Researches *on the* Development *of the* Gregarinæ. By Edouard Van Beneden, D. Sc., Professor of Zoology and Comparative Anatomy in the University of Liege.

The development of the Gregarinæ has been the object of a great number of investigations, and has exercised the sagacity of a number of distinguished observers. Nevertheless, at the present time it is not fully elucidated. The relation existing between the Gregarinæ and the psorospermic vesicles was first perceived by von Siebold,[1] Henle,[2] and von Frantzius,[3] and definitely demonstrated by the beautiful researches of Stein,[4] Kölliker,[5] and Lieberkühn.[6] It appears well established that, although sometimes two Gregarinæ conjugate, fusing subsequently into a common mass in one and the same cyst (Stein), yet the conjugation does not necessarily precede the encystment, and often a single Gregarine transforms itself into a vesicle (Bruch, Frantzius, Leuckart, and myself), to give birth, quite as in the first case, to a great number of psorosperms. There are certain Gregarinæ in which the conjugation is never observed; others which one finds always apposed (Zygocystis, Didymophyes), either by their analogous extremities, or by their opposite extremities (Gregarinæ).

The granular contents of the cysts may divide, and the capsule common to the two globes thus produced may disintegrate and become transformed into a viscid and granular substance, after a new membrane has developed round each of the new globes of the second generation. These again may divide in their turn, and there will be thus presented series of cysts. enclosing some a single granular mass, others two similar masses enclosed in a single capsule. All these cysts, which may be compared as far as their mode of multiplication is concerned, to the corpuscles of cartilage, are held in suspension in a common fundamental material resulting from the disintegration of the original capsules (Edouard Van Beneden).[7] In this manner we can explain the presence of those linear series of cysts which are met with in the thick-

[1] Von Siebold, 'Beiträge zur Geschichte wirbelosser Thiere,' 1839, p. 69.
[2] Henle, 'Müller's Archiv,' 1845, p. 574.
[3] Von Frantzius, "Observationes quœdam de Gregarinis," Berol, 1848.
[4] Stein, 'Müller's Archiv,' 1848, p. 204.
[5] Kölliker, 'Zeitschrift fur wiss. Zool.,' t. i, p. 1.
[6] N. Lieberkühn, "Evolution des Gregarines," 'Mem. Acad. Roy. de Belg.,' c. xxvi.
[7] Edouard Van Beneden, 'Quarterly Journal of Microsc. Science,' New Series, No. XXXVII, 1870.

ness of the walls of the intestine of the lobster or, indeed, the existence of strings of vesicles bound together by a homogeneous substance, like those which MacIntosh[1] found in *Borlasia octoculata.* In this way, also, is explained the fact that the cysts are often much smaller than the Gregarinæ to which they have to be referred. We know also through the researches of Stein,[2] of Bruch,[3] and, above all, of Lieberkuhn,[4] what is the mode of formation of the psorosperms at the expense of the granular masses; but the question as to the manner in which the psorosperms are developed later into Gregarinæ, remained an enigma until the day when Lieberkühn[5] established in a decisive manner that a body exhibiting amœboid movements comes out of the psorosperms, and moves itself in the same way as the corpuscles which occur in suspension in the blood of the earth-worms, and which were observed and described for the first time by Morren.[6] According to Lieberkühn the globules of the perivisceral liquid of the earth-worm are true Amœbæ, which must be connected with the development of the Gregarinæ. We find in this cavity structures which present characters intermediate between those of Amœbæ and those of Gregarinæ; and Lieberkühn admits the direct transformation of the Amœbæ into Gregarinæ. But it is very necessary to remark that the exactitude of the observation has been contested by Schmidt,[7] and at the end of his work Lieberkühn says himself: "I am far from maintaining that all the Amœbæ are born from psorosperms, or that all the Gregarinæ develop from Amœbæ.[8] The observations which I have had the opportunity of making on the successive phases of the development of the Gregarinæ of the lobster serve to fill up the gaps which the history of the development of these mono-cellular beings hitherto presented, and to elucidate some points which have remained obscure in this evolution. I have been able to follow step by step in the *Gregarina gigantea* all the successive transformations of the little protoplasmic mass which

[1] "On the Gregariniform Parasite of Borlasia," 'Quart. Journ. of Mic. Sci.,' 1867.

[2] Stein, 'Müller's Archiv,' 1848.

[3] Bruch, 'Zeitschr. fur Wiss. Zool.,' Bd. ii, p. 110.

[4] Lieberkühn, loc. cit.

[5] Ibid., p. 16, "Ueber die Psorospermien," 'Müller's Archiv,' 1854; "Notice sur les Psorospermies," 'Bull. de l'Acad. Roy. de Belg.,' c. xxi, No. 7.

[6] Morren, "De Structurâ Lumbrici terrestris," 'Acta Akad. Gandar,' 1825, p. 170.

[7] Schmidt, "Beiträge zur Kenntniss der Gregarinen," 'Abhandl. der Senkenberg Gesellschaft,' 1854.

[8] Lieberkühn, "Evolution des Gregarines," p. 27.

comes out of the psorosperms, up to the complete Gregarina, which may attain a length of sixteen millimeters.

