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On the Characters and Behaviour of the Wandering (Migrating) Cells of the Frog, Especially in Relation to Micro-Organisms

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VII. *On the Characters and Behaviour of the Wandering (Migrating) Cells of the Frog, especially in relation to Micro-Organisms.*

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[Plate 29.]

Introductory.

INTRODUCTORY. (Added September, 1893.)

[The most salient feature of the wandering cells of the body is their marked increase in numbers in inflammation. VIRCHOW, originally, in his great work, 'The Establishment of the Cellular Pathology,' traced the "Rundzellen Infiltration" to a proliferation of connective tissue cells. Later, however, RECKLINGHAUSEN and COHNHEIM showed that the white cells of the blood could penetrate the walls of the

blood capillaries, and so pass to an inflamed area. Thus, the first step was taken towards defining the morphological position of the wandering cells as an independent portion of the body.

The fact that the wandering cells ingest discrete particles has long been known. Claus, Schultze, Haeckel, Hoek, and others described the process as occurring in both Vertebrates and Invertebrates. But the genius of Metschnikoff* first suggested the importance of this process to the body. He showed that ingestion was, in the case of bacteria and digestible ingesta generally, followed by digestion. This discovery enabled Metschnikoff to attain to some conception of the real significance of the inflammatory process, and the part the wandering cells play therein.

While Metschnikoff was working at this question, startling additions were being made to our knowledge of the life-history of vegetable micro-organisms and the part they play in disease. The enormous importance of his discoveries, as suggesting an explanation of the resistance of animals to the invasion of these bodies, became at once obvious, and Metschnikoff was led to formulate a theory of the immunity of animals in which the resistance to the disease germs is referred solely to the phagocytic activities of the wandering cells.

From this time onwards the wandering cells have been regarded almost solely from the relatively narrow standpoint of their relation to the conflict with pathogenic germs.

The next advance in our knowledge was the direct outcome of the labours of those bacteriologists who refused to accept the phagocytic theory of immunity as a complete solution of that problem.

The discussion of the origin of immunity, like the earlier discussion of the origin of pus corpuscles, led to the enunciation of extreme theories. Those observers who found themselves in opposition to Metschnikoff, went so far as to deny to the wandering cells any direct participation in the processes which attend the development of immunity, and, without attempting to explain how the plasma gained such properties, they gave to the world one or other form of a "humoral theory."

In this way two distinct schools arose, one which attributed all to the phagocytic activity of the cells, another which regarded the fluid plasma as all important.

The result of an attempt to effect a compromise between these extreme views was the formation of a third school, which regards any special activity of the plasma as being due to substances derived from the cells, and its adherents find their support in the work of Wooldridge, of Hankin, and of Buchner.

In order to see how this led to a wider appreciation of the functions of the wandering cells it is necessary to trace briefly the main lines of the discussion.

In 1887, Fodor, Nissen, and others established the fact that the blood serum

* 'Annales de l'Institut Pasteur,' 1887, pp. 321–336; *ibid.*, 1889, p. 25, *et. seq.*; *ibid.*, 1889, p. 289, *et. seq.*; *ibid.*, vol. 4, p. 65, *et. seq.*; *ibid.*, vol. 4, p. 465. Virchow's 'Archiv,' vol. 97, p. 177; *ibid.*, vol. 107, p. 209; *ibid.*, vol. 109, p. 176; *ibid.*, vol. 113, p. 63.

possesses marked bactericidal powers, and the result of this discovery was the enunciation of a humoral, as opposed to a cellular theory of immunity.

METSCHNIKOFF, and other supporters of the cellular theory, soon proved that this bactericidal power was not possessed by blood in the body, but was developed as a result of *post mortem* changes, and, in 1888, BUCHNER clearly correlated the development of the bactericidal power with the breaking up of the leucocytes in shed blood, and, in 1890, HANKIN succeeded in isolating a bacteria-killing substance from lymphatic glands and the spleen. Thus, the idea that some, at any rate, of the wandering cells were concerned in the production of a peculiar substance characterized by its bactericidal power was suggested.

The nature of this substance, and especially the nature of its action on bacteria, whether, for instance, it destroys them or merely hinders their growth, are questions which have engaged the attention of many workers, but which do not concern us here.

Turning to the development of our histological knowledge of the wandering cells, we find that the existence of more than one kind of leucocyte in Vertebrates was recognized at a very early period. But it was not until EHRLICH,* in 1878, drew attention to the specific granulation of these bodies, and, with the help of the aniline dyes, provided us with a method of histo-chemical analysis, whereby the different forms could be readily recognized, that any great advance in the direction of determining differences of function became possible.

The striking histological advances made by EHRLICH and his school were, until quite lately, completely ignored in the discussion which raged around the cellular theory of immunity, and the wandering cells still continued to be spoken of indifferently as phagocytes, with no recognition of the possible existence of diverse forms endowed with diverse functions.

It is difficult to determine when or how the attention of those concerned in the discussion came to be attracted to the granulation of the cells, but it may be traced mainly to the work of BUCHNER, HANKIN, and those others who derived the bactericidal substance from the leucocytes.

Although the observations which we are about to record have, we venture to think, a direct bearing on the theory of immunity, we would ask that they might be regarded, not in this light only, but as some contribution to the more general natural history of the wandering cells.]

On investigating the histology of the body fluids of certain Invertebrates and Vertebrates, we find that animals widely separated in structure and habits possess the same kinds of wandering cells. But we also find (1) that, within the limits of a single group of animals, the simplest forms possess only one kind of wandering cell, while those with greater structural complexity have all the three typical forms sharply and completely distinct from one another; and (2) that during the fœtal

* EHRLICH, 'Farbenanalytische Unters.,' Berlin.

period a Mammal has only one kind of wandering cell. These facts suggest two ideas: firstly, that a certain fixity of type must be accorded to each kind of wandering cell, that the different forms found in the more complex animals must be regarded as distinct from one another in their development and life history, even if they be regarded as having a common origin; and, secondly, that, corresponding with this divergence and fixity of type, there must be divergence and fixity of function. We have endeavoured to demonstrate a disparity of function comparable to the disparity of form by comparing the behaviour of the different kinds of wandering cells towards various substances when added to the lymph or blood.

Since the wandering cells of the Frog retain their vitality for a long time after removal from the body, that animal has been mostly used. The lymph is obviously most serviceable for examination, but the cells of the blood are similar and offer similar phenomena.

Some observations have also been made on *Astacus*, the Rat, and the Rabbit.

The experiments on the cells when out of the body have been controlled by parallel experiments on the cells while still within the body.

We have to thank many friends for willing help and criticism, but special acknowledgment is due to Miss GREENWOOD for allowing us to see her preparations, and follow the results of her later and still unpublished work on digestion in Protozoa.

SECTION I.

Methods Employed.

In determining the identity of the wandering cells, we have had regard to differences as to shape, whether the cell be resting or amœboid, as to the texture of the cell-substance, as to the nuclear type, and as to the presence or absence and the histo-chemical nature of the cell-granules. The shape and appearance of the cells when resting is not more serviceable for their identification than their appearance when active. The manner of emitting pseudopodia, and the appearance of the cell when it has thrust out these appendages is markedly different in different cells.

Differences in the texture of cell-substance are brought into marked prominence by the use of iodine, and this reagent cannot be too highly praised in this connection.

The nuclear type of the various cells has been studied with the aid of a solution of methyl-green, slightly acidulated with acetic acid, and to which a trace of osmic acid has been added.

Nuclear characters are also shown by treatment with an alkaline alcohol-osmic acid solution of methylene-blue, which is practically LOEFFLER's solution with much less methylene-blue present and with a trace of osmic acid added. With this solution eosinophile* granules remain entirely uncoloured and unchanged. Amphophile

* EHRLICH, 'Farbenanalytische Unters.,' Berlin.

granules are stained blue, or rarely a very dull violet when viewed with yellow light. And the basophile granules appear violet with daylight, and brilliant rose with yellow light. Nuclei and microbes are blue with both lights. The substance which produces the rose-coloured modification of methylene-blue does so whether it be present as granules in the cell-substance, or dissolved in the surrounding fluid. The reaction also survives with unaltered intensity when the preparation is dried at the temperature of the air and mounted in Canada balsam.

The study of the living cells, and their behaviour towards noxious or innocuous substances has been carried out (1) by injecting various substances into the lymph spaces of the Frog, and withdrawing drops of lymph for examination at varying intervals of time, and (2) by hanging drops. The hanging drops were suspended on the under side of a cover-slip in moist chambers sufficiently large to provide air enough for the needs of a small drop of lymph for about ten hours, without introducing a fresh supply. In this simple way we have been enabled to continuously observe the processes taking place in a drop of lymph or blood after inoculation with microbes, poisons, &c., for periods up to ten hours. Discontinuous observation has been kept up for 40 to 50 hours. The cover-slips used were always carefully cleaned with acid and absolute alcohol, and then sterilised by heat immediately before use.

The study of these drop-cultures was controlled by examining lymph taken from the lymph sac and peritoneal cavity of a Frog, into which microbes, &c., had been injected. In all cases the most complete accord was found, frequently extending to the element of time.

To study the effects of temperature, the drops were placed on the ordinary metal stage, through which warmed or cooled water was circulating. In these experiments it is essential that the whole chamber and the cover-slip should be brought to the requisite temperature before the lymph is added. Otherwise the earlier stages will occur before the temperature has either risen or fallen to the required point.

It is most important to note that, in order to obtain satisfactory results, it is necessary to use only freshly captured Frogs.

Section II.

Histology of the Frog's Wandering Cells.

