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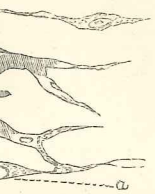

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jection forces out the coloured mass in little drops on the surface of the cord, and it seems probable that these make their exit by the *stomata* here described; but this point could not be distinctly seen. Were this clearly made out, it might at once be concluded that the stomata are the openings of the plasmatic channels; as it is, the author does not venture to pronounce a positive opinion on this point.

On a New Species *of* Gregarina *to be called* Gregarina gigantea. By Edouard Van Beneden, Doctor of Natural Science, Louvain.[1] With Plate VI.

The little organisms known as Gregarinæ have much attracted the attention of naturalists during late years. Their organisation is exceedingly simple; they consist of merely a single cell, but the history of their development, of their reproduction, and of their metamorphoses, has exercised the sagacity of a great number of eminent observers, and the profusion with which they are spread through nature has caused them to come under the notice of many naturalists. The literature relating to these inferior organisms is very rich, but, nevertheless, several points relating to their organisation and to their development are still completely unknown.

The Gregarinæ live as parasites either in the intestine or in the perivisceral cavity, or in the reproductive organs of animals belonging to various classes. They have been pointed out in the various groups forming the class of worms—from the Turbellaria (P. J. Van Beneden and Claparède) and Sagitta (Diesing), as also from the Nematodes (Walter), and the Annelids, where they are extremely abundant. The Gregarinæ of the earthworm have chiefly served as the subject for the numerous works of which these animals have been the subject. Schneider has described a species peculiar to the Holothuriæ; some are known among Molluscs, even among the Tunicata (*Salpa*, Diesing). They are very abundant in a great number of insects; they have been described as infesting Myriapods; both Scolopendra, and Julus, and the Crustacea also are sometimes infested with these minute organisms. Cavolini, who was the first to find Gregarinæ, observed them in the appendicular organs of the stomach of *Cancer depressus*; Von Siebold a long time ago made known the fine Gregarina of *Gammarus Pulex*; Lach-

[1] Read before the Academy of Brussels, and communicated by the author.

mann has described a Gregarina from *Gammarus putaneus;* and Kölliker made us acquainted with the *Gregarina Balani* which he had observed in *Balanus pusillus.*

I have the honour, on the present occasion, to present a description of a new species of Gregarina which lives in the intestine of the Lobster. It is chiefly remarkable for its great size, and it is on this account that I propose to call it *Gregarina gigantea.* Thanks to its size I have been able to ascertain some new facts relative to the organisation of these animals, and some of these observations are not without importance in relation to the theory of the cell. I have found besides some facts relative to the development of this Gregarina which will contribute to complete our knowledge relative to the history of the development of these singular parasites.

Description.—The Gregarina of the Lobster has a very much elongated form, which gives it a superficial resemblance to a Nematode. The largest individuals I have had under my observation did not measure less than sixteen millimètres in length, with a breadth of ·15 of a millimètre. The body presents nearly the same breadth throughout, excepting in its posterior part, where it diminishes progressively. The anterior extremity, on the contrary, rounded in front, is slightly swollen out into a globular form: the *Gregarina gigantea* belongs to the division which M. Kölliker characterised by these words—"Eingeschnürte Gregarinen, mit einem einfachen abgerundeten Vorderende."

The structure of these Gregarinæ is very simple; I consider them with M. Kölliker as monocellular animals; in them are found all the constituent elements of a cell, and it is impossible to find in their structure a fact which is of such a nature as to throw doubt on their monocellular constitution. Here we have, therefore, an animal cell which can reach the length of sixteen millimetres. I believe that, as far as simple cells are concerned, there are only the eggs of birds and of some other animals which surpass in their dimensions those of the cell which concerns us here. And there is this difference between the egg of these animals and our monocellular organism, that in the egg of the bird one has to distinguish a living, active part (the cicatricula), and an inert passive part (the yellow), which forms almost the whole of the egg. For this inert part of the egg-cell does not form an integral part of the cell. It is found within the cellular membrane truly (vitelline membrane), but the only living part of the cell is the cicatricula; it alone divides and gives rise to the cells of the embryo. In the Gregarina, on the contrary, all the parts

are living, active, and contractile, and I believe that, from this point of view, one may say that the *Gregarina gigantea* is the largest simple cell which is known.

In our Gregarina a membrane with a double contour can be very clearly distinguished, perfectly transparent, and without structure. It presents no opening, and its thickness is throughout the same, excepting, however, at the anterior part of the rounded bulb which terminates the body of the animal in front. This structureless membrane represents the "cell membrane" of this monocellular animal.