In the month of May of the past year I found in the small intestine of the lobster little protoplasmic masses entirely naked, devoid of nucleus as well as of membrane, and which, in respect of their finely granular aspect, their continual changes of form, and their entire constitution, may be compared with the *Protamœba agilis* or the *Protamœba primitiva* of Haeckel. They differ from these solely in the fact that fine molecular granulations are met with even at the periphery of the body, and in the fact that the forms scarcely depart from those of a globular body more or less irregular at its surface (Pl. XII, figs. 1, 2, and 3). I have never seen pseudopodia projected to a distance.

As we shall see, these little protoplasmic globes are the point of departure of the development of the Gregarinæ; they are distinguished from true *Amœbæ,* which always possess a nucleus, and often also a contractile vacuole by the absence of both one and the other. From a morphological point of view these little protoplasmic globes, devoid of any nuclear structure, are true Gymnocytods.

By the side of these little living masses devoid of all organization, we find here and there other little protoplasmic globes, which only differ from the first in the fact that they have lost the faculty of moving themselves and of changing their form (fig. 4). On the surface is observed a somewhat thick layer of a brilliant protoplasm, highly refringent, perfectly homogeneous, and absolutely devoid of all granulation, whilst the central protoplasmic mass holds numerous molecular granulations in suspension of which some appear as points of the extremest tenuity, whilst others have dimensions appreciable by the microscope. These last granules are probably only nutritive elements. I have been able to establish, as will be seen further on, the greater fluidity of the central granular matter; but the line of demarcation between the peripheral perfectly homogeneous zone and the central granular mass is not sharp and defined; the small protoplasmic mass is not delineated by a membrane properly so called, but rather by a layer of condensed protoplasm, if one may thus term that which acts as a membrane in such a way as to preserve the spheroidal form of the cytod.

In consequence of this tendency to the separation of the protoplasmic mass into two distinct layers, a cortical substance and a medullary substance, these globes rise to a position above the Monera. The latter never exhibit this separation, although it is general in the other lower Protista.

By the side of these sharply circumscribed and entirely motionless globular forms are to be observed certain cytods quite similar to those just described, excepting that they carry either one or more often two prolongations in the form of arms, which I should call pseudopodia, if they did not exhibit entirely peculiar characteristics which separate them very obviously from the pseudopodia of the Monera, the Foraminifera, and the Radiolaria. I should be more inclined to compare them to the mobile appendage of the Noctilucæ, chiefly on account of the constancy of their form and of the nature of their movements. These cytods with prolongations I shall call *generating cytods.*

Firstly, as to the characters which were presented by the prolongations of the cytod which I have represented in figs. 6, 6″, 6‴. The prolongations to the number of two are inserted at a little distance one from another on the same hemisphere. They are not only of unequal length, but they differ notably from one another in all their characters. That which is the shorter is at the same time the thinner, more delicate, with paler outline, and almost completely devoid of mobility. If in a displacement which the corpuscle undergoes either in virtue of its own vitality, or in consequence of a current which carries it along—this arm comes in contact with a resisting body, it becomes reflected, bent back, and I have seen this bend, produced accidentally, persist during more than three quarters of an hour. The protoplasm which constitutes this arm is pale but slightly refringent, very finely granular, and almost devoid of granules of appreciable dimension. I consider these last granules as being nutritive, combustible elements; and the almost complete absence of mobility in this arm may be explained by this fact that the combustion—that is to say, the liberation of the force necessary for mechanical movement—does not operate except with extreme slowness in this inert arm.

The other arm is notably longer, and also a little broader; its contours are darker, and the protoplasm which compose them is more refringent. Besides the almost imperceptible molecules which distinguish the protoplasmic matter, in this arm opaque granules are remarked. These granules are chiefly abundant at the slightly enlarged and very mobile extremity of this arm. It is thus very granular, and this character is sufficient to enable one to distinguish, at first sight, the second arm from its neighbour. It differs further from the first-mentioned prolongation by its extreme mobility. Two modes of manifestation of this mobility are distinguishable. Firstly, the arm can vibrate, very much as does the "lash" of the Noctilucæ. In the second place, a peculiar mode of movement is observed, which has probably, as end and result, the progressive elongation of the arm. The extremity of the prolongation is spontaneously reflected, and then one observes the reflected part gradually elongating; whilst at the same time the point of flexion gradually approaches the body of the cytod (fig. 6′, 6″). The straight part of the arm appears to contract at the same time, and a very slight transverse striation is seen to appear in this part of the prolongation (6″ and 6‴). Then suddenly and briskly the entire arm recovers its position, as though it were made of some eminently elastic substance; and at the same time the granular and fluid protoplasm of the centre of the cytod rush, forming a sort of current, into the interior of the arm. It is clear that these movements, which succeed at short intervals, ought to result in the progressive elongation of this arm. I have been able to establish the fact of this gradual elongation by observing the same cytod during several hours. The only other modifications which appear in the character of the prolongation are the pinching in of its basilar portion, and the accumulation of nutritive granules in its terminal portion, which I shall designate "*cephalic.*"

When the mobile arm has attained a certain length, it detaches itself from the body of the cytod, and, becoming free, executes undulatory movements in the manner of a Nematoid worm. I have not seen this arm actually detach itself from the cytod, but quantities of these filaments are found moving freely in the intestine by the side of the cytods, on to which they are also found fixed by one of their extremities.