In the body of the Frog three kinds of wandering cells occur. These are (1) the eosinophile cell, (2) the hyaline cell or phagocyte, and (3) the basophile cell with rose-reacting granules. These, together with the red corpuscles and platelets, constitude the sporadic mesoblast of the Frog, thus constituting a tissue whose elements, unlike the elements of the other tissues, have no coherence, and but little fixity of place. Like the other tissues of the body, however, this particular tissue increases or decreases in bulk in correspondence with certain bodily needs, the

increase in bulk being largely due to the multiplication of the cells, whether eosinophile, hyaline, or rose-staining, by binary fission. This occurs freely in the body fluids, and may be watched outside of the body in a hanging drop.

(1.) The *eosinophile cell* (Plate 29, figs. 1–3, 10, 14), when resting, is spherical in shape. The more central portion of the cell is occupied by a greater or less number of highly refractive granules, each granule having a yellow-green lustre. These granules were identified by EHRLICH with his α, or eosinophile group.* With very high magnification the individual granules sometimes appear to have a short and long axis, and to be slightly spindle-shaped. The central portion of the cell, or endosarc, which contains the granules and the nucleus, is clearly distinguished from the delicate ectosarcal layer of very transparent mobile protoplasm, though, in the Frog, owing to the smaller size of the cell, the distinction is not so beautifully shown as it is in the larger eosinophile cell of *Astacus*.† The nucleus of the eosinophile cell is exceedingly characteristic. It is elongated and bent to a horse-shoe shape. The chromatin filaments are either irregular or radiate from two nodes, situated towards either end of the nucleus. Sometimes the nucleus is trilobed. When proliferation of the eosinophile cells is taking place they are to be found with more than one nucleus. Under appropriate stimuli the eosinophile cell becomes very active. Such stimuli are, normally, of a chemical nature and may be regarded as a change in the surrounding fluid. This may be produced either by clotting, or by the introduction of foreign substances, such as microbic poisons. If the cell is floating freely in the fluid, then the activity is confined to the thrusting out of the ectosarc as short filiform pseudopodia, which radiate from the still spherical endosarc, and do not necessarily result in locomotion. If, however, the cell is in the neighbourhood of a chain of active microbes, then the pseudopodial activity becomes so far modified, that the cell progresses towards the chain. Lastly, when the cell effects actual contact with the microbe the pseudopodial activity becomes suddenly changed into violent streaming movements, which result in the extension of the cell along the chain (figs. 10, 14, 17). So-called indifferent particles of any kind or shape, such as Indian ink, in no wise affect the activities of the cell, even though accidental contact occur. Contact with an active microbe, however, not only stimulates the cell to increased movement, but also produces a new activity of a glandular nature. The granules are thrust from the endosarc into the ectosarc, and travel towards that portion of the cell which is in contact with the chains; there they rapidly lose their brilliant refractive nature, shrink in size and disappear. (Fig. 10B.)

Complete discharge of the granules may occur, and then after an interval the cell may reform its granules which, at first, are different from the initial eosinophile granule. These processes are described in detail in a later section; enough has been

* EHRLICH, "Ueber die specifischen Granulationen des Blutes," 'Verhand. d. Physiol. Gesellsch. zu Berlin,' 1878.

† Compare figs. 1 and 5, Plate 7, 'Journal of Physiology,' vol. 13.

said here to substantiate the statement that in the eosinophile cell we are dealing with one which has both glanular and amœboid properties corresponding to differentiation of the cell's body into a central glandular endosarc, and a peripheral contractile ectosarc.

A change of medium also stimulates cell division. This process we have watched in a drop culture (Exp. 1), and in all cases the daughter-cell has been free from granules. In a comparatively short time granules commence to be formed at a point situated rather to one side of the centre of the daughter-cell.

The Re-formation of Granules.

The Amphophile Granule (fig. 17).—The glandular activity of the cell does not always result in a complete obliteration of the granule as a histological feature of the cell. Under the influence of poisons, *e.g.*, urari, or bacterial products, the granules may be so far altered that they stain with methylene-blue as well as with eosin. In other words they become amphophile in the sense in which EHRLICH uses the term. The relation of this change to the complete discharge of the granule can be better discussed after the different phenomena exhibited by the eosinophile cell have been more fully described. The amphophile granule is also produced in another way. The eosinophile cell will, after the complete or partial discharge of its granules, load itself with newly formed ones. These at first appear to be always amphophile, and there is, therefore, a stage in the elaboration of the eosinophilous substance at which this is amphophile.

(2.) *The hyaline cell* (figs. 1, 4, and 17) is the only one of the three elements which has been seen by us to manifest the phenomenon of phagocytosis, that is, which ingests and digests discrete particles. This cell is the "mononuclear" cell of METSCHNIKOFF.* Since, however, the other cells of the Frog are strictly mononuclear, we have thought it wise to adopt a different term. In the resting condition the cell is spherical. The cell-substance betrays no differentiation into ectosarcal and endosarcal portions. Therefore, when this cell becomes active, it may exhibit the wildest irregularities of form. There is no special pseudopodial character, the processes may be simple, or branched, they may be extraordinarily long and attenuated, or mere rounded eminences. Lastly, the cell may become excessively flattened until it is reduced to a protoplasmic film. The cell-substance is very clear and transparent, and free from specific granules. The nucleus is exceedingly characteristic. It presents, when stained, the appearance of a spherical bladder, formed by a very delicate staining membrane, and enclosing a sharply defined spherical nucleolus, which is placed at the centre of the sphere (fig. 4). Fine filaments may often be traced from the nucleolus towards the nuclear capsule, but they are seen with difficulty. When proliferation of these cells occurs nuclei may be found containing two nucleoli (fig. 17).

* 'Leçons sur la Pathologie comparée de l'Inflammation,' Huitième Leçon, p. 131.

The most prominent functional characteristic of the hyaline cell is its power of ingesting and digesting solid particles. At the same time this power is strictly limited, for the hyaline cell is absolutely incapable of ingesting, or even of effecting contact with, for instance, an intact and virulent anthrax bacillus. The bacillus must first be killed or maimed. In the presence of such substances as coagulated proteid, or dead bacilli, the cell exhibits the following well-marked phenomena. The food particle is ingested by pseudopodial activity in the ordinary way, and at first lies merely embedded in the cell-substance. A digestive vacuole is formed round the particle, which floats freely in the vacuolar fluid. The particle, if soluble (*e.g.*, the bacillus or proteid mass) is dissolved, and, lastly, the now empty vacuole closes. In other words, this cell accurately reproduces the ingestive and digestive phenomena of the carnivorous Protozoa.

Though we have not endeavoured to witness the fission of these cells, yet we have found them increase in number in a hanging drop. We have also seen a hyaline cell multiply in a hanging drop by a process of budding, rather than by binary fission. In this case the daughter-cell is about one-third the diameter of the mother-cell. Such young hyaline cells may be seen in hanging drops when leucocytosis, that is to say, an increase in the number of cells, has been induced by the presence, for instance, of some microbe (fig. 7).

The hyaline cell is much more easily killed by change of medium than the other two kinds of cells. It, however, exhibits considerable differences in this respect in different Frogs. If the cells be asphyxiated, as, for instance, by putting a cover-slip down on to a drop of lymph, they frequently burst in a way precisely similar to the bursting of the "explosive" or hyaline cells of *Astacus*.* Rarely a similar fate befalls some of the hyaline cells in a hanging drop, and it is probably due to the formation of a very dense clot. In the relative instability of its cell substance the hyaline cell recalls the similar ingestive cell of *Astacus*. In *Astacus*, however, the cell is so unstable that it has been called the "explosive cell." The hyaline cell of *Astacus* is similarly the phagocyte of that animal.

(3.) *Rose-reacting Basophile Cell* (figs. 4 and 5).—This cell, like the similar cell in *Astacus*, is characterized by its relative immobility. We have never seen any signs of active pseudopodial movement. In normal lymph the rose-reacting cells are very few in number (about two per cent. of the total number of cells), and those present are also small as compared with the eosinophile and hyaline cells. As a result of the presence of foreign matter in solution in the plasma, these cells increase both in number and in size, and become highly charged with granules. A similar increase is frequently associated with an œdematous condition of the animal. A comparison between figs. 5A and 5B will render clear the great difference between what may perhaps be called the resting and active condition of the rose-staining cells. The transition from the resting to the active condition will take place in hanging drops.

* Hardy, 'Journal of Physiology,' vol. 13.

In the resting condition the cells are small, spherical, and have a large spherical nucleus. The cell-substance is very scanty, but is charged with granules which are much smaller and very much less refractive than the eosinophile granule. It was in these cells that EHRLICH first described the existence of the fine basophile granule, or δ-granulation.* In the latter character, namely, the duller appearance in the living cell, they resemble the similar rose-reacting granules of *Astacus*. The spherical nucleus either stains uniformly, showing no chromatin filaments, or it encloses a central irregular chromatin mass, from which filaments stretch to the nuclear capsule.

The enlarged active cell is usually angular in shape, and the nucleus is then mostly oval. The cell-substance is highly charged with granules, which, as in the similar cell of *Astacus*, extend quite to the margin of the cell. There is never any trace of an ectosarc. Not infrequently the cell body contains a vacuole. We have never witnessed the manifestation of any kind of ingestive activity on the part of the rose-reacting cell, and the increase in size and number appears to be related solely to the presence of substances in solution in the lymph. Little is known of the distribution of these cells in the body.

In *Astacus*, as has been previously shown,† similar cells inhabit a peculiar tissue, which forms the adventitia of the anterior sternal artery.

