VII. *On the Cœlomic Fluid of* Lumbricus terrestris *in reference to a Protective Mechanism.*

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[Plates 4, 5.]

I. Preliminary.

Since the publication of Claparède's classical memoir on the earthworm, numerous writers have contributed to advance our knowledge of the morphology of this animal. In England, the writings of Ray Lankester, W. B. Benham, and F. E. Beddard have considerably increased the literature of the subject; but, with the exception of incidental references to function, the papers hitherto published have dealt chiefly with the morphology, taxonomy, or geographical distribution of the animal. Darwin long ago collected the observations of Claparède and other older authorities on the physiology of the earthworm, in his work on Vegetable Mould, which consists mainly of his own observations on the habits of the animal. An article "On the Retractile Cilia in the Intestine of *Lumbricus terrestris*," by M. Greenwood, in the 'Journal of Physiology,' vol. 13, is, I think, the only systematic contribution in English to our knowledge of the physiology of the earthworm in recent years.

The present communication contains the results of an investigation into the functions of the cœlomic fluid. The greater part of the research consists, therefore, of a detailed study of the morphology and physiology of the amœboid cells, which form the most important contents of the cœlom. But in the course of my observations, I was led to investigate some of the chemical characters of the cœlomic fluid, and also to examine certain other structures, such as the skin and the dorsal pores, which form, with the cœlomic fluid, a very remarkable protective mechanism.

The cells were studied both in the living state and after fixation. The re-agents used for the latter purpose were chiefly corrosive sublimate and Flemming's solution.

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To obtain reactions from the living cells, the substances used were either placed in a "hanging drop" or were injected into the cœlom, the contents of which were then from time to time removed and examined. The distribution of the cells was studied by sections in every possible plane. The details of the special methods employed to elicit particular functions will be described in their appropriate places.

II. The Cœlomic Fluid.

1. *Physical Characters of the Cœlomic Fluid.*

In a very large variety of animals there exists a perivisceral cavity containing a fluid rich in corpuscles. Among Annelids the perivisceral cavity or cœlom is subdivided into compartments by mesenteric dissepiments, and constitutes a very prominent feature. In all these animals the cœlom communicates with the exterior through the openings of the Nephridia, but in the Lumbricidæ and a few other forms of Annelids each chamber of the cœlom also communicates with the external world through the dorsal pore which will be described later on. The fluid which fills the cœlom of the earthworm is of a variable colour, the differences in tint being due to the nature of its cellular contents. As a rule the fluid has a milky-white appearance, and is opaque. Its specific gravity varies within very wide limits. When the fluid is tolerably thin and free from yellow granules, it is between 1007 and 1009. The fluid freed from corpuscles is quite transparent. It has an alkaline reaction, and contains albuminous and saline substances, besides the amœboid corpuscles. The amount of the concentration of the fluid depends entirely upon the humidity of the animal's environment. Specimens obtained from worms in moist localities are very watery, whilst those derived from animals purposely kept in dry places are much thicker. In the former case the cœlomic fluid is exceedingly copious. The fluid occurs in greatest abundance in the cœlom. It must be remembered, however, that that cavity is in free communication with the intermuscular spaces, so that the fluid not only occupies the cœlomic cavity, but also permeates the somatic musculature of the earthworm.

2. *Contents of the Cœlomic Fluid.*

The solid constituents of the cœlomic fluid are numerous. Putting aside for the moment the cellular elements peculiar to it, mention must be made of the occurrence of various crystals (chiefly calcium carbonate), pigmentary matter and various living organisms. In some individuals the cœlomic fluid resembles a culture of microbes. The organisms most commonly met with are certain bacteria, various species of Protozoa and a nematode belonging to the genus *Rhabditis*. If the fluid has been obtained by means of a capillary tube introduced into the cœlom, it will be found to contain great numbers of yellow refringent spheres, which have been derived from the yellow (or "liver") cells to which reference will be made.

Symbiotic Micro-organisms (figs. 38–41). That microbes may live in certain organisms without producing apparent evil results, is now a well-established fact. Indeed symbiotic bacteria have been described as occurring even in the protoplasm of a certain species of *Amœba* (BOURNE). In the cœlomic fluid of the earthworm three or four varieties of micro-organisms are usually present, but whether these are symbiotic in the strict sense of the term, or whether they are parasitic may be doubted. It is certain, however, that they occur in animals that are apparently healthy. The microbes are mostly bacilli, but there are also micrococci and spirilla (fig. 38). I separated the various forms by plate cultures in the ordinary way from specimens of the cœlomic fluid which were withdrawn with a sterilized pipette after the animals had been thoroughly washed with a solution of corrosive sublimate. The forms which occur in largest numbers, are large thick bacilli (fig. 39) many of which appear to have bevelled-off ends. On gelatine plates they form a discrete white translucent colony with central whitish opacity. They liquefy the jelly somewhat slowly. They are motile but their movements are very sluggish.

Another common variety is an oval-shaped actively motile bacillus in form indistinguishable from *Bacterium termo* (fig. 41).

A third species of bacillus has the form of a somewhat narrow rod with spore formation (fig. 40). With ordinary aniline dyes there is a central spot in each rod which remains unstained. These organisms grow in long chains. The rods are occasionally swollen in the middle, and may look not unlike the bacillus of tetanus. These bacilli are motile and form chrome-yellow colonies, which appear in four or five days. In addition to these, there occurs in the fluid a species of micrococcus (fig. 38), which grows as a bright yellow colony with a raised centre and sinuous margins. Even in broth cultures this yellow colony may be found at the bottom of the tubes. The bacilli produce turbidity in liquid cultures, which then have a strong peculiar odour not unlike that of earthworm pots. It is, therefore, possible that the peculiar smell of earthworms is due to the presence of some of these micro-organisms.