To elucidate completely this part of the evolution of the Gregarinæ, we ought yet to inquire whether all the body of the cytod is not employed in the elaboration of one of the free mobile filaments.

It follows from the facts which I am about to enumerate that one and the same cytod gives rise to two filaments, destined each to become a Gregarine, that is to say, that two Gregarinæ always are produced from a single cytod, which, on this account, I have called the "*generating cytod.*" The first to attain maturity is the mobile arm, it detaches itself from the cytod before the second—the right arm—attains the phase of mobility. On the other hand, all that remains of the body of the cytod is employed in the maturation of this second arm.

Among the cytods with two arms, one inert, and the other extremely mobile, some cytods are found which have only a single prolongation. Of these, some possess an inert arm, presenting all the characters of that which we have described above (fig. 9); others have, on the contrary, a mobile arm, and are devoid of the inert arm (figs. 10, 11, and 12). It is to be observed that in this latter case the body of the cytod has smaller dimensions than belong to those cytods with two prolongations. Among the cytods with only one prolongation, some are found with the arm presenting characters intermediate between those of the mobile and those of the inert arm. It results clearly enough, from the comparative examination of these various forms, that the inert arm of the two-armed cytods is destined to become in turn a mobile arm; after the original mobile arm has become detached from the cytod. The inert arm is then merely a still younger pseudopod than the mobile arm, destined to take on at a certain epoch the characters of the latter.

The fact which is regularly observed in the two-armed cytods, that the mobile prolongation thins away progressively at its basilar portion (figs. 7 and 8) when it has attained a certain length, proves that the prolongation tends to detach itself from the cytod; and this conclusion is confirmed by the existence of cytods, having only an inert prolongation.

But this now requires for its development all the rest of the body of the cytod. That is at least the conclusion which appears to be deducible from the occurrence of free filaments, having a vesicular enlargement at their posterior extremity; although no narrowing is observed between the body of the filament and the terminal enlargement.

These facts lead to the following conclusions:

1st. Each cytod gives rise to two filaments, destined to develop each into a Gregarine; but the development of the two processes takes place *successively*.

2nd. The filament which develops first attains its maturity, and detaches itself from the body of the cytod, before the other proceeds with its development, and before it attains the phase of "the mobile arm."

3rd. This latter does not detach itself from the cytod; it develops by gradually absorbing the body of the cytod, as the embryo of a vertebrate absorbs little by little the contents of the vitelline vesicle. It passes successively through the same phases of development as the mobile filament.

The protoplasmic filaments thus developed from the cytod move in the intestine with extreme activity (figs. 13 and 16). The only movements which they execute are undulatory movements, in every respect comparable to those of the Nematoid worms. In consequence of their resemblance to the Nematoid worms, I have termed these protoplasmic filaments *pseudo-filaria.* If these vermicular filaments had not been seen developing under one's eyes at the expense of a cytod, it would be difficult to believe that they were not young Nematoids. We know, in fact, that it is always extremely difficult to distinguish cellular elements in these little worms, and often it is not possible to detect, except with great trouble and in a very obscure manner, any trace of a digestive tube. It is the "pseudo-filaria" of the Gregarinæ of the earthworm in all probability which have been taken for young Nematoids, and we have here clearly enough the explanation of the very erroneous opinion which has prevailed, according to which the Gregarinæ are only a phase in the development of the Nematoid worms. This opinion has been defended by naturalists of the first rank, such as Henle,[1] Bruch,[2] Leuckart,[3] and Leydig.[4]

In 1845 Henle expressed himself thus as to these relations between the Gregarinæ and the Anguilluloid parasites of the earth-worm:[5]—"It has become my conviction that the Gregarinæ of the earthworm stand in the same relation to the Anguillula-like Entozoa of the same animal, as, according to Miescher, do the rigid chrysalids in the intestines of many fish to the *Filaria piscium.* I have detected a series of transition forms between Anguillula and the Gregarina, of which some have been already described by Dujardin[6] as *Proteus tenax,* and by Sarissay[7] as *Sablier proteiforme.* The Anguillula becomes stiff, and its intestine breaks up within the outer skin into a granular mass, whilst the form of the body is changed from an elongated into an oval or spheroidal form."

Whilst Bruch and Henle admitted the possibility of the transformation of worms similar to young Filariæ into Gregarinæ, Leydig, according to observations made on the parasites of a Terebella, was more inclined to believe in a metamorphosis in the other direction—that is, from Gregarinæ into Nematoids.

It is not to be doubted that it is the analogy between the forms and the movements of these protoplasmic filaments, which I have just described under the name of "*pseudo-filaria,*" with young Nematoids, which has caused these

[1] Henle, 'Müller's Archiv,' 1845.
[2] Bruch, 'Zeitschrift fur Wiss. Zool.,' t. ii.
[3] Leuckart, 'Archiv für Phys. Heilkunde,' xi, 1852, p. 429.
[4] Leydig, 'Müller's Archiv,' 1851.
[5] In his 'Jahresbericht für Histologie,' 1845.
[6] 'Annales des Sciences Naturelles,' 2nd series, t. iv.
[7] Ibidem, 2nd series, t. vi.

errors; and it clearly results from their mode of formation that they are no more Nematoids than are the whales—fishes.