Under this membrane can be distinguished clearly a layer of some resistance, formed of a substance which is perfectly transparent and devoid of granulations. It is this structure which Leidy and Ray Lankester first made known, and I have been able fully to establish in this species that it is truly in this layer that the parallel striæ arise, whence results that fibrillar aspect of the animal which one remarks on using high powers. It is when the Gregarina contracts that these striæ appear, and they disappear when it is in repose. I believe, with Leidy and Ray Lankester, that the substance which constitutes this layer possesses essentially contractility, and that it is this which is concerned in the production of the movements of which the animal is capable. It represents, physiologically, the muscular subcutaneous layer of many animals more elevated in organisation, and of Nematodes especially; but instead of being formed of distinct muscular fibres it is a continuous layer of contractile,—if you like, muscular—substance.

The consistence of this layer is much greater than that of the granular matter which occupies the centre of the cellular tube. The central granular matter is very mobile; it moves about in the interior of the cavity of the cell. This is by no means the case with the transparent and contractile substance of which we were speaking; this is fixed and intimately blended with the membrane of the cell. The limits between this layer and the granular matter of the centre are, however, not sharply marked. I imagine that the density of the layer decreases from the periphery towards the central axis of the tube.

The thickness of this layer is nearly the same throughout the whole length of the animal. It augments, however, a little at its anterior extremity, principally at the point of union of the globular enlargement with the rest of the body. There, this layer sends a prolongation inwards in the form of a transverse septum to the interior of the tube, in such a way as to divide the central granular mass into two parts, of

which the one, very small, occupies the cavity of the anterior globular enlargement, and the other fills all the rest of the body of the animal. The whole cavity of the body of the Gregarina is filled with a granular matter, formed by a viscid liquid which is perfectly transparent. This holds in suspension fine granulations of a rounded form, which are formed by a highly refractive and slightly yellow matter. The quantity of granules with which the liquid matrix is charged increases with the size of the Gregarinæ, and, moreover, the opacity of the animal is greater in proportion as its dimensions are larger. This granular liquid which occupies all the cavity of the cell is very mobile, and may be seen shifting about in the interior of the membrane whilst the animal executes its movements.

It is always easy to distinguish the nucleus of the cell in suspension in the granular liquid which occupies all its cavity. It has, normally, a regular ellipsoid form, and its dimensions vary with those of the Gregarina. In the largest individuals which I have found it measured ·13 of a millimètre across its major axis, ·08 to ·10 of a millimètre across its minor axis. This nucleus presents a membrane which is perfectly distinguishable, and the cavity of the vesicle is filled by a homogeneous, colourless, and transparent liquid. This nucleus is not a solid body destitute of a membrane, as M. von Frantzius thought it. It is easy to assure oneself of this by isolating the nucleus, and subjecting it to pressure. One may then see, at a given moment, a bursting of the membrane take place, and the liquid contents of the nucleus escape by the aperture produced. The membrane of the nucleus is, however, very thin, and it is this which explains the modifications of form which the vesicle undergoes when external pressure is brought to bear on it. I have seen a nucleus affect successively the forms represented in fig. 6, *a*, *b*, *c*, *d*, in a Gregarina which glided on the glass slip placed on the microscope-stage, picking its way among the various solid contents of the intestine of a lobster; but the form of the nucleus never changed itself spontaneously, and apart from the influence of external causes.

The most important fact of all that are put out in this notice concerns the spontaneous apparition and the disappearance of the nucleoli of the nucleus in a very short space of time. If one of these Gregarinæ of moderate size is observed, the nucleus is seen at first provided with a single nucleolus, presenting, some seconds later, a great number of little refracting corpuscles, of very variable dimensions, which are also nucleoli. Some of these enlarge considerably, whilst

the primitive nucleolus diminishes in volume little by little, finally disappearing. The number of nucleoli varies at every instant. Some disappear whilst others are forming; they commence in the form of a minute point scarcely perceptible. This point grows to a certain limit; it becomes a veritable corpuscle, formed of a homogeneous, highly refractive substance, then the corpuscle diminishes in volume; it refracts the light less and less; finally it disappears. It even happens that all trace of nucleolus disappears in the nucleus, and some instants later one or several nucleoli can be distinguished there, which undergo afresh all the variations which I have just described. This fact of the successive apparition and disappearance of the nucleoli in a nucleus of a cell, and the modifications which occur in the nucleus as to number, dimension, and character of the nucleoli, has never yet been pointed out to my knowledge. It appears to me to have a great importance in relation to the cell theory. The idea of the existence of a membrane round these little bodies (the nucleoli), and of their vesicular nature, is by no means reconcilable with the rapidity of their formation, and the modification which they undergo in the course of some minutes; and if it is demonstrated that the membrane is not an essential part of the cell, and that the *nucleolus* is sometimes, if not always, devoid of membrane, may it not be presumed that the *nucleus* of a cell is not necessarily a vesicle, and that, contrary to the generally received opinion,[1] a nucleus of a cell may be equally devoid of membrane?