In the Frog they are normally present in the connective tissues, and in the Mammalia, probably, identical cells are found in the peculiar adventitia of the arteries of the spleen and grouped about the capillaries, especially of the splanchnic area. Dr. SHERRINGTON exhibited specimens to the Physiological Society, in May, 1891, which showed very similar cells grouped about the blood-vessels of the intestine in cases of cholera.‡

Giant Cells (figs. 11 and 16).—These do not normally occur in the Frog, but are formed at a certain stage after the introduction of microbes into the lymph. Their formation may be followed in the hanging drop, and they are then seen to be very remarkable bodies, produced by the partial fusion of eosinophile cells or hyaline cells, or both. They are, therefore, of a plasmodial nature. As will be seen later, their formation recalls, in its mode and effects, the temporary conjugation of some Protozoa, and similarly these plasmodia ultimately disintegrate into their constituent cells (see Section V.). These plasmodia are produced either by the fusion of (1) eosinophile cells—in this case the fusion is limited to the ectosarc—or (2) hyaline cells, when the fusion of cell-substance is, so far as can be seen, complete.

* "Beiträge z. Kenntniss d. Granulirten Zellen, &c." 'Verhand. d. physiol. Gesellsch. zu Berlin.' 1878–79, No. 8.

† HARDY, 'Journal of Physiology,' vol. 13, p. 177.

‡ Since writing the above we have found that the basophile cell found in the peritoneal cavity of the Frog differs from the basophile cells found elsewhere in the body. The differences are of two kinds: (1) the peritoneal cells are larger, and (2) the basophile granules which they contain are larger and are well-defined spheres. The peritoneal cells thus show granules belonging to EHRLICH'S group γ.

Lastly, there is also (3) a plasmodial mass, formed of both eosinophile and hyaline cells. With regard to the third case we are not prepared to say whether the fusion is complete, a single plasmodium resulting, or whether the plasmodium is double, the eosinophile cells being in contact with an inner hyaline plasmodium.

Various observers have noted the fact that the eosinophile cells are relatively more numerous in Frogs during winter. Our observations have been confined to the summer months, and we have found that the relative number of the different classes of cells varies in different parts of the body. In the lymph from the subcutaneous lymph spaces the eosinophile variety form from 17 to 25 or 30 per cent. of the total number of cells. The basophile cells form about 2 per cent. In the peritoneal fluid the percentage of eosinophile cells is higher, ranging from 30 to 50 per cent.

The histological structure of the sporadic mesoblast of the Frog, excluding the red corpuscles and platelets, which stand on a different footing from the rest, may be summarized as follows :—

Normal	I. Cells normally free in the blood and in the lymph.	(*a.*)	Eosinophile cells, nucleus horse-shoe shaped or lobed; do not ingest particles; but are motile unicellular glands.
		(*b.*)	Hyaline cells, free from specific granulation: nucleus round with central nucleolus. Phagocytic, *i.e.*, they possess the power of ingesting and digesting discrete particles.
	II. Cells are few in number and small in normal lymph. Normally present in the lacunar spaces of areolar tissue.	(*c.*)	Basophile cells, spherical, with scanty protoplasm when small, angular, rounded or flattened when large, cell-substance charged with tiny basophile granules, which give a vivid rose colour with methylene-blue. Large oval or round vesicular nucleus, sometimes containing irregular chromatin mass and filaments.
Abnormal	III. Large amœboid cells, vacuolate, with ingesta frequently in the vacuoles, multi-nuclear, very active and phagocytic.		Giant cells formed by fusion of hyaline cells similar to the large phagocytic cell of *Astacus*.
	IV. Small bodies either round and quiescent or amœboid.		Nucleated cells budded off from the eosinophile or hyaline cells.

Section III.

Leucocytosis.

The most constant phenomenon of inflammation in a vascular part is the appearance of an increased number of wandering cells in the tissue outside the blood-vessels. Some of these cells appear on the scene by means of migration from the interior of the blood-vessels, others find their way thither by migration from neighbouring tissues along lymph paths, but the increase is also largely due to the direct multiplication of the cells by fission on the spot. Whether these cells are attracted by means of "chemotaxis" or not, does not concern us here, and we shall abstain

from offering an explanation of the process or cause leading to this collection or infiltration of cells. A question which was of much greater importance to us is the nature of the cells, and the sequence in which these cells appear. On injecting substances like anthrax culture under the skin of the Frog, do the three kinds of cells appear simultaneously, or does one class of cell appear before another?

This is a point which, so far, has been neglected by those who have made "chemotaxis" a subject of special investigation. Being satisfied, or assuming, that the attracted leucocytes are phagocytic in property, they have considered it sufficient to prove that, in certain animals, by means of certain substances, or bacilli, leucocytes are attracted, and positive chemotaxis was considered to be of special use to phagocytosis, because it was tacitly understood that the leucocytes attracted to the spot were always active phagocytes. We shall now show that this conception is true in part only, and that the usefulness of the wandering cells in the conflict against injurious substances does not lie wholly in their phagocytic powers.

We have seen that the Frog possesses three kinds of wandering cells. Of these, the eosinophile cells are never phagocytic, the hyaline cells, on the other hand, are so. If, therefore, the usefulness of "positive chemotaxis" in the battle against micro-organisms lies solely in the fact that thereby phagocytes are attracted, the hyaline cells should be the ones to appear on the battle-field. We found, however, that the cells which first collect in greatest number (or are attracted) are the eosinophile ones, and that it is not until some time has elapsed that the hyaline cells become evident.

On injecting a fresh culture of anthrax bacilli into the subcutaneous tissue of a pithed Frog, and keeping it at the ordinary temperature, which was 12° C. at the time these experiments were performed, and removing drops of lymph with a capillary pipette at intervals of half an hour, it was noticed that the leucocytes which first appeared were the eosinophile ones. They rapidly increased in number until the third or fourth hour after inoculation, and collected in masses around the bacilli. From the third or fourth hour onwards hyaline cells become conspicuous, increasing gradually in number. The eosinophile cells still further increased, and after eighteen or twenty-four hours their number was very great, and often masses of bacilli could be seen surrounded by those cells, and wherever the number of eosinophile cells was greatest, there also the hyaline cells were most conspicuous, and phagocytosis most marked.

The first phenomenon noticed, therefore, after an inoculation with anthrax, is the appearance of eosinophile cells, or, *sit venia verbo*, an eosinophile leucocytosis. Many of these cells certainly find their way to the field of action by migrating from the vessels, others from the neighbourhood, eosinophile cells being always found free, *i.e.*, outside the vessels, in the lymph spaces. Undoubtedly also the cells multiply *in situ*. For, as we shall show later, a multiplication of the eosinophile cells may be easily demonstrated on the slide in a hanging drop; again, all the above phenomena may

be watched in the amputated leg of a Frog, and also if after stripping the skin of a Frog's thigh from the muscles, and tying it at both ends so as to convert it into a closed tube, we inoculate this tube with anthrax bacilli, and keep it at the ordinary temperature. "Chemotaxis," therefore, cannot by itself explain this leucocytosis, for besides an attraction, if such exist, we also have an active proliferation of eosinophile cells.

On repeating the above experiments with cultures of *Bacillus pyocyaneus*, or with beer-yeast, or even with inorganic irritants, such as nitrate of silver, the same result was always obtained: the cells to appear first were invariably the eosinophile leucocytes, and it was only later that the phagocytes increased at the seat of lesion.

Other methods also were employed to show that the eosinophile cells are the first attracted. On placing small sponges dipped in cultures of anthrax, pycyaneus, or yeast, or even in a very dilute solution of nitrate of silver, either under the skin or into the peritoneal cavity of a Frog, after two to four hours eosinophile cells were almost exclusively found in the meshes of the sponge. Lastly, on placing capillary tubes filled with the same substances under the skin, or into the peritoneal cavity of a Frog, after two to four hours the same result was obtained.

It is only after the eosinophile leucocytosis is established that the hyaline cells begin to appear in appreciable quantity, and they now rapidly increase in number. At the same time, however, the eosinophile cells do not cease to increase, so that there is both an eosinophile and a hyaline leucocytosis. The hyaline cells at once become active, and begin to take up the bacilli, and, as we said above, are most active where the eosinophile cells are most numerous. This has been observed so consistently, that even in the absence of better evidence, a correlation between phagocytosis and the eosinophile leucocytosis suggests itself.

The hyaline cells now rapidly increase in number, and after eighteen to twenty-four hours are often very numerous; they may eventually outnumber the eosinophile cells, and they mostly contain bacillary remains in their interior.

When phagocytosis is at its height we notice yet another change. The rose-reacting cells, which under ordinary conditions are but sparse, are now greatly increased, so that at one time (about the eighteenth to twenty-fourth hour) all the three cellular elements are numerous.

We have then the following changes after inoculating a Frog with anthrax. At first, the eosinophile cells appear and collect around the bacilli (eosinophile leucocytosis); then the hyaline cells appear (hyaline leucocytosis) and soon show a keen phagocytic action, the eosinophile cells increasing, however, at the same time; and latest of all the rose-reacting cells also increase (rose-reacting leucocytosis). We shall attempt to show later the specific value of each of these groups of cells. One form of leucocytosis merges into and overlaps the other, and it is difficult to separate them from each other by any time limit. This much, however, is certain, that we have

never observed phagocytosis with virulent anthrax without previous eosinophile leucocytosis.

If, for some reason or other, the eosinophile cells do not appear, the hyaline cells are apparently powerless. Thus, on warming a Frog which has been inoculated with anthrax, the eosinophile leucocytosis is absent, and though phagocytes appear at times in large numbers, the bacilli will thrive, being left unharmed. It cannot be objected that heat "paralyses" the phagocytes, because some of them did contain bacilli, and they will not refuse Indian ink or vermilion particles, though exposed to a temperature of from 25°–30° C.