We have now to set forth the modifications which the pseudo-filaria undergo during their transformation into Gregarinæ.

The pseudo-filaria, simple threads of protoplasm, attenuated at one extremity, slightly swollen at the other or cephalic extremity, which is always richly charged with refringent granules, move about freely in the intestine during a certain time. Then the movements languish, and the length of the body diminishes little by little at the same time as the breadth, especially in the anterior portion (figs. 13 to 18). Soon all undulatory movement ceases, and the pseudo-filarium becomes quiescent. This is at least the conclusion derivable from the comparative examination of individuals, which are found in great numbers in the intestine. Some are seen which are very long, very thin, and extremely agile, side by side with others which are rigid, shorter, and obviously broader, especially in the anterior part of the body. At the same time there is seen to appear, near the middle of the long axis of the body, a dark circular spot, which is formed of a more refringent matter than the protoplasm (figs. 15 to 17). The dimensions of this spot vary very slightly, but its limits become more distinct. This is the *nucleolus* which appears directly in the protoplasm, probably as the result of a deposit, around an ideal point, of certain peculiar chemical elements, previously diffused in the protoplasmic mass.

I can only explain this formation to myself by comparing it, as Schwann has done, in describing the free formation of cells in a blastema to a crystallisation. In the same way as given chemical elements in solution in a liquid can dispose themselves around a fictive point, so as to form a crystal, so here the elements of the nucleolus, diffused at first in the protoplasm, aggregate to form a *globular* body, a veritable nucleolus.

The cell taken in its entirety appears to be an organic combination, comparable to those mineral combinations formed by crystals imbedded one in another.

The nucleolar layer is of a different chemical nature from that of the nuclear layer, just as this also itself differs from the cell-substance. This nucleolus is formed of a substance which differs from the primitive protoplasm by its physical and chemical properties, and these elements of the nucleolus have evidently a special function (as yet unknown) to perform in the life of the cell.

These elements, primitively scattered in the protoplasm, unite into a small distinct corpuscle, in virtue of the law of localisation, all the while continuing to perform in the economy of the organism the same functions as when they were scattered in the nucleo-cellular layer. It is this same law which is apparent in the progressive complication of any cell whatever, of a muscular cell, for example, when the myosin, at first scattered in the protoplasm, accumulates at a special point of the cell, in which one can then distinguish a protoplasmic body, and a part formed of contractile substance.

It is the same law, again, which presides over the formation of organs by division of labour; the biliary cells, scattered in the lower animals among the epithelial cells of the digestive-tube, continue to fulfil the same function when they have become united in such a way as to form a particular organ—the liver—which presides over the secretion of the bile.

All around the nucleolus can soon be distinguished a perfectly transparent zone, free from molecular granulations; but it is not possible to determine the limits of this zone (figs. 18 and 21).

The pseudo-filarium continues to shorten itself, and the protoplasmic filament soon becomes a body of more or less oval form (figs. 20 to 22), presenting often towards its middle a slight attenuation (sometimes the pseudo-filaria take on the biscuit-form, fig. 19). This body is limited by a dark contour, except at its anterior extremity, where this contour is much more pale. In some individuals the protoplasm bulges out at this point in such a way as to form either a discoid flattened eminence (figs. 19 and 20), or a hemispherical protuberance (figs. 21 and following). Sometimes this is situated in the main axis of the body, at other times it is placed a little on one side (fig. 25). It is in this anterior, somewhat prominent part, that the refringement-granules are always found in greatest number. They are to be distinguished also, but less numerously, in all that portion of the body situated in front of the nucleus. But it appears that all these granules have a tendency to pass to the anterior extremity of the body, and accumulate in the terminal enlargement.

Beneath the dark outline which demarcates the body of the young Gregarina is found a homogeneous and transparent layer of protoplasm, in which not a trace of granulation is discernible. The medullary substance alone is finely granular (figs. 20 and following).

The nucleolus is always very distinct; it is a refringent corpuscle, always rather large, but with dimensions varying in different individuals. In some there is to be observed in the nucleolus a small vacuole (figs. 25 and 26).

The layer of the nucleus tends to acquire more and more sharply defined limits; in all the nucleolus is surrounded by completely transparent zones, of a very variable thickness, and more or less sharply delineated (figs. 20, 22, and following). In small Gregarinæ, of the same size, very notable differences are to be observed in this respect. By the side of small Gregarinæ, whose nucleolus is surrounded by a transparent thin layer sharply circumscribed, others of the same size are found, where the nuclear layer is, on the contrary, thick, but with very vague outline. The position of the nucleus is not more constant than its dimensions. Sometimes it is situated in the middle of the body, and in its narrowest part ((fig. 19); at other times it is situated in front, in the broadest part of the cell; more rarely it is situated in its posterior half.