3. *Histology of the Cœlomic Corpuscles.*

The cœlomic fluid, is, as we have said, rich in amœboid cells (Plate 4, figs. 1–8, 11, 15, 16). Broadly speaking, two types of cells may be recognized if mere differences in size be not taken into consideration. As W. KÜKENTHAL has pointed out, the cells may be described as (1) granular (fig. 2) and (2) non-granular (figs. 6, 7). It is, however, essential to premise that all the granular cells are not physiologically or morphologically identical; although it is true that we are unable to say at present whether the varieties of granular corpuscles are *sui generis* separate groups or whether one form may pass into another. In addition to these amœboid cells, there are often found in the cœlomic fluid large cells packed with yellow spheres (figs. 10, 31) these being the so-called "liver cells" of the older morphologists. Turning first to the

varieties and characters of the cells as seen in a living state, it is convenient to discuss those characters which are common to all the cells, before attempting to describe the different corpuscles individually.

(*a*.) *Amœboid Movements and Plasmodia.*—Although the essential features of the amœboid movements of these cells do not differ from those of Protozoa, yet it is desirable to refer to the movements, because they are both interesting and important. If examined as soon after removal from the body as possible, the majority of the cells will be found in great activity. Delicate filiform pseudopodia radiate from the surface of the cell-body giving to the cell a star-like appearance (fig. 23). As a rule, these protoplasmic processes are straight, but sometimes they may be branched or irregularly disposed so that the cells seem to be remarkably granular, the whole having the appearance of an irregular network of coarse fibrils. Under abnormal conditions, the radiating filiform pseudopodia disappear, and the cells then show a central granular mass containing the nucleus and a marginal zone of hyaline protoplasm (figs. 8, 11), from which project very delicate and short processes. The non-granular cells emit more or less lobate pseudopodia but may also be seen with filiform processes, though these are never very numerous (figs. 6, 17, 36).

Modifications of the grouping of the filiform pseudopodia are interesting. They may be aggregated at one pole of a cell, so that the latter with its pseudopodia may be likened to the capitulum of a Scotch thistle (fig. 5). Through the movements of the processes the corpuscle is dragged along.

Another remarkable character of some of these cells must be noticed. This is peculiar to certain granular spindle-shaped cells (figs. 15, 35), which, in other respects also, are not to be distinguished from the other granular cells. They possess the peculiar power of being able to project their two ends to great distances. The extremities may then swell out or become branched, or may be broken off. A spindle cell may in this way, and with great rapidity, stretch itself across the whole breadth of the field of the microscope and its ends may fuse with other cells, thus leading to the rapid formation of plasmodia.

The different forms of cells very readily fuse with one another to form dense plasmodia (fig. 22), and thus, as METCHNIKOFF has shown, they build up, as it were, hugh giant cells in order the better to succeed in their struggle with parasitic organisms. From the periphery of the large colonies of cells, corpuscles having a slender stalk and resembling various infusoria in shape, project at intervals; while all the cells on the surface of the mass have vacuoles or long pseudopodia (figs. 16).

(*b*.) *The Cell Substance and its Contents.*—The cell substance of the cœlomic corpuscles is very viscid and resistent, and may be either opaque or perfectly transparent. In the former case there are present great numbers of amorphous granules or minute spheres; but apart from this granulation the cell substance appears to be thicker and is not transparent. The corpuscles in *Lumbricus* are

white or yellowish in colour, but in some species of earthworms they are pigmented. The contents of the cells are very variable and deserve detailed consideration.

(i.) Under certain conditions, minute opaque spherules make their appearance in the non-granular cells (fig. 13). At first these bodies are small and whitish, but, as they increase in size, they acquire a yellowish tint. They may remain deep-seated in the cell body or may project from the surface (fig. 34). They may even be cut off and discharged. What conditions are most favourable to their development, and what their meaning is we shall consider when we come to the functions of the cells. When the spheres are mature, they are distinctly yellow and could not be easily distinguished from the chloragogen granules of the so-called liver cells. These bodies, which we have been considering, are very irregular in size and in shape; so that the word "sphere" is not strictly applicable. They are readily stained by acid fuchsin, taking on a pink colouration. They may occur in such numbers as to obliterate other features of the cells.

Some of them are yellow and are probably the maturer forms of the spheres. Others are brown, and these may be irregular in shape and possibly represent various stages in the degeneration of the spheres. The ultimate result is the production of pigmentary débris, which may occur as amorphous granules or irregular masses. Sometimes the spheres are extruded or cut off from the protoplasm, instead of undergoing reduction within the cells.

(ii.) *Granules.*—More than half of the corpuscles are granular, and, in dried films, these may be readily distinguished from the non-granular cells by staining with fuchsin. There are two kinds of granules (figs. 2, 3, 4, 5, 32, 33)—the one highly refringent and discrete, the other irregular in shape—variable in size and more numerous. These granules are readily stained with fuchsin with varying tints; the same kind of cell now staining one tint, now another. At the present stage of our knowledge it is inadvisable to lay too much stress on the action of aniline dyes, and we shall, on this account, merely classify the granules as coarse or fine as the case may be. It would seem that the one variety of granules may be derived from the other, although we have not sufficient evidence to prove it. It seems clear, however, that the granules belong to cells which are quite distinct from the non-granular corpuscles, both from a morphological and a physiological point of view.

(iii.) *Vacuoles.*—It is easy to distinguish two kinds of vacuoles, which may be called (1) deep (fig. 17) and (2) peripheral (fig. 1) in relation to the periphery of the whole cell. We shall reserve further remarks on the vacuoles until we come to discuss the physiology of the cells.