Again, on narcotizing a Frog with a mixture of chloroform and ether, and inoculating it, while under the influence of the anæsthetic, with anthrax baccilli, the latter, as KLEIN and COWELL have shown, will often grow well. In some cases, however, even under these conditions, they will refuse to grow. In the latter cases, the eosinophile leucocytosis is always extremely well marked, in the former, the number of eosinophile cells is very small. Here also it cannot be claimed that the chloroform-ether mixture paralysed the phagocytes, because the latter, at times, were present in fairly large numbers, and, though they left the bacilli untouched, they invariably took up spores.

The same was observed when the circulating blood was replaced by saline solution (·75 per cent.). A cannula was tied in the conus arteriosus and the vena cava ant., and the NaCl solution was allowed to circulate under a low pressure for 2–4 hours, and then the Frog was inoculated in its subcutaneous tissue. The bacilli grew in most cases, and then the absence of an eosinophile leucocytosis was marked, although the phagocytes were often present in large numbers, containing, in many instances, spores.*

No doubt here, chloroform and ether and the artificial circulation cause changes in the chemistry of the tissues, but it must always remain a significant fact that in the absence of eosinophile cells the bacilli thrived excellently. The result of warming a Frog for a long time, keeping it for instance at 25° C. for a week or more, is quite different from that of a brief warming. If, at the end of the longer period, the animal be inoculated with anthrax, it is found to be immune as in the normal condition, and now, unlike the case of the brief warming, an abundant eosinophile leucocytosis takes place. Lastly, it may be mentioned here, that in many instances a cluster of bacilli was seen to be closely surrounded by a mass of eosinophile cells, with hardly a phagocyte near. The bacilli were then extremely degenerate and broken up, or forming spores, staining very badly or indifferently with methylene-blue.

All these observations tend to show the great importance of the eosinophile cells in the conflict against the bacilli or micro-organisms.

Leucocytosis in a Hanging Drop.—The three stages of leucocytosis can also be demonstrated outside the body on the slide, by means of a hanging drop. On

* Compare footnote to page 296.

removing a drop of lymph from a Frog and inoculating it with anthrax, and selecting a suitable spot for examination, it is seen that the eosinophile cells collect in numbers around the cluster of bacilli. In a successful specimen there is a well-marked increase of these cells, and it is possible at times to watch the division of these leucocytes.

Later on, the hyaline cells approach the cluster, so that at this time the mass of bacilli is surrounded by both eosinophile and hyaline cells. The former appear to be present in larger number. On examining the hanging drop 12–18 hours after inoculation, staining it previously with a rapidly fixing solution of methylene-blue, a decided increase in the number of rose-reacting cells is noticed. The latter cells are found in all stages of development, from the small round form to the large and very granular one.

On the slide therefore, as within the body, the phenomena are identical, and in each case the inoculation with bacilli is followed first by a collection or aggregation of eosinophile cells, next the hyaline cells appear in appreciable numbers, and the rose-reacting cells also increase at the scene of action.

We have, in the hanging drop, also seen that whenever the initial eosinophile leucocytosis was absent, the hyaline cells did not exert any activity and were not attracted in numbers. On the other hand, whenever the eosinophile cells had collected around the bacilli in large numbers, in all typical cases the hyaline cells streamed towards the bacilli and ingested them eagerly. Here, therefore, we again find that there exists a distinct correlation between phagocytosis and eosinophile leucocytosis.

The correlation between the increase in number of the basophile cells and the other kinds of leucocytosis is much more difficult to prove, because it seems that on simply keeping a drop of lymph over night in a moist chamber these cells will increase, though not in the same measure as after a previous inoculation with anthrax.

Again, on inoculating a drop of lymph with a solution of albumen, the rose-reacting leucocytosis is well marked, though an increase in the eosinophile cells may not be observed.

The question naturally arises as to how far the three kinds of leucocytosis are really distinct, as to how far they may occur independently.

There appears to be little doubt that an increase in the numbers of one or other of the three types of cells may occur in the absence of a corresponding increase in the numbers of the remaining kinds.

In œdematous Frogs the lymph is usually highly charged with rose-reacting cells, and this may occur with a diminution in the number and vitality of the eosinophile and hyaline cells. Similarly the injection into a lymph sac of finely divided and sterile coagulated proteid, such as is produced by boiling a solution of egg albumen, leads to an enormous increase in the number of hyaline cells, without a corresponding increase in the eosinophile cells. On the other hand, we have never witnessed an

eosinophile leucocytosis unaccompanied by a subsequent increase in the numbers of the other cells.

Phagocytosis.

Having thus far described the general changes which follow on an inoculation with bacilli, and the order in which the various cellular elements appear at the seat of injection, it remains now to discuss the minute changes and the parts which the different cells play in the conflict. Before doing so we must indicate what we consider to be the proper phenomena connoted by the term "phagocytosis."

M. Greenwood's study of the Protozoa, and particularly of Amœba, has made it possible to give to this word a precise meaning.* The phenomena which follow the ingestion of a particle by one of the Protozoa are as follows:—(1) If the particle is digestible it at first lies embedded in the cell-substance, then a vacuole is formed about it so that the food mass now floats freely in a digestive fluid. Solution of the particle is more or less completely effected, and lastly, the vacuole closes, and if there be any insoluble remnant it is extruded. (2) If, however, the ingested body is insoluble the physiological reaction is, as it were, incomplete, and, as in the stomach of the highest animals in similar circumstances, there is no secretion of a digestive fluid, and therefore no formation of a vacuole.

Thus the salient feature of the process is the inclusion within the cell's body of discrete particles. If the ingesta be of a nutrient nature then certain well-defined phenomena follow, namely, the formation of the digestive cavity and the digestion of the fragment. If, on the contrary, the ingesta are insoluble, then the phenomena stop short at the ingestive act. Following these lines we would define phagocytosis as being primarily the inclusion of discrete particles in the body of a cell. This may be followed by the formation of a digestive vacuole and the solution of the particles This is the complete process of phagocytosis; or the included particles may simply remain embedded in the cell's body, and to this incomplete act the term must also be applied. In brief, phagocytosis implies an intra-cellular process. Extra-cellular digestion may occur, but it is not phagocytosis.

Section IV.

(1.) We shall now proceed to describe the appearances and changes in a hanging drop of Frog's lymph, kept on a moist stage without being previously inoculated.

On preparing a hanging drop as described above, and examining it under a high power (Zeiss D or E, oc. 4) we see that it is rich in cellular elements. These consist of the wandering cells with which we are dealing, together with some red blood corpuscles. The eosinophile cells at first sight usually appear to be most numerous, owing to the striking appearance presented by their refractive granules. the rose-

* 'Journal of Physiology,' vols. 7 and 8.

reacting cells are by far the least numerous. It is, however, by no means easy to recognize the latter in the fresh unstained condition; but we have, by means of stained control-specimens, convinced ourselves of the great scarcity of these cells at this time.*

It may here be remarked that good and healthy lymph clots rapidly, and that this property may serve as a criterion of the excellence of the lymph. It was often found that when our Frogs were diseased their lymph refused to clot, and, on the other hand, whenever lymph refused to clot, the changes and phenomena to be described later did not appear.

The drops were placed under the microscope as speedily as possible. It was then noticed that the eosinophile cells at once became active, throwing out pseudopodia. Their movements are rather sluggish, and the change of form is also a slow one. The cell will soon again retract its pseudopodia and become spherical, and then once more throw out pseudopodia. It will thus alternately change its shape, and cease to do so only when it is apparently dead, being then spheroidal and regular in outline (fig. 3).

The hyaline cells are more numerous, but are less readily seen. As a rule they are very active, and are seen to wander about the field and to throw out long and thin pseudopodia.

It is, as we have just said, extremely difficult to recognize the rose-reacting cells in unstained drops, the regular spherical nucleus surrounded by a granular protoplasm being the only guide. In an ordinary drop they are apparently quite inert. They, however, increase in number and size.

Effect of Inoculating a Hanging Drop with various Substances.

By noting the different effects produced by different substances, when added to a hanging drop of Frog's lymph, one is soon convinced that these substances might be divided into classes according to whether they affected more particularly one or other of the different cells. In one class would be grouped those substances which are completely unnoticed by the eosinophile cell, while they were readily ingested by the hyaline cell. Indian ink, anthrax spores, and coagulated proteid are instances. Another class would include those bodies which attract both kinds of cells, such as vermilion, which has a slight action on the eosinophile cells, and is readily incepted by the hyaline cells, and yeast cells, which have a very pronounced attraction for the eosinophile cells, and are taken up by the hyaline cells to a relatively limited extent. Yet another class would embrace substances which at first profoundly attract the eosinophile cells, and only after they have been subject to their influence can become the prey of the hyaline cells, such as active growths of *B. anthracis* and *B. filamentosus*. Lastly, there would be the soluble substances, the action of some of which, at any rate, is, like that of egg albumen, mainly limited to the rose-reacting cells. But

* Compare the relative numbers of the different cells given at the close of Section II.

the completion of such a series can only be contemporaneous with the attainment of a full knowledge of the functions of the various wandering cells, and, for the present, we can only note the differential action of various substances when introduced into the body, or into a hanging drop of lymph.

The effect of some of the substances, which we have so far examined, may now be given in detail.

