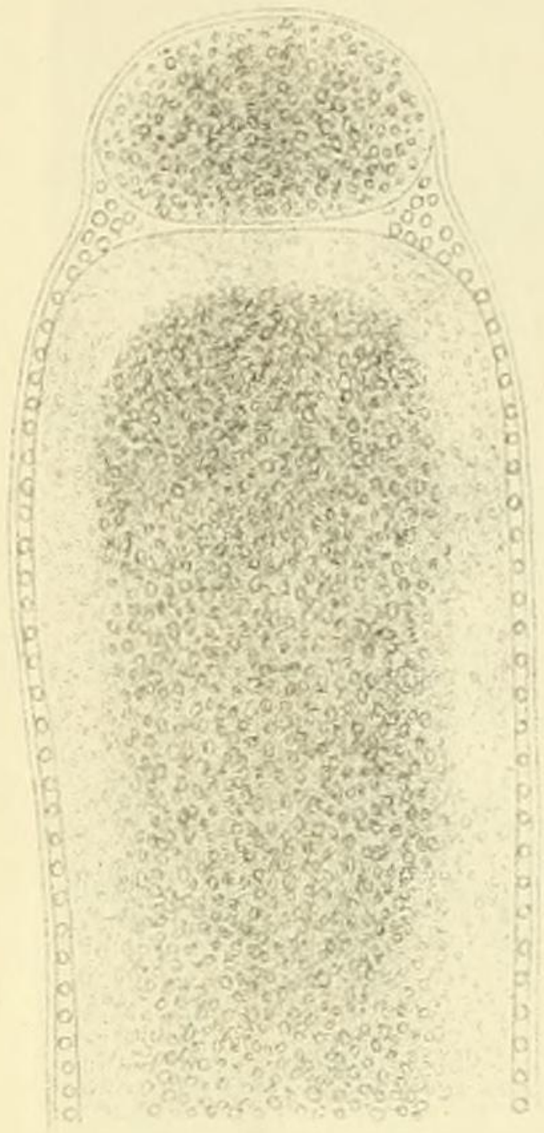
Fig. 7.—Posterior part of the body of a Gregarina which had rolled itself into a ball surrounded with a cyst of connective tissue in the coats of the rectum. It had still kept its form and all its characters of structure.

Figs. 8 and 9.—These figures show highly magnified the part of the body of a young Gregarina near the nucleus; figure 8 shows the optical section, 9 the surface of the body.

Fig. 10.—Monerian condition of the Gregarina.

Figs. 12 and 13.—The nucleus appears; the body has changed form and has considerably enlarged. All vermicular movement has stopped at this phase of the evolution.

Fig. 14.—Ulterior phase of development.



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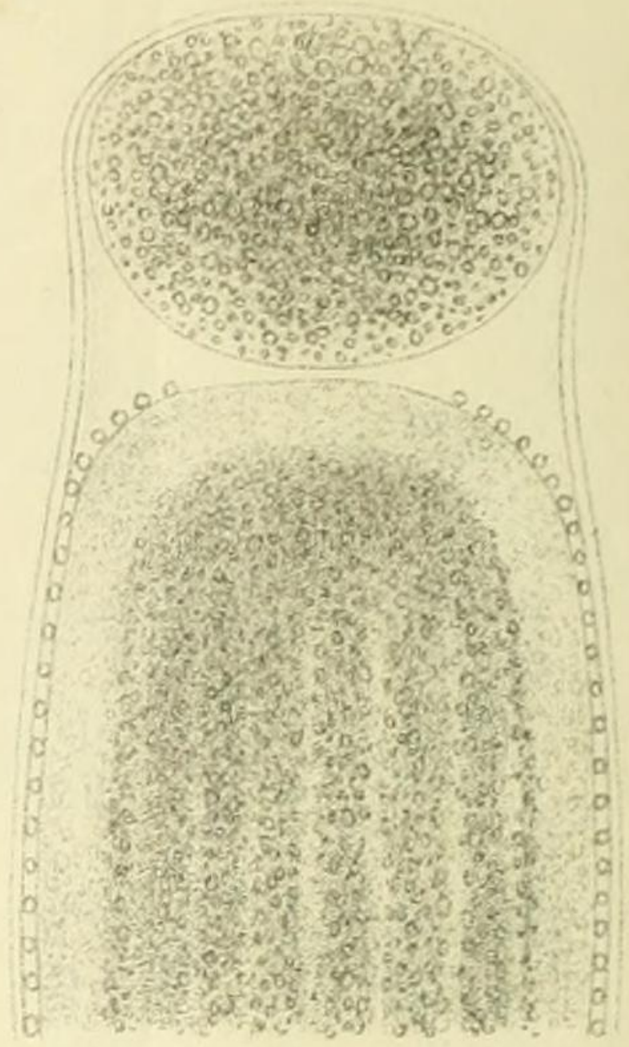