(*c.*) *Nucleus.*—All the cells, with a few exceptions, have a spherical nucleus, which very often contains a pair of nucleoli (figs. 1, 2, 9). As a rule the corpuscles are uni-nuclear, but cells in various stages of direct division may easily be found (figs. 25, 26, 27, 29, 30). In some cells the nucleus is crescentic (fig. 26) and presents a partition dividing it into two halves. Occasionally, one of the daughter-

nuclei has undergone further fission before the protoplasm has divided, so that we have apparently a cell with three nuclei (fig. 28). More rarely irregularly-shaped nuclei are seen, but they are usually found in small and unhealthy-looking cells. The dyes which I have found most useful in the study of the nuclei are dahlia, Bismarck brown, and methyl-green.

4. *Description of the Different Forms of Cells found in the Cœlomic Fluid.*

Any given specimen of cœlomic fluid contains cells that at first sight appear to be quite distinct varieties, but further study of the cells themselves and of their life-history leads to the conclusion that they are either modifications of one kind of cell or are derived from two forms. Histologically, however, it is convenient to classify the cells according to their morphological characters. In a later paragraph, reasons are given for the belief that the corpuscles are derived from two kinds of cells, granular and non-granular, which may be regarded as the analogues of the hyaline and granular cells in the endoderm of Hydra. The following classification is introduced for the sake of convenience, but it also enables one to describe more fully the different forms which the cells may assume.

(1.) *Small Non-granular Cells.*—There are, in the cœlomic fluid, small cells with relatively large nuclei, having all the essential characters of ordinary lymphocytes (fig. 7). The nucleus is generally ovoid, and stains very distinctly with dilute-methylene-blue, of a blue colour. It often contains two nucleoli. The cells exhibit comparatively sluggish movements. They probably give rise to the next variety of corpuscles, namely :—

(2.) *Large Hyaline Cells.*—These are large non-granular cells (figs. 6, 17) which occur in great numbers, forming a fifth to a third of the total number of cells. In a few instances they have been found in insignificant numbers. The nucleus is spherical or ovoid, or more rarely crescentic. The cells emit short thick pseudopodia, which are more or less lobate. They are actively phagocytic.

(3.) *Small Granular Cells.*—The small granular cells are very similar to the eosinophile corpuscles of other animals (figs. 32, 33). Their granules, which are discrete and closely packed, stain with dilute eosin. With fuchsin, the granules stain bright red in some cells, but in other cells they take on a dull slaty colour. The nucleus is spherical. These cells are actively amœboid, and move about by means of delicate filiform pseudopodia.

(4.) *Large Granular Cells.*—These constitute the most numerous form of all, and they are doubtless derived from the above-mentioned small cells (3), because forms are found which are intermediate in character. The large granular cells can be divided into two groups, (α), cells having refringent discrete granules, all of which are of about the same size (fig. 2), and (β), cells having granules which are irregular in size and shape (fig. 5). Large granular cells of the first type are present only in

small numbers, except under special conditions. It is by no means certain whether these two varieties are distinct kinds of cells, or merely modifications of the same kind. The granular cells are very actively amœboid. They project filiform pseudopodia from the surface of their protoplasm in all directions (figs. 3, 4, 5, 8, 11, 23).

(5.) The blood vessels of *Lumbricus* are, as is well-known, invested closely by yellow cells, the "chloragogen" cells of CLAPARÈDE. The large yellow spheres which stud the colourless protoplasm of these pyriform cells, appear at first as minute specks and, when mature, show some complexity of structure. Under pressure they form discs with broken margins; among stains, dahlia, hæmatoxylin and methyl-green colour them deeply, and solution of the yellow pigment (identified as a lipochrome by MACMUNN) leaves the shape of the spheres unchanged. Degeneration follows maturity in the cycle of development, and the perfect sphere passes into an irregular mass of pigment.

The yellow cells are generally regarded as modified epithelial cells, but some evidence has been brought forward in support of the view that they are derived from cœlomic corpuscles, and that they come to anchor by means of delicate filiform processes which branch slightly and are free from pigmentary formation. Be this as it may, the cœlomic corpuscles do at times hold typical yellow spheres in their protoplasm, and there is clear evidence that degenerated yellow cells are thrown off into the perivisceral cavity. So usual indeed is their presence that I mention them in this list of the cells which characterize the cœlomic fluid, acknowledging at the same time that they may occur accidentally rather than inevitably.

(*d.*) We have already called attention to the presence of spindle shaped cells in the cœlomic fluid. When the plasmodia are formed, these cells, or cells resembling them, project from the margin of the cellular masses (figs. 16, 22). Thus, through the agency of these rapidly elongating corpuscles, different colonies more or less distant from one another may become united.

When water or any fluid substance or particles of any foreign body come in contact with the cells, they at once form plasmodia, and very soon develop vacuoles. These are at first small, but after a time assume enormous sizes (fig. 37). Very dilute acetic acid, caustic potash, alcohol, quinine, &c., are all capable of producing the vacuolation.

5. *Action of the Cœlomic Corpuscles on Bacteria.*

Since METCHNIKOFF called the attention of biologists to the universal occurrence in the animal kingdom of cellular ingestion of microbes and certain foreign bodies, much work has been done to establish the doctrine of phagocytosis. But whilst it must be at once conceded that the ingestion of microbes is an important fact to be observed in the struggle between cells and bacteria, yet it is obvious that phagocytosis alone constitutes only a link in a more or less obscure series of phenomena. In the case of the cœlomic corpuscles it is very easy to demonstrate that all the cells

are not phagocytic. In making this statement, I have specially kept in mind the possibility of a negative chemiotaxis, and have made allowance for what has been called "discrimination in the choice of ingesta." As already stated, there are in the cœlomic fluid, even normally, great numbers of bacteria which are rarely or never ingested by the cells under normal conditions. Under certain conditions, however, these bacteria are ingested and digested within vacuoles. If, for instance, a drop of anthrax culture in broth is added, then it is usual after an hour or so to see beautiful examples of phagocytosis (figs. 17, 43). We may suppose that the symbiotic bacteria for some reason or other do not stimulate the activity of the cells, and therefore are left untouched. When anthrax is added, however, the cells are intensely affected, and consequently ingest anything in contact with them.