(*a.*) *Inoculation with Anthrax Spores.*—When a drop of lymph is inoculated with anthrax spores obtained from an old agar-agar culture, many of the spores are at once taken up by the hyaline cells present.* A single cell may take up several of them. The spores are apparently destroyed by the phagocyte, for, on allowing the specimen to rest at the temperature of the room (15–20° C.), while many of the spores developed into bacilli—a process which can easily be watched under the microscope—these spores were invariably extra-cellular; in no case did we observe spores, which had been ingested, develop into bacilli. Food vacuoles were, in many cases, observed within the cell around the spore, showing that these bodies were being digested. The hyaline cells seemed to take up the spores immediately without the intervention of the eosinophile cells, there being thus, as we shall show, a great difference between the ingestion of bacilli and the ingestion of spores. It is difficult to say whether or no the eosinophile cells exert any influence on the spores, but this much is certain, that the spores may be taken up and digested by the unaided hyaline cells. Nor did we notice a movement of the eosinophile cells towards a mass of spores collected on the cover-glass, like that which occurs in the case of the bacilli.

On the other hand, when the spores had grown out into bacilli, the eosinophile cells were at once seen to move towards them and attack them in the manner we shall describe later when we come to discuss the conflict between the cells and the bacilli.

(*b.*) *Inoculation with Indian Ink.*—The particles are completely unnoticed by the eosinophile cells, but are readily ingested by the hyaline cells.

(*c.*) *Effect of Finely-divided Coagulated Proteid.*—The results are most interesting, and may be mentioned here, though they are most useful as aiding us to interpret the phenomena of repair.

The coagulated proteid was prepared by dissolving white of egg in tap water, filtering, and then boiling the filtrate. An exceedingly fine precipitate may be thus produced. If this be injected into a lymph sac of a Frog, or added to a hanging drop, it is found to induce an excessive leucocytosis, which is almost limited to the hyaline cells. These ingest the exceedingly fine proteid particles, and mass them into balls which superficially resemble a specific granulation, and stain blue with methylene-

* The observations of Ward, on the destructive action of light on anthrax spores, which have been published since the MS. of this paper was completed ('Roy. Soc. Proc.,' vol. 54, p. 472) make it possible that the spores, which were so readily ingested by the hyaline cells, were already dead. It will be noticed that the spores used by us were obtained from old dry (agar-agar) cultures.

blue. For the present, the important point to notice is that the injection causes a proliferation, or leucocytosis of the hyaline (ingestive) cells.

(*d.*) *Effect of Solution of Egg Albumen.*—This has been only partially investigated, and one result will alone be emphasized here. If a sterile solution of egg albumen be injected into a Frog, or added to a hanging drop of Frog's lymph, it results in an abundant leucocytosis characterized by an increase in the number and size of the rose-reacting cells.

(*e.*) *Inoculation with Vermilion.*—In the hanging drop we have observed that the phagocytes may take up vermilion particles without the intervention of the eosinophile cells. But we never saw a destruction or digestion of the pigment particles, so that it is quite possible that the hyaline cells simply take up vermilion granules in order to carry them away without destroying them. We can, however, safely conclude from our experiments that the vermilion particles may be taken up by the hyaline cells without the intervention of the eosinophile cells.

These observations were controlled by experiments in the body. Anthrax spores and vermilion particles are rapidly taken up and carried away, without a previous eosinophile leucocytosis at the seat of inoculation. It is often stated that the spores first develop into bacilli and are then taken up by phagocytes. Observations on the living (pithed) Frog, however, show that most of the spores are taken up by cells, and at once destroyed. Many of the extra-cellular spores, however, develop into bacilli and are subsequently destroyed.

Saline solution injected subcutaneously does not lead to any apparent changes, the eosinophile cells are not increased, and the hyaline cells seem to be unaffected.

Section V.

The Behaviour of the Cells towards Active Growths of Bacillus anthracis *and* B. filamentosus.

We shall now proceed to describe, in detail, the phenomena observed in a hanging drop following on an inoculation of bacilli (and their products). *Bacillus anthracis* and *Bacillus filamentosus** in fresh cultures, readily grow into long chains, which form very convenient objects for continued observations. These bacilli are non-pathogenic, so far as the Frog is concerned, but they by no means react as indifferent substances, since when injected under the skin they always lead to a typical inflammation.

It will be seen that the phenomena observed when these bacilli are used are widely different from those noticed when innocuous substances are employed. With very slight differences the appearances are the same, whether anthrax or filamentosus bacilli are employed.

* This bacillus has been separated by Klein, who kindly gave us a culture. It grows in beautiful filaments, being, however, non-pathogenic.

On inoculating a drop with a trace of bouillon culture (the latter should be fresh, preferably not older than twenty-four hours), and observing a suitable chain of bacilli, we find the eosinophile cells which happen to be in the neighbourhood travel towards the chain, performing slow amœboid movements during their progress. Some of the cells apparently come from some distance. This may be an attractive influence exercised on the cells by the bacilli and their products, or it may be the expression merely of the active movements induced by the change of medium. When the cells reach the chain or individual bacilli, they at once become extremely active, extending and retracting their pseudopodia, and a constant streaming of the granules may often be observed. The granules, moreover, may be discharged, or at least wholly disappear, so that the same cell at one time appears highly granular, at another almost hyaline. Then suddenly the cell will apply itself along the bacillus and, so to speak, pour itself along it, being in some cases applied to one side of the rod or chain, in others surrounding it, and yet in others fixing itself at the extremity of a bacillus. (Figs. 10, 13, 14, 15, 17.)

At first sight it seems as though these cells had taken up the bacilli, but careful or continued examination will easily prove that this is simply an appearance. The bacilli can, in most cases, be clearly seen to lie on or under the cell, or the cell is, so to speak, folded around the bacillus, and, if from any cause the cell contracts to a sphere, the sphere does not enclose the bacillus but only touches it. Moreover, as we shall show later, the eosinophile cell eventually leaves the bacillus, handing it over to the hyaline cells to be devoured. In fact, almost numberless observations have proved to us the correctness of METSCHNIKOFF's* statement that the eosinophile cells are never phagocytic. We have tempted them with charcoal, vermilion, Indian ink, with many kinds of bacilli and cocci, with curare and other substances, but they refuse all things alike.

Thus the eosinophile cell comes into apposition with the bacillus and streams along it, losing its previous spherical shape. It is now more or less fusiform, so that we have the appearance shown in figs. 10B and 17. In many cases by focussing one can detect the bacillus and determine with ease that it does not lie *in* the substance of the cell, but in other cases it is impossible to recognize any trace of the bacillus in the area occupied by the cell.

The latter will remain motionless for some time, the only change noticed being a continual fluctuation in the appearance and number of the granules. After a time the cell may again change its shape, becoming once more spherical, soon to assume another shape, and once more to "pour itself out" along the chain.

During this time other cells apply themselves along the chain, passing through the same phases. Others, on the other hand, simply swarm around the chain, so that at one stage the latter is both actively attacked by some eosinophile cells and closely

* 'Leçons sur l'Inflammation.' Paris, 1892, p. 136.

surrounded by others. In the case of the later arrivals the loss of granules is not so complete. This may be said to be an inverse function of the number of cells attacking the chain. The mass of eosinophile cells gradually contracts, so that the result is a "plasmodial" mass hiding the chain (figs. 11, 11A, &c.). It should be stated, however, that some of the eosinophile cells may leave the bacilli soon after they have poured themselves along the chain.

The next phase is the approach of the hyaline cells or phagocytes. Several of these come up and become hidden in the plasmodial mass, which now presents the appearance of an opaque rounded or nodulated mass with separate heaps of granules on the surface (fig. 11B). The contact of a fresh cell, as it comes to lose itself in the mass, acts as a stimulus, and causes active streaming and writhing movements. These become fainter and fainter until they are re-awakened in their first intensity by the arrival of another cell. Now gradually one eosinophile cell after another separates from the mass and moves off. Eventually we find that all which is left behind is a plasmodium of the hyaline cells containing fragments of bacilli enclosed in vacuoles, thus showing that an actual digestion is going on (fig. 16B). The chain of bacilli has thus succumbed. The digestion of the bacillary fragments is completed, and finally, at any rate in some cases, the plasmodium of hyaline cells breaks up into separate cells.

This is the description of a typical conflict. We have thus two stages—(1) the attack by the eosinophile cells; (2) the ingestion and digestion by the hyaline cells.

The attack by the eosinophile cells is an active one, and one which must be carefully distinguished from phagocytosis. Phagocytosis it is not, because we have neither ingestion nor digestion. There can be no doubt that the eosinophile cells have a distinctly harmful action on the vitality and growth of the bacilli, for it is seen that in cases where a chain is attacked by a sufficient number of these cells, without the subsequent access of hyaline cells, all growth is suspended. The bacillus apparently dies, or, at any rate, nutritive activity is impaired, because, ceasing to grow, it will either form spores or become absolutely unstainable. An actual solution or destruction of the bacillus by the eosinophile cells has not been observed, but a chain will often be seen to be broken up into fragments, or to become diminished in size.

In many specimens, where the number of eosinophile cells was large and the hyaline cells sparse, no growth was noticed anywhere in the drop, but many plasmodial masses were observed, within which the bacilli were hidden.

The eosinophile cells proliferate, cell division being not infrequently observed, and young stages being by no means uncommon. They also seem to possess a high degree of vitality, for eosinophile cells which apparently had done their duty were seen to leave the plasmodial masses or the chain of bacilli, and to direct themselves to another field of action.

At the end of the process the number of rose-reacting cells is greatly increased, cells in all stages often being found in great numbers.

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We have, by numerous observations, convinced ourselves that these phenomena are not simply stage phenomena, but that they also occur in the organism. By removing lymph at different intervals from the seat of inoculation of a Frog injected with anthrax, all the various stages can be easily obtained.