We have henceforward under our eyes a young, well-characterised Gregarina, which has only to grow in size to become that fine cell of sixteen millimètres in length, which well justifies the name of *Gregarina gigantea,* which I have given to it.

The body elongates progressively, assuming more and more clearly the shape and the characters of a cylindroid sac, a little enlarged only in its anterior fourth. But the posterior part of the body elongates more rapidly than that which is situated in front of the nucleus, and from this it follows that the latter, which in all the young Gregarinæ occupied generally the middle of the body, exhibits itself now constantly at the line of junction of the anterior third of the body, with the two posterior thirds, as in the adult (fig. 26 and following).

The little enlargement of the anterior extremity of the body, which is often hemispherical, has also developed itself; only it is no longer circumscribed by a so clearly marked form. It is continuous almost insensibly with the rest of the body, from which it is no longer separated, except by a slight constriction (26 and 27).

The refringent granules which have accumulated in this terminal enlargement have agglutinated themselves into a mass separated from the granular protoplasm of the axis of the sac by a perfectly transparent layer of protoplasm. This layer forms, in the interior of the sac, a transverse partition, which divides the cavity of the sac into two chambers—the one, anterior, very small, is filled with refringent granules, which were at first scattered in the anterior portion of the body, at the period when the two chambers were not separated; the other, posterior, embraces the larger part of the body of the cell (fig. 26 and following). It is remarkable that from the very commencement of the development of the protoplasmic filament on the surface of the "generating cytod," its free end was more charged than the rest of the body with opaque granulations. The cephalic extremity or "anterior compartment" of the body of the adult Gregarina was already indicated.

The partition between the two chambers which is in continuity with the hyaline protoplasm of the periphery of the body differentiates itself little by little as it gets rid more and more completely of molecular granulations.

Another modification which manifests itself in the constitution of the body of the Gregarina is the more and more complete delimitation of the most external portion of the protoplasm, which soon appears under the form of a membrane with double contour. This membrane, which becomes more and more distinct, can be compared to the cuticle of the Infusoria, and, consequently, may be distinguished as the cuticular membrane.

At the same time as the body elongates, it broadens notably, and the quantity of semi-fluid granular protoplasm which fills the greater part of the sac augments rapidly whilst the external protoplasmic layer, always hyaline and resistent, augments but slightly in thickness.

The nucleus takes on a perfectly regular oval form; it enlarges at the same time as the cell, and it surrounds itself with a membrane, the presence of which, indicated by a double contour, can be demonstrated by making the nucleus submit to a transverse pressure. When the pressure has attained a certain degree of intensity, the nuclear membrane becomes rent (figs. 28 and 29).

I have not recognised in the young Gregarinæ the successive disappearance of the nucleoli, so easy to observe in the adults. In the young Gregarinæ the nucleus never encloses but a single large nucleolus, in which very generally is observed a small vacuole.[1]

To complete this work it is necessary to compare the observation which I have above recorded with the most recent researches, of which the lower organisms have been the

[1] Since the publication of my first work on the Gregarina of the lobster (this Journal, January, 1870), where I announced for the first time this fact of the successive disappearance and reappearance of the nucleoli in the nucleus of a cell, M. Svierczweski, Assistant in the Physiological Laboratory at Kiew, has made known analogous facts observed by him in the ganglionic cells of the frog.—*Centralblatt für die M. W.*, 1869, No. 41.

object, and consider them from the point of view of the cell theory and of the protoplasm theory.

Professor Ernst Haeckel[1] has made, in these last few years, a discovery of great importance, in demonstrating the existence of a whole series of lower organisms, devoid of all organisation, of all appreciable structure, of all determinate form. In all phases of existence they consist of simple little masses of protoplasm, without any membrane, and without any nucleus. He has formed them into a special group, which he has called the group of the Monera. These beings are not only the simplest organisms known, but they are the most simple beings which one can imagine. Their existence demonstrates that there are beings to be met with simpler than the monocellular organisms. In fact, the Monera are not cells; life manifests itself in small masses of albuminoid material, without form and without organisation. One cannot distinguish in them any differentiation of parts, any organ, any trace of nucleus. Cienkowski[2] had observed and described, nearly at the same time as Haeckel, organisms of this group—the Protomonas and Vampyrella; but it is Haeckel who first demonstrated that it is necessary to separate these organisms from all the groups hitherto known; it is he who has demonstrated their extreme importance from the point of view of general morphology; it is he who has proposed to constitute the group of Monera, and who has made known the greater part of the creatures belonging to this group.

The Monera, not being cells, Haeckel proposes to distinguish them, histologically, under the name of Cytods, and he distinguishes the Gymnocytods and the Leptocytods according as these little living masses are devoid of or provided with an enveloping membrane.

The substance which constitutes these organisms is identical, as far as its physical characters are concerned, with the sarcode of the Rhizopods; this is itself nothing more than that protoplasm which one finds in every living organic element, cell, or cytod, whether belonging to a protiston, a plant, or an animal.