When anthrax bacilli are injected into an earthworm the cells with large refringent granules increase. The ordinary granular cells also become very active. They approach the chain of bacilli and place in contact with the microbes a series of vacuoles and also the endosarc which contains the granules (fig. 12). Phagocytosis, in the sense of intracellular digestion, has never been seen. The granular cells may surround a thread of anthrax for 5 to 24 hours without any obvious change being observable in them (figs. 14, 18).

Sometimes, as KANTHACK and HARDY have described in the eosinophile cells of the frog, the refringent granules are discharged. The other kind of granules may also be extruded, and spheres may be cut off and discharged, but what interpretation it is safe at present to make of these phenomena must be left undecided. The cells are apt to develop a system of superficial vacuoles which come in contact with bacteria and invest them. In some cases I thought I had observed granules disappearing when the vacuoles came to view (fig. 24). The loss of granulation might be due to solution of the granules or to their escape. Hyaline cells may be watched digesting bacteria in vacuoles, but the granular corpuscles, as already shown,—though they approach bacteria—have never been observed to ingest them and to contain them in vacuoles.

The observations on the reactions of cells to microbes may be briefly summarized by saying that there are two forms of cellular digestion, (1) the most primitive type—the intra-cellular digestion within vacuoles by the hyaline cells, and (2) the more specialized method—the extra-cellular digestion by matter secreted by the granular cells. This generalization is of universal application, inasmuch as the two kinds of processes may be observed throughout the animal kingdom. The best instance among the fixed tissues for purposes of comparison is to be found in the earthworm, in which, either in the epithelial lining of the skin or in that of the intestine, there is a well-marked differentiation into granular and non-granular cells. The respective functions of these are, as M. GREENWOOD puts it, (1) "Secretion, which takes origin in the granules of unicellular glands," and (2) absorption "by the cells which surround the glands." The researches of KANTHACK and HARDY would seem to show that

these observations apply also to the leucocytes of vertebrates. The plasmodium forming powers of the cells are called into play when larger parasites come into contact with them. METCHNIKOFF, in his 'Leçons sur la pathologie comparée de l'Inflammation,' has carefully described the phenomena then seen.

As usual, the cells form plasmodia in contact with foreign material of any kind. Gregarines become encysted in the cœlomic cavity and surrounded by a mass of amœboid cells and then undergo degeneration (fig. 21). When in the motile form, the gregarines are generally free from cells, owing, as METCHNIKOFF pointed out, to their violent movements preventing the corpuscles from adhering to them.

Ciliated infusorians are commonly seen in the fluid, and again the same phenomena are observed. When the parasite becomes quiescent, amœboid cells of all kinds surround it. Sometimes an infusorian may be seen in a large vacuole formed by the coalescence of vacuoles which are developed near the surface of the different corpuscles attacking it (fig. 42). It is somewhat important to note that around some objects vacuoles are developed, but not around others. It is, however, probable that, as in the endoderm of *Hydra*, the vacuoles appear only during digestive activity. At all events, these large parasites, when entombed in vacuoles, lose their histological characters and are ultimately reduced to irregular masses of débris.

Another enemy of the earthworm is a species of Nematode worm (fig. 44), which infests its perivisceral cavity, inhabiting chiefly the region of the generative organs and of the yellow cells. When these parasites are active, they are generally free from cells, but in hanging drops of cœlomic fluid the amœboid cells are seen to attach themselves to their bodies when their movements become sluggish. The only positions on which they can at first remain are the head and the tail ends. They are soon swept off other regions by the twisting of the worm. One part of its body rubs against another, but in its contortions the head and tail usually go past without friction. On these parts, therefore, the cells aggregate—and soon they are united to the pseudopodia of the plasmodia around them. The worm, in struggling to free itself, may pull out the plasmodia to protoplasmic threads, which only render its escape more difficult. When the worm rests, the cells contract and, at last, the parasite is embedded in the midst of a very large colony of cells.

It must be noted that all forms of cells are united in the plasmodia. This can be best made out by staining with fuchsin and methyl-blue, as prescribed by Professor EHRLICH. Lastly, it may be mentioned that in the plasmodial masses it is very usual to see pigment derived mainly from the yellow (chloragogen) cells.

6. *Origin and Distribution of the Cœlomic Cells.*

W. KÜKENTHAL has written an exhaustive paper on the histology of the lymphoid cells of annelids, and it is not necessary therefore to go over with much detail the ground covered by his paper. The cœlomic cells are reproduced not only in the

cœlomic cavity, but also in the inter-muscular spaces. They are also probably in part derived from the epithelial lining of the cœlom. In transverse sections of earthworms, spaces can readily be detected between the bundles of muscular fibres, and an abundance of loose connective tissue is seen to occupy the interval between the external and internal layers of muscles, and to lie between the fasciculi of each muscular stratum. The wandering cells occur in large numbers, both in the spaces and in the loose connective tissue (fig. 19). It will be remembered that the internal muscular layer presents a peculiar resemblance to a series of feathers placed in a row. This appearance is due to the fasciculi of the longitudinal bundles of muscles having a bipinnate arrangement. The areolar tissue is most abundant in the neural region and around the bases of the setæ. The nerve-cord also is invested by a muscular sheath, which is largely composed of loose areolar tissue packed with granular cells (fig. 20). The cells that are found in the connective tissue and spaces possess very large granules. If the conjecture of HANKIN turn out to be correct, and the observations of KANTHACK and HARDY, as well as those embodied in the earlier part of this paper, bear the interpretation which has been put upon them, then one is justified in believing that the granular cells around the nerve-cord act as a protection against the encroachment of the symbiotic bacteria, at least, if not also against that of other parasites. I have purposely abstained from using the terms employed by Professor EHRLICH, because I am not sure if cells in different animals having the same reaction to aniline colours, have also the same functions, and because I am not by any means certain if the different granular cells be not derivable one from another. But this much may be affirmed, that between the non-granular and the granular cells there is a well-marked distinction which we can safely make both on morphological and on physiological grounds.