These experiments then throw new light on phagocytosis, showing that previous to the ingestion and digestion of the bacilli there is an attack by the eosinophile cells, which apparently prepares the bacilli to be taken up by the hyaline cells. That this is actually the case, observations in cases where the bacillus is victorious will show.

In most hanging drops there are always some parts where the bacilli are not destroyed or impaired, but develop typically. Now here there has been either a total absence of an attack by the eosinophile cells, or a very incomplete one, the cells, after a feeble attempt, leaving the bacillus to its own fate. A part of a chain actually in contact with or surrounded by an eosinophile cell, however, seems to be hopelessly beaten and refuses to grow, though rapid growth may occur in the unattacked parts of the same chain. Lastly, we have never seen a hyaline cell take up and digest a bacillus or chain of bacilli that had not previously been attacked by the eosinophile cells.*

The following is a detailed description of a few among our many observations :—

Experiment I.

Drop I.

Two drop cultures made from lymph from thigh of a Frog.

Hanging drop inoculated with a small quantity of anthrax. A moist chamber was made, as described in Section I., and over this was placed a sterilized cover-slip, with a tiny drop of bouillon from an anthrax culture on the under surface. Lymph was then taken from subcutaneous spaces of the thigh with a fine pipette, a drop rapidly added to the anthrax, and the cover-slip again inverted over the chamber. The preparation was then as quickly as possible brought on to the stage of a microscope and examined with Zeiss ob. D, oc. 4. From time to time sketches were made (*cf.* figs. 11, 11A, and 11C).

Watched a long chain of bacilli in the centre of the field. Two eosinophile cells came to each end, and one to the middle of the chain. Five cells in all.

As soon as the cells reach the bacillus rapid streaming movements occur, and spread the cell-substance along the chain. Rapid discharge of granules.

Half hour.—Eosinophile cell at one end divided. Daughter-cell free from granules. It moved away from the chain. Within the next half-hour the same cell was seen to divide again. Also one of the cells at the other end of the chain was seen to divide.

Two more eosinophile cells have reached the chain, on which there are now seven cells.

Chain broke in two. Two eosinophile cells are on the north piece, and this was watched.

1 hour.—A hyaline cell has now moved to the chain and ingested one end. It has pushed out a long pseudopodium to the other end of the chain. (The chain is slightly bent, fig. 11.)

* This is only true when freshly made cultures of virulent bacilli are used. Compare Section IV., p. 295, and Section VI., on the conflict with yeast torulæ.

The pseudopodium is being contracted, thus slowly bending the chain.

The hyaline cell is displacing the eosinophile cells and more completely ingesting the chain; part of the chain still attacked only by eosinophile cells.

A third eosinophile cell rapidly moves towards the mass, and then throws out a delicate fringe of pseudopodia, which touch the mass, finally fusing with it. The contact of the new cell provokes violent streaming movements. Another eosinophile cell fuses with the mass (fig. 11B).

The mass has now become rounded, the eosinophile granules have been re-formed, and we have four heaps of granules representing the four eosinophile cells on the surface of the mass. The hyaline cell and bacillus are completely hidden.

N.B.—The last two eosinophile cells, and all those which join the mass later, do not completely discharge their granules.

2 hours.—The whole field of the microscope has now become very full of cells, all of which, both eosinophile and hyaline, are in active amœboid movement.

Churning streaming movements of the mass slacken. A fifth, and then almost immediately a sixth eosinophile cell fuse with the mass, their onset, as before, causing violent streaming movements. Blunt pseudopodia are also thrust out.

Sometimes the mass becomes lobed, and one sees a heap of very numerous, large, and very refractive granules in each lobe. Again all outlines fade, and the spherical mass has a remarkable refractive, curdled appearance, and is very opaque (fig. 11C).

2 hours 20 minutes.—A second hyaline cell has fused with the mass.

$2\frac{1}{2}$ hours.—Preparation accidentally shifted. Low power put on to find the mass again. This is quite impossible, for the whole drop is now studded with similar masses. There are also a great number of free eosinophile cells. An enormous multiplication of these cells has taken place. They are all heavily laden with granules. No free bacilli.

3 hours.—Another mass chosen for watching; a large one (fig. 11D). Hyaline cells very extended and numerous.

$3\frac{1}{2}$ hours.—Hyaline cells exceedingly numerous and exceedingly irregular. Some which have wandered into the field are very large, and show vacuoles which contain fragments of bacilli. They are *very* active (fig. 12).

The mass all this while assuming more and more the appearance of a heap of cells. All pseudopodial and streaming movements ceased.

The mass now looks like a heap of eosinophile cells. These are separating from one another and are moving away.

4 hours.—Twenty-five minutes later the eosinophile cells are gone, and disclose a large central hyaline cell. This stage carefully drawn with camera lucida (fig. 11E).

$4\frac{1}{2}$ hours.—Drop examined, and shows general phagocytosis. Observation discontinued.

Drop II.

Contained, in addition to scattered chains, one large mass of anthrax filaments.

Cells very much less abundant than in Drop I.

Three eosinophile cells attacked a cluster of anthrax filaments. Owing to relative paucity of cells the greater part of the anthrax in the field remained unattacked.

One cell watched attacked a mass of bacilli, and streamed along a chain showing active movements.

Movements gradually ceased, and the cell contracted into a ball, *which did not enclose the bacillus but remained just touching it.*

After a prolonged period of absolute quiescence the cell again became active, and now moved away from the bacilli. It twice changed its line of movement to avoid bacilli, and finally came to rest in an isolated position.

During the third and succeeding hours the anthrax grew with immense rapidity.

The notes of another drop-culture experiment, in which the phenomena were followed to the final dissolution of the plasmodium of hyaline cells and the close of phagocytosis, are as follows :—

Experiment II. (figs 16, 16A and C).

Drop culture made with subcutaneous lymph and observation started at 11.45.

11.45.—Eosinophile cells moved up to a chain.

A hyaline cell is near the south end of the chain.

There are now six eosinophile cells on the chain.

12.30.—The granules are constantly changing. Only four distinct cells now, three having fused.

12.35.—The movements of the eosinophile cells have bent the chain.

12.45.—Fusing of the eosinophile cells only partial. Can now count seven cells on chain.

A phagocyte at the south end passes into the mass and is soon lost sight of, as the eosinophile cells went all round it, moving *en masse* in a churning manner.

1.0.—Another phagocyte came into the field at the north end but wandered off again.

1.15.—The eosinophile cells are turning round and round, so that at one time it appears as though there were three or four cells, and then shortly eight or more.

1.40.—The eosinophile cells are separating. One already gone. A minute later another eosinophile cell went off with a sudden jerk and then came back.

1.45.—Central hyaline cell seen now. A phagocyte in the field has ingested a pigment mass. It is vacuolated and the vacuoles contain fragments of bacilli.

The same eosinophile cell left again and again came back.

2.12.—The eosinophile cells are leaving. The hesitating cell gone at last. Only one eosinophile cell left.

2.17.—Two hyaline cells have come from outside and fused.

2.35.—A fourth hyaline cell has joined.

3.35.—The hyaline cells now form a mass with food vacuoles (16B).

5.45.—The hyaline cells separating. Digestive vacuoles collapsing.

6.25.—Vacuoles collapsed.

As an instance of a case where the bacteria have ultimately triumphed, owing to their being present considerably in excess of the eosinophile cells, we may take the following experiment. In all such cases the conflict may be followed up to a point where the accumulation of bacterial products produces a complete paralysis of the eosinophile cells. Naturally this may occur at any stage in the conflict, and we have witnessed instances where the initial dose, so to speak, has at once paralysed the cells and instances which might aptly be styled drawn battles. In the latter the conflict will endure for hours, the eosinophile cells killing some of the bacteria, while in others the bacteria are so far worsted that they have to resort to spore formation.

Experiment III.

Drop Culture made containing a Large Quantity of *Bacillus filamentosus*.

11.15.—Scarcely any eosinophile cell with granules. In one case saw the granules shrink in size, leaving vacuoles.

11.30.—One long chain in the field stretches up from the bottom of the drop where cells mostly are into an almost cell-free region. Three cells on this chain. When first seen they were quite free from granules. Now they are re-forming them.

11.35.—Cells are moving up the chain and others are approaching the lower end.

11.38.—The eosinophile cells generally are re-loading themselves with granules.

11.40.—The appearance of the hyaline and eosinophile cells in the preparation is very striking (*cf.* fig. 18). Upper part of the chain still unattacked. A fourth cell is streaming up from the lower end. Another chain in the field, which lies lower down in the drop, is already closely surrounded by 12 granular eosinophile cells.

From this a short branch chain extends upwards which is free from cells.

The cells are all moving very sluggishly.

11.55.—The last cell to come on to the chain (No. 4) is hopelessly defeated. It bursts up and only droplets are left.

12.45.—No further attack has taken place. Cell No. 3 looks very bad and curdled. The bacilli are not growing. Two cells still on the chain are spherical, as are also the free cells.

1.10.—Some of the 12 cells on the deeper-lying chain are still extended. Others are rounded.

2.30.—Bacillus growing now where it has been unattacked. The chain which was attacked by 12 cells appears quite dead, but the short chain projecting upwards has increased in length.

Later.—Very rapid growth of bacilli. The chain attacked by the 12 cells, however, is completely dead. Of the other chain part was killed, and part which was exposed for only a short time to the attack of the eosinophile cells grew.

(The growth of the bacilli was always determined by making a plan of the chains and numbering the rods.)