The Gregarinæ move, and three kinds of movements may be distinguished in them:

1st. They present a very slow movement of translation, in a straight line, and without the possibility of distinguishing any contraction of the walls of the body which could be considered as the cause of the movement. Further, it is very difficult to account to oneself for the cause of this movement of translation, at least to admit—what is difficult to demonstrate—that the Gregarina acquires an adherence to the surface on which it moves. Invariably the appearance of this movement recalls completely that of the Turbellaria; but it is impossible to distinguish the least trace of vibratile cilia on the surface of the body of these animals, even with the highest magnifying powers.

2nd. The Gregarina of the Lobster presents another kind of movement, consisting in the lateral displacement of every part, taking place suddenly, and often very violently, from a more or less considerable part of its body. Thus the posterior part of the body may be often seen to throw itself out laterally

by a brusque and instantaneous movement, forming an angle with the anterior part. At the vertex of the angle the body then presents a regular fold, and the animal forms a broken line. Folds can be thus formed in a great number of points, more or less approximated; and it results from this that the animal can describe a spiral, if all the folds occur on the same side, or twist itself about in various ways. It is probably due to the contractility of the transparent subcuticular layer that the Gregarina has the power of executing these movements.

3rd. In consequence of the different contractions which are produced, and by the action of which the Gregarina folds itself so as to form broken lines, the granular liquid which occupies the cavity of the cell is seen to move, and the granulations to shift about in the interior of the body of the animal.

I have found as many as twenty-five Gregarinæ in the intestine of a single Lobster, and at certain times every Lobster presents this parasite. I have observed them in the months of May, of June, and of August, in lobsters coming from the coasts of Norway. It is probable that they will be found equally in those of the coast of Brittanny. I have found no traces of these parasites on Lobsters kept for a long time in captivity in the piscicultural parks at Ostend. Is it the same with the lobster confined in these parks as with the animals of our zoological gardens and the fishes of our aquariums? May the loss of their parasites be due to their captivity?

At the end of the month of last September I examined a great number of lobsters freshly arrived from Norway, with the object of refinding this beautiful Gregarina. Not a single one contained in its intestine the parasite I was seeking; but I perceived that all presented, in the walls of the rectum, little white grains of the size of the head of a small pin. These were the *cysts* of Gregarinæ, situated beneath the epithelium; and, what is remarkable, the cysts were disposed one by the side of another, forming little rectilinear series of 3, 4, 6, and even of 7 cysts.

By the beautiful researches of Von Siebold, Henle, Kölliker, Bruch, Stein, Lieberkuhn, and other eminent naturalists, the evolution of the Gregarinæ has been in great part elucidated. We now know that a single Gregarina may become encysted, and that the frequent fact of the existence of two granular masses in the same cyst is explained by the division of the contents of the encysted Gregarina, and not by the conjunction of two Gregarinæ in one and the same cyst, as Stein and other naturalists had supposed. It is

chiefly to Bruch, Lieberkuhn, and A. Schmidt that the honour of demonstrating this fact belongs. It is known also that after a kind of cleavage of the granular masses of the cysts these masses become transformed into little vesicles, which in turn give rise to the psorosperms or pseudo-navicells. Lieberkuhn has shown that the psorosperms produce amœboid forms, and he thinks these amœbæ themselves are developed into Gregarinæ, or give origin to Gregarinæ. But this last phase of the evolution of these little beings is still problematical, and will require further serious investigation. But what is perfectly established now, thanks to the labours of Stein, Kölliker, Lieberkuhn, and several other naturalists, is that there exists no relation of filiation whatever between the Gregarinæ and the Filariæ, and that the opinion maintained on this question by Henle, Bruch, and Leydig, must be finally abandoned.

I have not been able to observe these different phases of the evolution of the Gregarinæ of the lobster; I have not even succeeded in establishing the transformation of the granular masses of the cysts into psorosperms; but I have fully recognised, in confirmation of the observations of Bruch, Lieberkuhn, and A. Schmidt, that the contained granular matter of the cysts is at first a simple sphere, always devoid of nucleus, and that the two rounded masses which one observes frequently in the cysts come from the first in consequence of a sort of cleavage, in fact from a division. A groove appears at first at the surface of the granular sphere, into which the wall of the cyst immediately is applied. This fissure advances progressively towards the centre of the sphere, and eventually divides it into two parts. Each of them has the form of a hemisphere, and they are applied to one another by their plane surface; but soon the diameter of the cyst increases, a space which is filled with a limpid, colourless liquid as fast as it forms appears between the wall of the cyst and the surface of the two granular masses, which lose little by little their hemispherical form, becoming gradually rounded. The diameter of the cyst continues to increase, and the two masses at last become each a perfectly spherical globe. I have seen all these changes take place on the stage of the microscope. But what has not yet been observed is, that after this division of the primitive sphere into two spheres, the wall of the cyst formed by several concentric layers of a diaphanous material decomposes into a soft granular matter, whilst each of the two globes surrounds itself with a new membrane. Soon the traces of the envelope