III.

(i.) *The Structure of the Skin.*—The superficial covering of the earthworm consists of an epithelial layer of cells resting on a basis of connective tissue and protected by a well-developed cuticle (fig. 19). The latter has been figured by BENHAM, and is in reality a fenestrated membrane, having on its free surface delicate markings. The pores belong to the mucin glands of the skin, and their diameter is much less than that of the mucin globules which are usually seen in the gland-cells. The cells of the skin are generally of two kinds: (*a*) columnar cells, and (*b*) mucin cells. The former have well-defined nuclei, and are like other columnar cells in their characters. The mucin-forming cells are unicellular glands. They have a delicate membranous wall and one or more nuclei, which are not readily made out. Such are briefly the chief features of the epithelial covering of the earthworm, but it ought to be mentioned that in the clitellar region there are found two other varieties of cells, which are, I believe, merely histological modifications of the columnar and mucin cells respectively.

(ii.) *The Dorsal Pores.*—At the anterior end of every somite except the first seven or eight, there is dorsally a minute aperture lying evidently in the groove separating this from the segment in front. The apertures are known to morphologists as the dorsal pores. They are of some importance as diagnostic characters, inasmuch as, with few exceptions, they are absent in aquatic Annelids. They are, however, by no means present in all terrestrial forms, although in the Lumbricidæ it is usual to find them. Unfortunately no very definite account is to be found as to the habits of those animals in which the pores are absent.

These pores are usually described as simple apertures leading into the cœlomic cavity. But it is possible to make out in fresh preparations that strands of muscle fibres pass across the apertures longitudinally and transversely.

The layer of circular muscular fibres is extremely thin in the region of the grooves, which therefore appear as narrow white bands, in contradistinction with the broad brown bands representing the somites. The dorsal pores are situated in the mid-dorsal region of the grooves. The transverse muscular fasciculi separate into two bundles to embrace the apertures; the longitudiual bands of muscle similarly encircle them. The epithelial covering of the worm may be traced downwards to the deepest part of the dorsal pore which is visible, and no doubt this epithelium is continuous with the endothelium of the cœlomic cavity. The dorsal pore is therefore the external opening of a cavernous canal through the muscular wall of the earthworm, which communicates not only with the cœlomic cavity, but also with the numerous large spaces that exist between the fasciculi of muscles. When the dorsal portion of the body wall of an earthworm is placed on a slide and flattened down with a cover glass, wandering cells may be seen (with a ZEISS D Objective) between the muscles. If now the cover glass is pressed upon, streams are induced, which converge to the dorsal pore, and in consequence numerous corpuscles accumulate in it. Muscle fibres surround each aperture. These have been called the sphincters. In addition radiating bundles may be seen diverging from the margin of the dorsal pore and getting lost in the muscular wall. These muscular fibres are no doubt concerned in the opening and closing of the individual pores, whilst it is to be remembered that the general musculature of the animal plays an important part in rendering the functions of the pores effective. In short, the sphincter and radiating fibres prevent or permit, as the case may be, the discharge of the cœlomic fluid by the contraction of the muscular wall.

IV. EXPERIMENTS.

We must now consider certain experiments, which were made with the view of finding out the behaviour of earthworms towards irritation of any kind. Inasmuch as all kinds of irritants, if at all efficacious, produce the same results, it becomes unnecessary to describe separately the actions of the various substances used; any difference that may be observed is only in the degree of intensity of the reaction.

The experiments fall into two groups: (1) the irritation of the surface of the worm; (2) the injection into the cœlomic cavity of irritating fluids.

(i.) A drop of distilled water placed on the body of an earthworm produces no appreciable effect. On the other hand, if the skin of the animal is somewhat roughly scratched, it is at once made to wriggle about and to secrete a large amount of slime. The same thing is noticed if worms are handled with the fingers. Touching the animal lightly with a camel hair brush or pin-head does not lead to secretion. If the pin-head, however, is dipped in 1 in 1000 corrosive sublimate, and, after removal of surplus fluid, applied to the worm, such is the sensitiveness of the skin to certain chemical stimuli that the animal at once manifests signs of uneasiness. The part to which the irritant has been applied becomes swollen up owing to a contraction of the circular muscles in front of and behind the point of irritation. At the same time large quantities of milky cœlomic fluid are poured out through the dorsal pores. In this way the animal is provided with a good covering of slimy matter. Dilute acids, alkalies, and other substances with irritant properties are capable of producing the same results, and electrical stimuli induce the same phenomena.

Histology of the Slime.—That the slimy exudation is rich in cells is one of the important facts to be kept in mind, and the cells are those of the cœlomic cavity. They are all most actively amœboid, having moving pseudopodia radiating from almost every available point on the surface. But one can only see free wandering cells if the fluid is collected as soon as it exudes from the dorsal pore and is immediately examined. If the examination is delayed, great numbers of the cells are found to have already united to form plasmodia. The white frothy slime contains only comparatively few free cells, the great mass of them having already formed plasmodia around peculiar rope-like structures, which present irregular nodosities. To the naked eye these colonies appear as minute opaque white specks. The slime becomes opaque and turbid on the addition of dilute acetic acid, and on the addition of dilute caustic potash the rope-like structures disappear, leaving air bubbles in the regions of the nodosities. These appearances are due doubtless to the air in the grooves between the somites becoming enveloped in a thin coating of mucous substance. In some specimens these rope-like bodies form an irregular network around which the cœlomic corpuscles form dense plasmodia.