From the chemical point of view there ought to be a difference between the protoplasm of the Monera and Cytods generally and the protoplasm of cells. The sarcode of the

[1] E. Haeckel, "Der Sarcode Körper der Rhizopoden," 'Zeitschrift für wiss. Zool.,' 1865, Bd. xv. 'Generelle Morphologie der Organismen,' 1866.

[2] Cienkowski, 'Beiträge zur Kenntniss der Monaden;' Max Schultze's 'Archiv für Mikr. Anat.,' 1865, t. i.

Rhizopods, which appears to be identical with the protoplasm of cells, differs from the protoplasm of the Monera and Cytods generally, in that the chemical elements of the nucleolus and of the nucleus diffused uniformly throughout the entire substance of the body of these latter, occurs in the cellular beings separated into distinct organs, the nucleolus and nucleus.

The protoplasm of the Monera, from the chemical and physiological point of view, represents the protoplasm of cells plus the nuclei and the nucleoli. The two substances being different in spite of the identity of their physical characters, and the apparent similitude of their physiological properties, there is ground for distinguishing them, and to distinguish them efficiently it is desirable to designate them under different names. Haeckel has remarked, with reason,[1] that the word "protoplasm" signifies not *formative* substance, but much more *formed* substance (το πλασμα). The word plasson (το πλασσον) would serve better to designate the material which is *par excellence* formative, that which constitutes those living beings devoid of organization—the monera and the cytods. I propose the introduction of this word *plasson* into the scientific vocabulary, to designate the substance of cytods, which is capable of becoming, either in ontogenetic course or in phylogenetic course, mono-cellular elements after that the chemical elements of the plasson have been separated to constitute a nucleolus, a nucleus, and a protoplasmic body, and to preserve the word protoplasm to designate the substance of the body of a *cell*.

Protoplasm is really relatively to plasson a formed material, which has undergone a first differentiation by the formation of the nucleus and nucleolus. The plasson, on the contrary, is the formative substance *par excellence*, at the expense of which have been formed in due phylogenetic order all living beings.

Plasson differs from the "germinal matter" of Beale, in that Beale gives this name to the living elements of the cell, whether the nucleus be differentiated or not. Plasson cannot exist in a cell; it ceases to exist from the moment when the cellular element has become characterised as such; it is then broken up into protoplasm, nucleus, and nucleolus. Plasson and protoplasm present the same physical characters; they can both manifest the phenomena called "vital."

The existence of the Monera and of cytods demonstrates that life is connected with the existence of a determinate chemical composition, much more than to a form; and the

[1] Generelle Morphologie,' vol. i, p. 276.

question of spontaneous generation, which has for so long a time been bound up with the question as to whether a cell can take origin independently of a pre-existing cell, becomes now an inquiry as to whether it is possible artificially to engender plasson, and to cause vital phenomena to appear therein. It is quite certain that the Monera—simple fragments of plassic matter—manifest their vitality quite as do the most elevated organisms by the phenomena of nutrition, of multiplication of movement, and of irritability.

Every small living mass of plasson is a cytod, and the cell differs from the cytod in that a nucleus is differentiated in its interior from the surrounding matter. It clearly results from the theory of evolution, that plasson must have existed before monocellular beings, and the latter take their origin in cytods.

The ontogenetic evolution of the Gregarinæ represents the history of the genealogical or phylogenetic development of the cell. The psorosperms give rise to the globules of plasson, devoid of all nucleus, vacuole, or membrane; they may be compared to the simplest Monera. The Gregarinæ are originally then simple naked cytods (Gymnocytods). But soon a clearer and denser peripheral layer appears around the cytod, whilst the central part of the globule remains formed of a more fluid and more granular plasson. The Gymnocytod tends to elevate itself above the Monera, which are always devoid of a cortical layer; whilst we find regularly such a layer in the Protoplasta, viz. the Rhizopods, the Myxomycetæ, and above all, in the Infusoria. In speaking of *Protomyxa aurantiaca* (see this Journal, 1869), Haeckel says clearly, "Nothing is to be observed of a separation into a thicker cortical layer and a thinner fluid medullary layer, as is found in many Rhizopods and Myxomycetæ."

But the Gregarina in course of development remains still in the cytod condition, and on the surface of the cytod the two pseudofilaria develop as buds formed at the expense of the material of the cytod, as described above. In the gradual formation of nucleolus, nucleus, and cortical substance, we see a gradual differentiation and localisation of chemical elements, primitively united in the plasson of the cytod.

There is not a general agreement as to what must be understood by the endogenous multiplication of cells. It has been long admitted that endogenous generation consists essentially in the division of the cellular contents without the cell-membrane taking part in this division; but since we have learnt the true nature of the cell-membrane, we know that it never takes part in the process of cell-division. The only rational distinction which can be made between endogenous cell-formation and cell-division consists in this, that in the multiplication by division the nuclei of the daughter cells form at the expense of the nuclei of the pre-existing cell; whilst in the multiplication by the endogenous method (which the botanists call "free cell-formation"), the nucleus of the daughter cell develops in the body of the mother cell without the participation of a pre-existing nucleus.