The fact that the bacilli are attacked at first exclusively by the eosinophile cells was clearly shown in the following way:—About 0·2 cub. centim. of a fresh bouillon culture of anthrax was injected into the peritoneal cavity of a pithed Frog. When 20 minutes had elapsed the cavity was opened and film preparations made, and stained with eosin and methylene-blue. On counting the cells in these preparations, it was found that no less than 82 per cent. of the eosinophile cells were in contact with bacilli. On the other hand, only 2·6 per cent. of the hyaline cells were even in contact with bacilli.

In a second experiment of a similar nature it was found that 42 per cent. of the eosinophile cells were in contact with bacilli, and only 2 per cent. of the hyaline cells.

The cells were counted with Oc. 10, Ob. $\frac{1}{12}$ apochromatic oil immersion, by Powell and Lealand.

The phenomena seen in a hanging drop of lymph inoculated with anthrax or *Bacillus filamentosus* show clearly that the intruding organism is always attacked first by the eosinophile cells before the hyaline cells attempt to ingest them or their remains. But we are able to go a step further than this and say that the hyaline cell is incapable of ingesting an anthrax or filamentous bacillus before it has been killed or maimed by the eosinophile cell.

The sequence of events observed in experiments similar to those described in detail would afford strong presumptive evidence for this statement, and we find further support in noticing the different behaviour of the two kinds of cells towards the

bacteria and indifferent particles when they are present together in the hanging drop.

We have already seen that the eosinophile cells are not at all phagocytic, and that they are completely unaffected by the presence of indifferent particles in the plasma. On the contrary, the hyaline cells are attracted by such particles as vermilion, Indian ink, or coagulated proteid. They ingest them, and, if possible, digest them.

Now, in the drop cultures, we find that the phagocytes are never attracted by the fresh bacilli. They are even repelled by them. We have seen a freshly budded phagocyte come into the near neighbourhood of an anthrax chain (fig. 8) and there exhibit active pseudopodial movements, which neither resulted in locomotion of the cell, nor in effecting contact with the bacillus. After a while the movements slowed and ceased. Then they were renewed and now the cell moved away from the bacillus.

Bacilli and Indian Ink or Vermilion.—In a drop of lymph inoculated with bacilli and with Indian ink or vermilion, we see strikingly displayed the differential activity of the two kinds of cells. The phagocytes will readily ingest the indifferent particles, while the eosinophile cells with even greater readiness attack the bacilli.

Bacilli and Spores.—This is only true of the kinetic phase of the life-history of the bacillus. The potential form, the spores, are at once ingested by the phagocytes,* and in a hanging drop, inoculated both with bacilli and spores, we witnessed the eosinophile cells attacking the bacilli, while the phagocytes ingested the spores. In illustration of this we may quote the notes of the following experiment :—

Experiment IV.

A drop of Frog's lymph was inoculated with fresh bouillon culture of anthrax, and also with anthrax spores from an old agar culture.

3 P.M.—Spores taken up rapidly by hyaline cells without intervention of eosinophile cells—quickly vacuoles are formed, and some of the spores are being digested. At the same time the usual attack of eosinophile cells on the bacilli took place.

7.45.—Many extra-cellular spores have become bacilli.

9.30.—Eosinophile cells have attacked the newly-developed bacilli and everything is proceeding as before. There appears to have been a proliferation of eosinophile cells.

But to prove the statement that the destruction of anthrax and *Bacillus filamentosus* by Frog's lymph is due primarily to the eosinophile cell, it was necessary in some way to paralyse the eosinophile cell while leaving the phagocyte unharmed, and then show that under such conditions, namely, in the absence of the eosinophile attack, the phagocyte is powerless and the bacteria grow freely in the lymph. We have been enabled to do this in two ways.

Action of Heat.—It has already been shown (Section III.) that when a Frog is warmed to a temperature of 25–35° C. it becomes susceptible to anthrax. We there-

* *Cf.* note, p. 296.

fore tested the effect of heat on the processes taking place in our drop cultures, and found that the eosinophile cells are completely paralysed by a rise of temperature, and became quite incapable of attacking the bacteria. *At the same time the phagocytes retain their activity and freely ingest Indian ink particles or spores present in the same drop.* The thermometer of the warm stage indicated a temperature of 35 to 37°·5 C. in our experiments.

Action of Urari.—Turning to poisons, we obtained a similar differential action with urari. This drug produces a marked alteration in the eosinophile cells: their eosinophile granulation becomes changed into an amphophile granulation. This change occurs both in the body and out of the body with a sufficient dose of the drug. In the body, after a while, recovery takes place, and eosinophile granules are again found. In the same way, owing probably to the elimination of the poison, the neuro-muscular mechanism will, at length, recover, and the animal regains the power of movement. Out of the body in the hanging drop the cells appear incapable of recovery, and the amphophile granulation persists.

If a drop of lymph, taken from the body of a urarised Frog while the amphophile granulation persists, be inoculated with anthrax bacilli, we find that the bacilli are not attacked by the eosinophile cells, and after a time they grow.

Abnormal Lymph and Bacilli.—Lastly, among Frogs which have been long kept in confinement in the laboratory and starved, individuals are sometimes found more or less œdematous, and whose lymph contains few eosinophile cells incompletely charged with granules which are sometimes largely amphophile. *B. filamentosus* has been found to multiply in the body of such animals, and, ultimately, to kill them, and both filamentosus and anthrax bacilli will grow freely in drop cultures of lymph taken from such an animal. At the same time hyaline cells are present, and have not lost their phagocytic power. Take, for instance, the following experiment.

Experiment V.

A drop of non-clotting lymph from a Frog which had been a long time in the laboratory was inoculated with anthrax bacilli and anthrax spores.

The eosinophile cells did not act at all, and therefore the hyaline cells made no attempt whatever to ingest the bacilli.

At the same time the spores were ingested as usual by the hyaline cells.

The bacilli grew rapidly.

Ingested spores were seen to be lying in distinct food vacuoles, and many were undoubtedly digested.

How essential the removal of the dead bacterial remains by ingestive solution on the part of the phagocytes must be in the body, we see when we watch hanging drops made from lymph either abnormally deficient in hyaline cells or in which the hyaline cells are abnormally sensitive to a change of medium. The relative number of the two kinds of cells varies in lymph drawn from different parts of the body. Occasionally in hanging drops made with peritoneal lymph hyaline cells may be almost absent.

Under these circumstances the eosinophile attack takes place and the bacilli are killed, but there are no scavengers to clear away the traces of the fray, and the field remains strewn with bacilli, which, if they are not dead, have at any rate completely lost the power of growing in the drop.

The Changes in the Specific Granulation of the Eosinophile Cell.

The Amphophile Granule (fig. 17A).—The facts discovered by following the phenomena exhibited in a hanging drop, were supplemented by the examination of such drops, or of lymph taken from the body, when fixed and stained with the methylene-blue solution, or with eosin and methylene-blue at different intervals after inoculation.* The same phenomena, the attack of the eosinophile cell, the formation of plasmodial masses, and the final phagocytosis, are again seen, but new light is thrown on the glandular nature of the activities of the eosinophile cell. In the attack on a bacillus the cell discharges, more or less completely, its granules. The amount of discharge depends on the strength of the stimulus. If, for instance, a considerable number of cells attack a bacillus, then the discharge will be only partial in many of the cells. The process of the discharge of granules was followed with high powers. If a cell attacking bacilli be watched under Oc. 4, Ob. $\frac{1}{12}$th, the granules will be seen to travel either singly or in groups of two, three, or four, from the central mass of granules to those parts of the cell which are immediately in contact with the bacillus. There they will be gradually seen to shrink in size until they disappear. This phenomenon, namely, the diminution in size and loss of granules, can be demonstrated in preparations fixed with the methylene-blue solution, or in films which have been rapidly fixed by heat, or by corrosive sublimate. As compared with the rate of loss of granules in, for instance, the secreting cells of a salivary gland, the process is very rapid. After the discharge the cell often re-forms its granules. Tracing these events by means of the basic and acid dyes, we find that the re-formed granules are at first amphophile, and only towards the close of the conflict do they become truly eosinophile.

But the amphophile substance is a stage, not only in the construction of an eosinophile granule, but also in its destruction. The action of urari may again be cited, for if this drug be added to lymph, the eosinophile granules alter to amphophile granules, and the change may be followed in a hanging drop of lymph to which has been added a little urari, and a trace of methylene-blue. We have watched the granules in a cell become slightly smaller and less refringent, and then colour with the dye. Similarly, when urari or bouillon containing a considerable quantity of bacterial products is added to the lymph, a change from eosinophile to amphophile granulation rapidly occurs. In the latter case, if the poisons be not present in too great quantity, the eosinophile condition is re-established.

* Preparations stained first with eosin and afterwards with methylene-blue were made according to EHRLICH'S film method.

When the conflict with the micro-organisms is carried out swiftly to a successful issue, the changes appear to be as follows :—

i. The initial sluggish eosinophile cell.

ii. The discharge of the granules, either completely, when the cell effects contact with a bacillus, or so far as to make them amphophile.

iii. The reconstruction of amphophile matter by those cells which have completely discharged, the amphophile matter being a stage in the elaboration of the eosinophile granule.

iv. The complete regeneration of the eosinophile granules. And this occurs in what may be spoken of as the plasmodial stage of the conflict.

The change of the granulation of the eosinophile cell from eosinophile to amphophile and back again to eosinophile may be illustrated by the following experiment.

Experiment VI.

A series of anthrax drop cultures were made with the lymph from the same Frog. These were stained at different intervals with the methylene-blue solution.