(ii.) *The Influence of Heat and Cold.*—When a large healthy worm is put in a test-tube and heated in an incubator at 35° C., the animal instantly perceives the change of medium, and exhibits the most active movements; at the same time the dorsal pores discharge an abundance of cœlomic fluid, and large quantities of slime accumulate on the skin. The discharge of cœlomic fluid depends upon the integrity and activity of the nervous system; and so we find that so long as the animal continues to struggle, the fluid continues to exude more or less. So great, indeed, may be the squeezing action of the muscular apparatus, that in the majority of worms

observed, hernial protrusion of the gut occurs through weak points in the somatic wall—commonly through the dorsal pore or external nephridial opening. But heat produces intense hyperæmia of the skin, leading to the escape of bloody fluid through the epidermis and to the production of multiple ecchymoses. If the worms are suddenly exposed to a very high temperature, *e.g.*, 500° C., there is an immediate discharge of cœlomic fluid, but the animal is rapidly paralysed. The heat causes in this case no further escape of cœlomic fluid through the dorsal pore, nor herniation, but only exudation of a clear fluid, deeply tinged with hæmoglobin. It thus seems conclusive that the herniation is due to over-action of a protective mechanism, recalling instances of evil effects following excessive activity of protective functions in human pathology.

Intense cold also produces a discharge of cœlomic fluid. Several big worms were placed on slabs of stone during an intense frost. The surface of their bodies very soon assumed a dry, glistening appearance, and the animals became torpid. From time to time cœlomic fluid welled out through the dorsal pores; the discharge of cœlomic fluid ceased before the animals were completely paralysed by the cold.

(iii.) *Injection of various Substances.*—Various coloured liquids, including cultures of microbes, were used for injecting into the cœlomic cavity. Owing to the high pressure of the cœlomic fluid, it is somewhat difficult to inject liquids into the cavity without using a force which is very apt to be greater than requisite. The fate of all sorts of liquids is the same: they are expelled through the point of injection as well as through the dorsal pores. Immediately in front and behind the seat of the injection the muscles undergo tetanic contraction, so that it is impossible, without laceration, to pass a pipette through the constriction produced. The somites into which the fluid has been injected are distended and paralysed, and they may become the seat of intestinal protrusions. Injection of anthrax cultures causes a profound momentary effect on the worm; but large healthy specimens survive. After the introduction therefore of any substance, and especially one of an irritating nature, there is set working a reflex mechanism to separate either completely or potentially the part affected from the rest of the body.

V. Certain Habits of the Common Earthworm.

It is well known that earthworms are very rarely found in dry sandy places. Darwin, among others, pointed out that the localities inhabited, at least by the ordinary species, would invariably be found to be humid. These animals not only prefer moisture, but also will crawl from a cooler to a warmer spot. These peculiarities in the habits of worms will go far to explain their habitats in different seasons, and their somewhat erratic journeyings. They account also for the presence of large numbers of earthworms in places where decaying vegetable matter is abundant, for apart from the food which the animals may derive from it, the fermentative changes occurring

from decomposition increase the temperature, which naturally in such quarters would be higher than in the uncovered ground. Further, there is always abundant moisture. But such localities, as a rule, team with myriads of putrefactive bacteria, besides infusoria, diatoms, and other low forms of life. Experimentally it is easily demonstrated that worms thrive best when they are kept in a warm and moist chamber. Exposure to dry heat, as, *e.g.*, by leaving the animals on a dry plate even at the ordinary temperature of the laboratory, usually kills them in several hours or more. When the earth in the pot in which the worms are kept is too much moistened, they invariably come up to the surface. On the other hand, when the earth is allowed to dry, the animals always lie at the bottom of the pots. It is also worthy of notice that worms in moist places are generally, if not always, thicker than those found in dry localities or in dry pots. When a large healthy worm is carefully cleaned, and its body is dried with a bit of blotting paper, it may remain quite dry if it is placed on a piece of moist paper. On the other hand, if it is deposited on dry sand or any dry surface, the body of the animal very soon assumes a bright moist glistening appearance, and in a short time its immediate surroundings will be found to have been wetted. On careful examination it will be seen that the greater part of the moisture has come from the dorsal region of the worm. If an animal is watched from the beginning, the fluid is seen exuding between the segments as minute drops. It is obvious that the liquid has come out of the dorsal pores, and that it is nothing more than the liquid contents of the cœlom. In my experiments I have found that under certain states the earthworm can be made to discharge its cœlomic fluid, and now we see that under natural conditions the animal also has power to pour out its cœlomic fluid. The action of the fluid in delaying desiccation is of great significance when one remembers how much worms dislike dry places.

VI. Summary and Conclusions.

We are now in a position to consider what may be the probable functions of the dorsal pore and the glandular structures of the skin together with the cœlomic fluid. First, it is clear that Annelids with dorsal pores differ from those which do not possess these structures in the possession of the power or discharging their cœlomic fluid on to the surface of the skin. We do not intend here to discuss the functions of the cœlom and of its contents, but with regard to the corpusculated fluid in the cœlom we may say at least that in its amœboid cells it possesses a remarkable mechanism for attacking bacteria. When we consider how the functions of this fluid are correlated with those of the skin, we may realise the existence in the earthworm of a complicated protective mechanism which may effectually exclude parasites. To a highly organised animal like the earthworm, inhabiting localities swarming with parasites, it must be an enormous advantage to have the power of discharging upon its assaulting enemy great numbers of phagocytes. Possibly the

cœlomic fluid when poured upon the skin of the worm stimulates the mucous glands to discharge by reason of its alkaline reaction. Be this as it may, there is formed from the cœlomic fluid and from the product of the mucous glands a slime possessing considerable complexity of structure, and which in addition to active phagocytes, contains sticky threads of mucin which form a complicated network ready to entangle any parasite that endeavours to bore its way through the skin.