Each of these two modes of multiplication can present itself in connection with a sort of budding. The multiplication by budding is only a particular case of the two fundamental modes of cell-multiplication. What distinguishes this particular mode of division is, that in the case of budding, a generating and an engendered element can be distinguished, a mother cell and a daughter cell; whilst in division, pure and simple, the two cells are derived from one mother cell; they are, both one and the other, daughters, and therefore sister cells.

It is undeniable that the formation of the nucleus in the body of the pseudo-filaria presents us with a true endogenous generation, following on a multiplication by budding of the generating cytod.

The only examples of endogenous generation which I have found mentioned are the endogenous formation of the blastodermic cells in the eggs of a great number of insects, especially of the Diptera;[1] the development of an entire layer of cells in the interior of the vitelline membrane of the ovarian egg of the *Ascidia canina*, without the germinal vesicle participating in the least degree in the formation of these cells;[2] and finally, the generally admitted fact of the formation of a nucleus in the egg of animals after fecundation, to replace the germinal vesicle.

The observations of Weissman on the formation of the blastodermic cells do not appear to me conclusive; they do not demonstrate that the nuclei which appear in the protoplasmic layer (Keimhautblastem) are not derived from the germinal vesicle. It is notorious that the opacity of the vitellus of the egg of insects generally, renders these delicate observations impossible. And Weissmann's interpretation is rendered doubtful by the fact that in the Cecidomyiæ and the Aphides, where the vitellus is nearly transparent, the nuclei of the blastodermic cells are derived from the germi-

[1] Weissmann, 'Entwickelung der Dipteren.'

[2] Kupffer, "Die Stammverwandtschaft zwischen Ascidien und Wirbelthieren," 'Archiv für Mikr. Anat.,' Bd. vi, 1870.

nal vesicle, as Metschnikow has demonstrated.[1] The much more recent observations of Kupffer on the development of the Ascidians have brought to light a most remarkable fact; it is the development in an endogenous manner of an entire layer of cells under the membrane of the ovarian egg, and that, too, before fecundation. These cells are formed at the expense of a continuous layer of finely granular protoplasm, and the nuclei are said to appear in the cells after their individualisation.[2]

I have elsewhere expressed my views as to the development of the nucleus in the fecundated egg in the place of the germinal vesicle.[3]

In the vegetable kingdom also, certain examples of this mode of cell-multiplication are known, to which botanists give the name of "freie Zellenbildung."[4] Such are the formation of the embryonic vesicle, and of the first cells of the endosperm.

Seeing that the cases are few in which endogenous cell-formation is demonstrated, my observations on the development of the Gregarinæ have interest from this point of view, since here certainly the nucleolus and nucleus are developed in the cytod by endogenous formation. When I observed, for the first time, the disappearance and reappearance of the nucleolus in the nucleus of the Gregarina, it seemed to me that these facts tended to diminish the importance which one is accustomed to attribute to the nucleolus as a constituent part of the cell. It is, therefore, with astonishment that I saw the nucleolus appear before the nucleus in the progressive development of the cell; and as a result, one must admit a stage intermediate between the cytod and the nucleus-bearing cell; this stage being that of the cytod provided with a nucleolus.

This fact of the appearance of the nucleolus before the nuclear layer confirms the view of the illustrious founder of animal histology, who held that the nucleolus appears first, then the nuclear layer, and finally the body of the cell.

The existence of the Monera, which have been the origin of all living beings, and whose extreme simplicity is found again in the youngest Gregarinæ, proves the existence of

[1] Metschnikow, "Embryologische Studien an Insecten," 'Zeitschrift für Wiss. Zool.,' Bd. xvi.

[2] Kupffer, *loc. cit.* This cellular layer persists during the entire embryonic development of the Ascidian, and is destined to become the test or external layer of the mantle.—*Kupffer.*

[3] Edouard Van Beneden, 'Recherches sur la Composition et la Signification de l'œuf;' 'Mem. de l'Acad. Royale des Sci. de Belg,' t. xxxiv.

[4] Sachs, 'Lehrbuch der Botanik,' p. 11.

the plasson as the primitive condition. But in the plasson the nucleolus appears before the nuclear layer. If we identify plasson with the blastema, such as Schwann understood it, we shall return to the views of the celebrated histologist who assigned to the cell a centrifugal evolution. The parts then develop from within outwards, and the nucleolus assumes as great an importance as, or one comparable to that of, the nucleus. It is not easy to reconcile this with the fact of the possible disappearance of this element of the cell, as observed by me in the Gregarina.

Haeckel has, with much reason, arranged the Gregarinæ by the side of the Amœbæ in his group of the Protoplasta; he considers the Gregarinæ as parasitic Amœbæ. "I regard the Gregarinæ as Amœbæ, which have become degenerate (*ruckgebildet*) by parasitism." Every parasitic animal is evidently derived from a form originally living in the state of liberty. It is clear that the Gregarinæ are at least as intimately related to the Amœbæ as are the Lernæans to the free Copepods. But whilst one observes generally in parasitic animals a retrogressive development, the Gregarinæ, instead of retrograding, appear to me to be raised in the scale by their parasitic life. Evidently the Gregarinæ are very high "Lepocellulæ," as the study of their entire organization proves.