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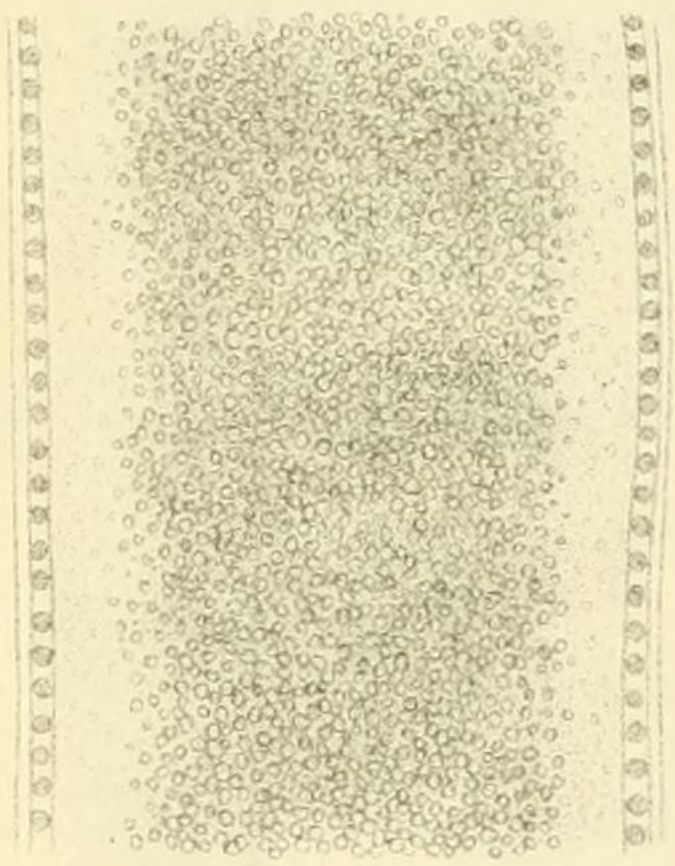

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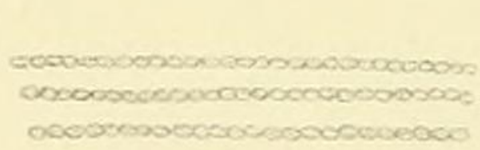

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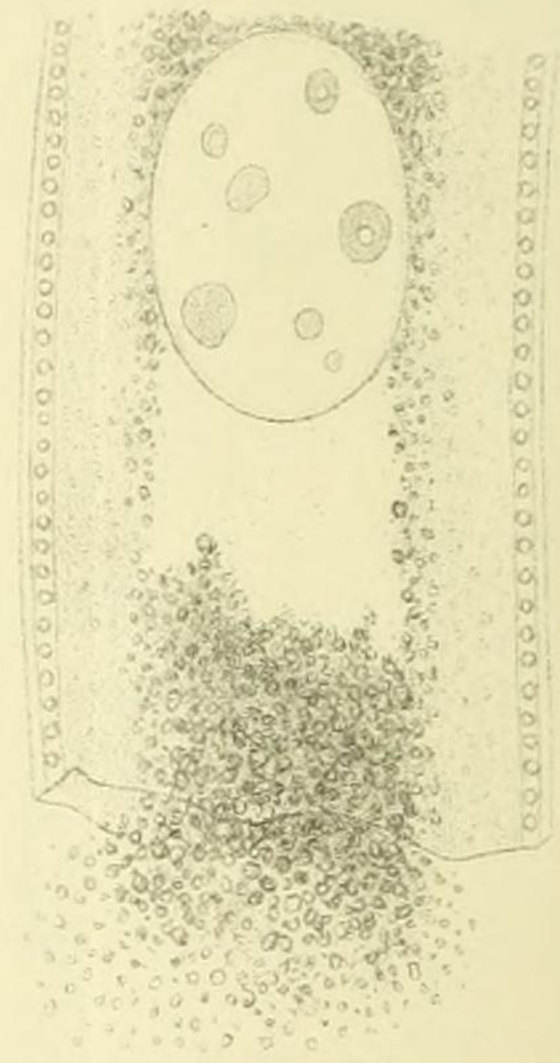

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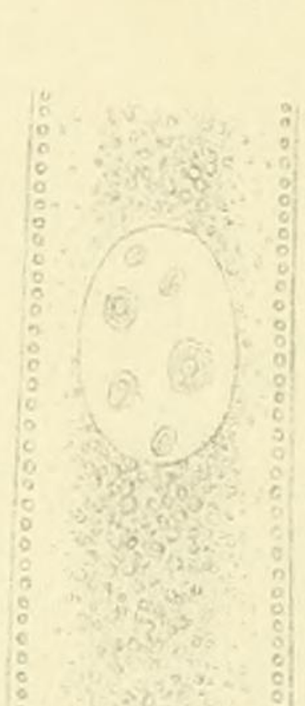

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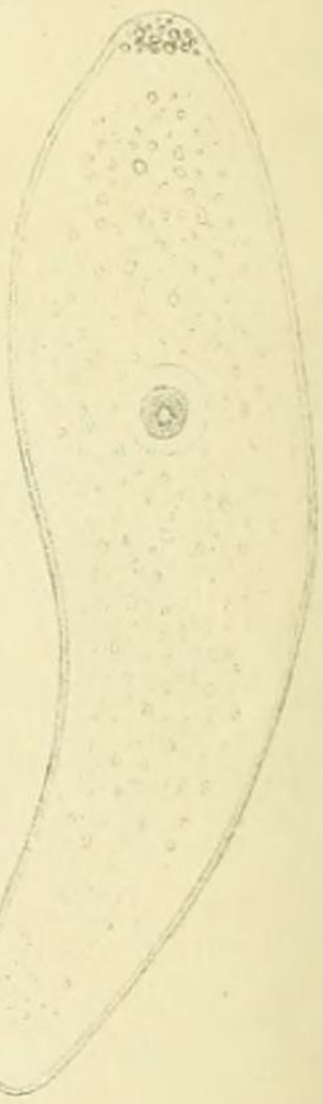

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Gregarina gigantea.