[1] Kölliker's 'Elements of Human Histology.'

of the primitive cyst can be observed, but very obscurely, and the two globes of the second generation only are distinguishable, surrounded by a common granular substance. The globes grow little by little, at the same time that their envelope thickens. Henceforward each of them is to be regarded as a new cyst, whose contents will divide in their turn, to give rise to two new globes or spheres, which will become cysts of the third generation after the absorption of their walls. The upshot of this is that the cysts of the Gregarinæ can multiply by division before giving rise to psorosperms, and the manner in which this phenomenon presents itself recalls completely the multiplication of the cells of cartilage. There, too, the cells multiply by division, and the capsules of the cartilage change little by little their character, and are transformed into intercellular substance. The granular globes of the Gregarina-cysts may be compared to cartilage cells, and the granular matter which surrounds them to the intercellular substance of the cartilaginous tissue. In this mode of multiplication of the cysts the reason of their rectilinear arrangement in the walls of the rectum of the lobster is at once obvious. It is hardly necessary to add that these observations on the multiplication of the cysts give the explanation of the fact, so often observed but not yet interpreted, of the existence of two granular masses in one and the same Gregarina-cyst.

It appears from what precedes that in certain circumstances—perhaps at a fixed period of the year—the Gregarinæ, which were living freely in the intestine of the lobster, migrate into the rectum. There they become encysted, after having penetrated under the epithelium of the intestinal walls, and these cysts multiply by division. It is not possible to doubt that, after a certain time, the cysts are resolved into psorosperms; but it has yet to be found out what becomes of these psorosperms, as also how the Gregarinæ arrive in the intestine again, and under what form they first appear there.

Note.—I may take this opportunity of remarking that the view entertained in the very interesting paper of my friend Dr. Van Beneden, as to the encystment of the Gregarinæ, can hardly be considered as yet demonstrated. It may be fully admitted that single Gregarinæ do become encysted; but there are facts which lead to the supposition that two are *usually* thus encased, and that the formation of pseudo-navicells thus presents a remarkable approach to the conjugation of Algæ, as remarked by Huxley. Not only are two masses most commonly seen in a cyst, but in a spherical cyst of *Gregarina Blattarum* I found two *nucleated* Gregarinæ ('Quart. Journ. Microsc. Sci.,' vol. iii, new ser., pl. vii, fig. 17). The habit of attachment

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of two individuals head to tail, as it were, common in many bilocular Gregarinæ (loc. cit., figs. 18, 19), and in *Monocystis* also (fig. 6), and *Zygocystis cometa* of Stein, indicates a tendency to conjunction which would favour the enclosure of two individuals in a single cyst. At the same time it is only right to state that this head-and-tail-attachment is commoner in small than in large specimens of Gregarina, and may be due possibly to some process of division occurring without encystment, though this is quite hypothetical.

E. Ray Lankester, Ed. 'Q.J.M.S.'

The Kinship *of* Ascidians *and* Vertebrates.

In 1867 Kowalevsky published, in the transactions of the Imperial Academy of St. Petersburg, some observations which are more profoundly interesting than anything of the kind which has appeared of late years, since, if correct, they indicate distinctly a bridge over the chasm, supposed to separate Vertebrates from all other animals. They prove the existence of *a bicavitary structure*, of a neural tube and a visceral tube, separated by an axial cartilaginous rod, in the early stages of development of Ascidians, an arrangement previously believed to be essentially characteristic of Vertebrates.

We are indebted to Professor Michael Foster for the following account of Kowalevsky's paper:—

The observations of Kowalevsky were made on several Ascidians; but in all the processes are remarkably similar. The earlier stages were chiefly studied in *Phallusia mammillata*, the metamorphosis of the larva into the sessile form in *Ascidia intestinalis*.

The ova of Ascidians possess, on leaving the parent, a somewhat complex structure. Each consists of a *vitellus*, devoid of any proper vitelline membrane, and surrounded by a layer of gelatinous material, in which are strewed yellow nuclei or cells. These nuclei or cells are small homogeneous vesicles, very similar in appearance to the blood-corpuscles of the higher vertebrata, and are probably purely material elements derived from the follicles in which the ova are developed. Their subsequent history is remarkable, inasmuch as they become transformed into the "white cells" of the mantle. Outside this gelatinous layer is a hard membranous capsule, which in turn is studded with peculiar structures, varying exceedingly in the various species. These, however, are of no importance and speedily disappear.

The vitellus varies a good deal. In *Phall. mammillata* it is highly transparent and refractive; in *Asc. intestinalis*, *Cynthia*, &c., it is opaque, brown or dark yellow. No nucleus