By elaborate researches on the chemical composition of protoplasm (analysis of the protoplasm of the Myxomycetæ), Kühne has demonstrated the complex nature of this material. Protoplasm is formed of a mixture of different albuminoid matters, among which are especially found myosin, lecithin, &c. Protoplasm contains, moreover, a substance very similar to vegetable cellulose.[1] In accordance with this, it is very evident that the progressive differentiation of cells and their characterisation from the physiological point of view, depends on the preponderating accumulation of one or other of these principles, and on the separation of this or that from the other elements of the protoplasm (law of localisation).

The muscular cell contains a larger quantity of myosin, able to separate itself progressively from the other elements of the protoplasm in proportion as it is formed. We know that in a monocellular being, somewhat elevated in organisation, this myosin tends to separate itself also, and to become deposited, in one form or other, under the cuticular layer, and to bring into existence in this way, in the cell, a locomotive system, comparable, in a physiological sense, to that of the nematoid worms. The cuticle in the Nematoids is a sort of

[1] Verbal communication from Professor Kuhne.

framework, able to act in virtue of its elasticity; under the cuticle is found a layer of contractile substance, formed of muscular cells.

We find also, in the Gregarinæ, this muscular layer. Leidy[1] was the first to recognise it, and he endeavoured to demonstrate that there exists under the cuticle a muscular membrane, which, when it contracts, becomes plicated longitudinally, in such a way as to produce a well-marked striation. Leuckart[2] and Ray Lankester[3] have arrived at the same conclusion. In studying, by means of reagents, the immense Gregarinæ of the lobster, I have quite satisfied myself of the existence of a veritable system of muscular fibrillæ, comparable to the muscular fibrillæ of the Infusoria. I hope to be able to demonstrate the existence of this system of fibrillæ in a further work on the intimate structure of the *Gregarina gigantea.*[4]

If we take into consideration only this single fact of the existence of a muscular layer, recognised since Leidy by all naturalists who have occupied themselves seriously with the Gregarinæ, we must recognise that these cells rise far above the Amœbæ. In my opinion it is impossible to consider the Gregarinæ as *Amœbæ which have undergone a retrogressive development.*

However that may be, the Gregarniæ of the lobster passes successively, in the course of its embryonic development, through the following stages.

1. Moner stage. 2. Generating cytod stage. 3. Pseudo-filarium stage. 4. Protoplast stage. 5. Encysted Gregarine stage. 6. Psorosperm stage.

It is certain that few of the higher organisms even have so complex an evolution.

Before finishing I have yet to examine the question as to whether one must admit a true alternation of generations in these beings. The solution of this question is entirely dependent on the question as to whether it is necessary to admit the existence of a true conjugation[5] in these organisms.

That certain species are always found attached end to end is incontestable. But we must not, therefore, conclude from

[1] Leidy, 'Transact. Amer. Phil. Soc. Philadelphia,' 1852, vol. x.

[2] Leuckart, 'Jahresbericht Archiv fur Naturgesch.,' vol. xxi, p. 108.

[3] Ray Lankester, 'Quart. Journ. of Micros. Science,' 1863.

[4] In a more recent work, Ray Lankester expresses the opinion that the longitudinal striation depends on the cortical protoplasmic layer.—Notes on Gregarinæ, Ibidem, 1865.

[5] By true conjugation, I understand a fusion having for its object fecundation.

them that there is necessarily conjugation. Certain Gregarinæ can become encysted without a foregoing conjugation; but when this conjugation does occur, is its object the fecundation of the two individuals, the one by the other, the Gregarinæ being sexual forms? or is it not rather an accidental phenomenon? What makes me rather inclined to admit this last interpretation is, first, that the conjugation is not necessary; secondly, that this apposition of Gregarinæ is observed in certain species in quite young Gregarinæ; thirdly, that this apposition does not always present itself in the same way. Sometimes the individuals are attached by their homologous extremities, sometimes by their opposite extremities; fourthly, that one sometimes finds several Gregarinæ attached, one behind the other (Von Siebold, &c.); fifthly, that often two Gregarinæ enclosed in one cyst do not fuse together into a single granular mass, but they give rise, each on its own account, to a brood of psorosperms.

I think it is more just to compare the supposed conjugation of the Gregarinæ to the fusion of Amœboid particles forming a *plasmodium*, as De Bary first observed in the Myxomycetæ, and Haeckel in the Monera (*Protomyxa aurantica*). For in these beings this fusion of elements has simply for its object the enlargement of the protoplasmic mass, in order to arrive more rapidly at the reproduction by Sporogonia.[1] In that case, then, the multiplication by division would be the only mode of multiplication in the Gregarinæ, and there would be no digenesis. The multiplication by division would be the only one possible; but this manifests itself at two distinct stages of their evolution:—1st, it follows upon the encystment, and results in the production of the psorosperms (sporogonia); 2nd, it takes place in the generating Cytod, to produce the pseudofilaria (budding).

Haeckel has characterised his kingdom of Protista by the absence of all sexual reproduction. The Gregarinæ find their place in this kingdom, side by side with the true Amœbæ.

[1] Haeckel, "Monograph of Monera," 'Quart. Journ. of Microscopical Science,' 1869.