That the cœlomic fluid is sometimes used by the earthworm to lubricate or moisten its body is very probable, and SPENCER has called our attention to this fact. But that this is its only function is very improbable. Thus we see that in most terrestrial Annelids* the cœlomic fluid possesses remarkable functions in virtue of the presence of dorsal pores. The latter structures are therefore of great physiological significance, and, with the cœlomic fluid and the mucin-forming glands of the skin, they constitute an important mechanism for the protection of the animals against the inroads of parasites.

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* For exceptions see works of BENHAM and BEDDARD.

Description of Plates 4 and 5.

PLATE 4.

Fig. 1. Finely granular cell with a system of superficial vacuoles. Zeiss, D × 3.

Fig. 2. Cœlomic corpuscle, with distinct discrete granules stained with eosin and methyl-blue. The cell is of the medium size among the granular corpuscles. Zeiss, D × 3.

Fig. 3. The drawing is either of a single cell with a dividing nucleus, or the union of two cells after addition of culture of anthrax to hanging drop. In one the protoplasm contains amorphous granules; in the other markedly refringent spheres. The vacuoles are well shown. Zeiss, D × 3.

Fig. 4. The same cell 30 minutes later. Note the disappearance of the superficial vacuoles. Zeiss, D × 3.

Fig. 5. A granular cell with a bunch of pseudopodia, the ends of which are packed with minute granules. Zeiss, D × 3.

Fig. 6. A non-granular corpuscle ingesting a diatom. Zeiss, D × 3.

Fig. 7. A young hyaline cell.

Fig. 8. A granular cell showing a vacuolated rim of clear ectosarc.

Fig. 9. A granular corpuscle contained within a large hyaline cell. Stained with Ehrlich's neutral dye. Zeiss, D × 3.

Fig. 10. A small chloragogen (" liver ") cell with yellow granules. This drawing is of tail-less cell. Zeiss, D × 3.

Fig. 11. A granular cell with filiform pseudopodia, highly magnified. The granules are very distinct. Zeiss, Apochrom. ? × 4.

Fig. 12. A finely granular corpuscle adhering to a chain of anthrax bacilli. Zeiss, D × 3, tube 160 millims.

Fig. 13. A hyaline corpuscle showing nutritive spheres (yellowish), and one bright granule (green).

Figs. 14 and 18. Two stages of a granular cell attacking anthrax bacilli.

Fig. 15. Spindle cell with active elongated pseudopodia.

Fig. 16. A cell stretching out from the margin of a plasmodium, having fused with another.

Fig. 17. A clear hyaline cell digesting a symbiotic bacterium, showing digestive vacuoles. Zeiss, D × 3, tube 160 millims.

Fig. 19. Transverse section of skin, showing cuticle, epithelial layer and underlying connective tissue.

Fig. 20. Section of nerve cord with its muscular sheath.

PLATE 5.

Fig. 21. Encysted gregarine, surrounded by amœboid cells. The details of the structure of the gregarine are omitted.

Fig. 22. Diagrammatic representation of a plasmodium in cœlomic fluid, to which anthrax culture has been added. Note the fragmentation of cells.

Fig. 23. Finely granular cell with filiform pseudopodia.

Fig. 24. Cell with vacuoles. All granulation gone; anthrax in contact for 18 hours.

Figs. 25 to 30. Cells showing varieties of nuclei and their division.

Fig. 31. A yellow cell with processes.

Fig. 32. A granular cell with large discrete refringent granules; increased after introduction of anthrax.

Fig. 33. Ordinary cell with discrete refringent granules.

Fig. 34. A hyaline cell with spheres.

Fig. 35. A spindle cell (cp. 15).

Fig. 36. Hyaline cell spreading out.

Fig. 37. Hyaline cell developing large vacuole.

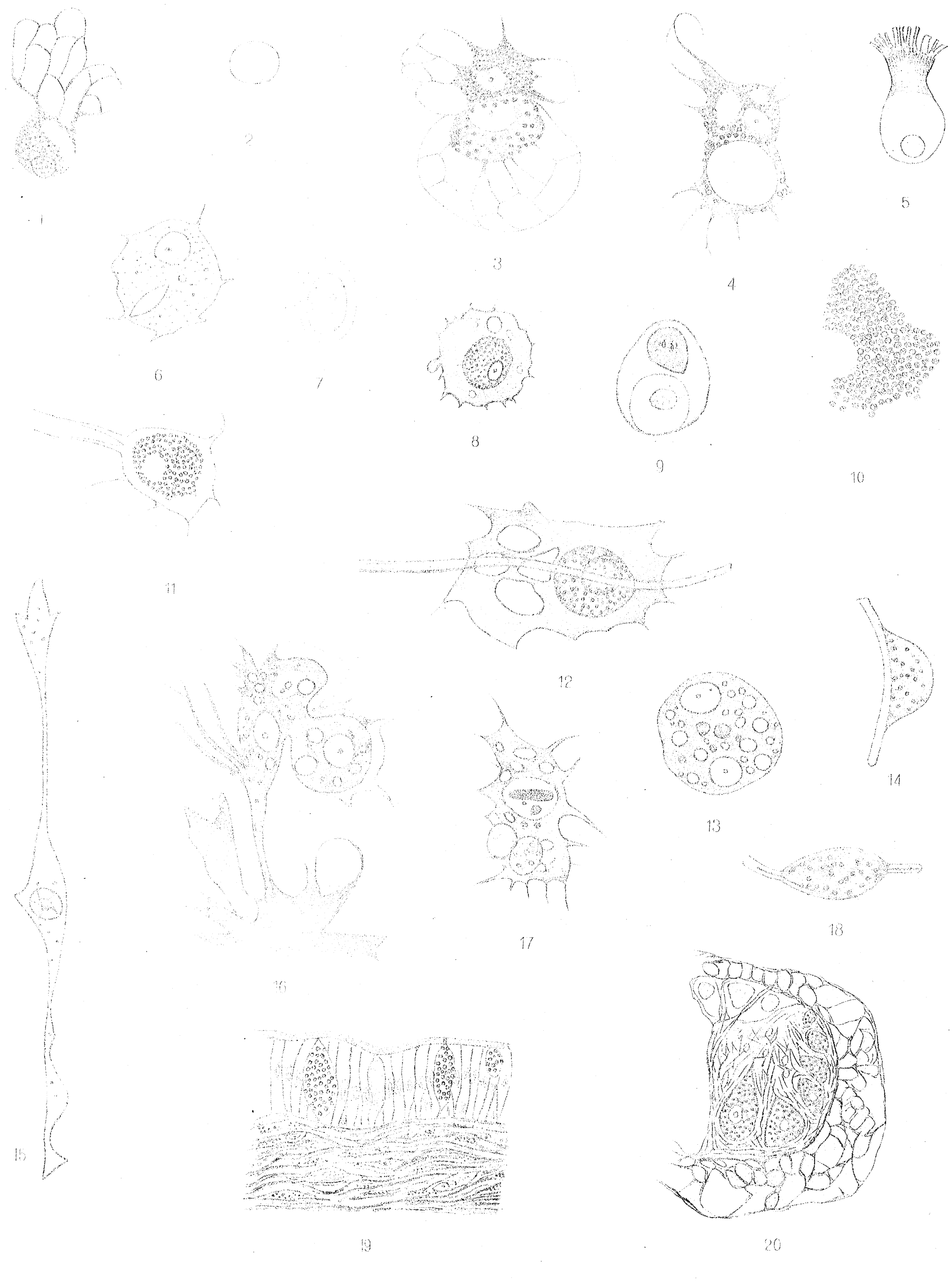
Fig. 38. Microccoci found in cœlomic cavity.

Figs. 39, 40, and 41. Bacilli found in cœlomic cavity.

Fig. 42. Infusorian in a large vacuole.

Fig. 43. Anthrax thread in a large vacuole within a hyaline cell.

Fig. 44. Nematode struggling with amœboid cells close to two plasmodia.



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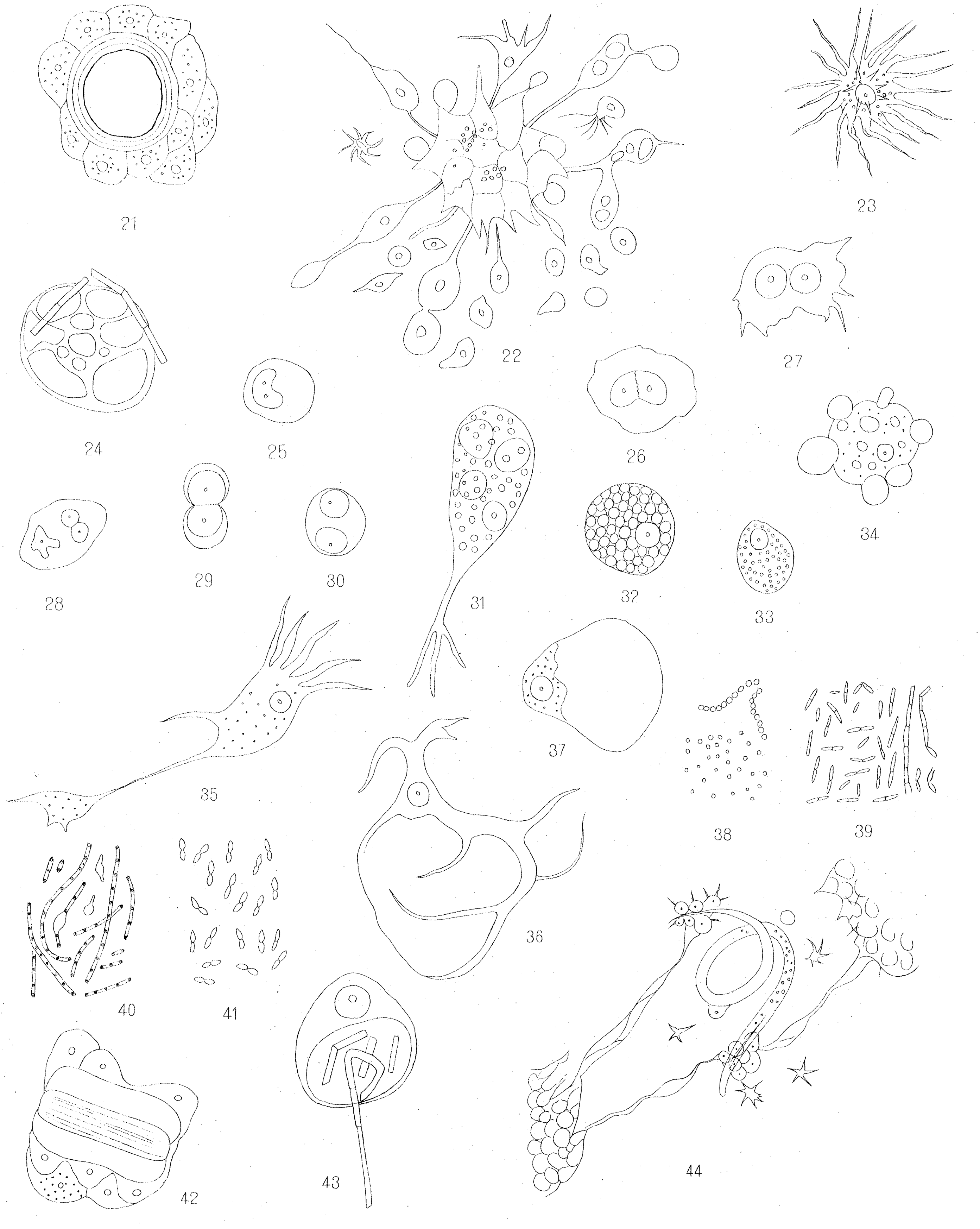

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