(*a.*) Stained as soon as possible after the addition of the lymph to the drop of bouillon culture. Eosinophile cells amœboid, granules amphophile or discharged. Many chains already attacked by eosinophile cells. Hyaline cells amœboid. Rose-staining cells present (fig. 17).

(*b.*) After fifteen minutes. Eosinophile cells with eosinophile granules present.

(*c.*) After thirty minutes. Eosinophile cells present, with perfectly eosinophile granules (*i.e.*, they absolutely refuse to stain). Where a cell is in contact with a bacillus it contains granules which are irregular in shape, are being discharged, and which stain.

(*d.*) After forty-five minutes. Where the eosinophile cells are in contact their granules are amphophile (blue-staining), where they are floating freely they contain eosinophile granules.

(*e.*) After one hour. Some eosinophile cells applied to chains are still quite free from granules. Contents of the bacilli no longer stain as a homogeneous blue substance. Disintegration has commenced, and the staining is in patches. Free amphophile cells rare.

(*f.*) After one-and-a-quarter hour. Rose-staining cells in marked abundance. Giant eosinophile cells (plasmodia) present, always with heaps of truly eosinophile granules.

The series was continued up to $3\frac{1}{2}$ hours, and the most prominent feature was the increase in the rose-staining cells.

Section VI.

Action of the Cells on Yeast Torulæ.

There are present then, in the body of the Frog, two kinds of wandering cells, the glandular eosinophile cell and the ingestive hyaline cell, and the most superficial study of these cells shows that the former is much more stable and can endure much greater changes in its environment than the latter. The eosinophile cells will persist as spherical bodies, with granules intact, for days in a drop of lymph, and for long after the other elements have disintegrated. They may or may not be dead, but

absence of movement is the only justification for supposing that life is extinct and not in abeyance. Similarly in *Astacus* we find that the eosinophile cell is very much more stable than the hyaline cell. Correlated with this fact we find that the change of environment due to the presence of bacterial poisons will call forth the most active manifestation of the special functions of the eosinophile cell, while the hyaline cell may be paralysed or destroyed. It is possible, however, that the poisons of all micro-organisms do not possess this discriminating value. It may be that if a sufficiently large number of micro-organisms were taken, they could be placed in a series commencing with those which acted towards the cells like indifferent particles, being ingested by the hyaline cells and unnoticed by the eosinophile cells, and ending with those like *B. anthracis* or *filamentosus*, which provokes the most profound activity of the eosinophile cell while the hyaline cell is incapable of attacking them. Bearing in mind the complex conditions of the conflict, we are forced to conclude that the organisms against which the Frog is not immune may be found either at one end or at the other end of the series, or at both.

Yeast, in its most virulent form, represents to a certain extent the middle of the series. Clusters of yeast cells are attacked by the eosinophile cells, just as are anthrax chains, and to these come hyaline cells; in fact, the story is the same as that which has already been related. At the same time hyaline cells will ingest stray yeast cells which have not been attacked by eosinophile cells. The notes of one experiment will illustrate this.

Experiment VII.

Bouillon yeast culture of great virulence when tested on Rabbits. Inoculated lymph drop with this. Frogs completely unharmed by large doses of this yeast.

1 *o'clock*.—Watched large mass of yeast. Three eosinophile cells came up and were lost in north end of mass.

Saw hyaline cell ingest stray torula.

1.12.—The same hyaline cell has now reached north end of mass and is attacking it. After three minutes it is lost in mass.

1.25.—Another eosinophile cell appeared and became lost in south end of mass.

1.30.—Another eosinophile cell, followed by hyaline cell, came in at north end and both fused with mass.

All this time the eosinophile cells have been covering the mass all over.

1.35.—At north end yeast has lost much of its distinctness. After some hours plasmodium formed, as with anthrax.

9 *o'clock*.—One eosinophile cell left plasmodium.

9.10.—Same cell came back and apparently fused with another.

9.15—Another eosinophile cell left.

Discontinued observation, and therefore did not see the general breaking up. Stained the preparation, and found many rose-reacting cells.

The hyaline cell is mostly, but not always, capable of digesting the yeast cells,

which it ingests. Similarly it can digest some, but not all the anthrax spores which it ingests, and we may classify the activities of the hyaline cells under two heads :—

(1.) The ingestion of particles which they can digest, such as coagulated proteid, dead micro-organisms, and certain living micro-organisms or spores. These the hyaline cells eliminate from the body in which they dwell by dissolving them.

(2.) The ingestion of particles which they cannot digest, such as Indian ink, or dust particles. These they do not eliminate from the body, but carry them to the connective tissues and there hide them away. Instances of this are found in the removal of dust particles by the phagocytes of the lung, and in the fate of Indian ink when injected into the body of *Dytiscus*, as shown by DURHAM.*

SECTION VII.

The Rose-reacting Cells.

It would appear, from the facts so far set forth, that the fate of the microbe in the Frog's lymph depends upon the activity of two kinds of cellular elements. Are we to attribute, therefore, no part in the conflict to the non-amœboid cells which are charged with rose-staining granules? This question is at once answered by the fact that one of the conditions apparently necessary to a successful issue to the conflict between the eosinophile cells and the micro-organisms, whether the conflict occur in the body or out of the body, is an increase in the number, and still more in the size and granulation of these cells. In the lymph of newly-captured, healthy Frogs, the rose-staining cells are usually very small, and so few in number, that they would be completely overlooked were it not for the brilliant rose tint which their granules take with methylene-blue. This condition is shown in figs. 4 and 5A.

The injection of micro-organisms is followed by a continuous increase in the number of these cells, and they now appear as shown in fig. 5B. The increase is a striking fact from the second to the fortieth hour. Beyond that time we have not followed them.

To state the same fact in other words, the rose-staining cells are continuously abstracting some substance or substances from the plasma and depositing it within themselves as rose-staining (basophile) granules. For the present we are content to suggest that the substances abstracted are foreign, abnormal substances in solution, such as, for instance, the poisonous products of bacterial activity. Frequently, in watching the processes taking place in a drop-culture, one sees the eosinophile cells at first vigorously attacking the bacteria, and then the whole process will come, sometimes almost abruptly, to a standstill, the eosinophile cells contracting and becoming spherical, while the hyaline cells disintegrate. This, we explain, by supposing that the bacterial poison in small doses stimulates the eosinophile cells, while in larger

* DURHAM, "On Wandering Cells in Echinoderms," 'Quart. Journ. Micros. Sci.,' vol. 33.

quantity it paralyses and ultimately kills them. If this be true, it is obvious that in the body some mechanism or mechanisms must exist for keeping the amount of bacterial poisons either taken in at once, or produced by the as yet unattacked or unkilled bacilli, below the limit at which it is fatal to the wandering cells or to the body at large. We suggest that this function is, in part, filled by the rose-reacting cells.

The evidence bearing on this question which we have so far obtained may be briefly summarised as follows.

The presence in the plasma of foreign bodies in solution, such as egg albumen or anthrax albumose, leads to an increase in size, number, and granulation of the rose-staining cells. This is true both of the Frog and of *Astacus*. Also, as has already been stated, if *Daphnia* is exposed to toxic substances, an increased formation of a rose-reacting (amphophile, in this case) substance results.

The alteration in the chemical composition of the plasma of a hanging drop of lymph due to clotting also leads to a similar result. Lastly, we may cite the absence, often noted by us, of rose-reacting cells in lymph in which the bacilli have grown, and this in spite of the fact that these cells are very resistent to the toxic substances. The presence pointed out by SHERRINGTON of similar cells in large numbers round the intestinal blood-vessels in cases of enteric disease suggests that they are there to intercept the toxic substances streaming in from the lumen of the intestines. The increase in the number of these cells in inflammation, in carcinoma, &c., and, generally speaking, in cases normal or pathological, in which there is an abnormal production of normal or abnormal products of metabolism was noticed by KORYBUTT, DASKIEWICZ, and EHRLICH.*

The importance attached to the removal of the bacterial poisons, and the endeavour made by the body to get rid of them, are strikingly shown by *Daphnia* and *Astacus*.

In *Daphnia* the whole blood stream may be watched on the stage of the microscope. The presence of bacterial poisons in the body is then seen to increase the adhesiveness of the corpuscles; they tend to adhere to the walls of the blood spaces, but they are mainly attracted to the excretory organs (the shell glands) around which they cluster in large masses. At the same time the epithelium of the excretory organs becomes more granular, vacuoles appear, and the inner surface of the cells becomes irregular, being pushed out into processes.

In *Astacus* the injection of very large quantities of *Bacillus filamentosus* into the pericardial sinus is followed by the almost complete disappearance of the filaments from the blood in half an hour. Drops of blood taken from the pericardial sinus, and from the ventral sinuses, both abdominal and thoracic, were stained and examined, but prolonged searching failed to reveal more than isolated rods or pairs of rods, and these only at the rarest intervals.

* EHRLICH, "Beiträge zur Kenntniss d. granulirten Zellen, &c.," *loc. cit.*

Finally, the animal was bled into some iodine solution, and the cells allowed to sink to the bottom. After twelve hours the sediment was examined, and only one or two cases of eosinophile cells attacking bacilli were found. The bacilli themselves were almost absent. The walls of the blood spaces were then examined and the missing bacilli discovered embedded in plasmodial masses clustering round the green glands, the excretory organs of *Astacus*.

It may be said in passing that the eosinophile attack appeared to precisely resemble that observed in the FROG's lymph.

SECTION VIII.

Preliminary Observations on Mammals (Rabbit and Rat).

(1.) The peritoneal fluid of these animals is full of cellular elements, these being, just as in the Frog, chiefly eosinophile and hyaline cells. On killing the animal (by decapitation), and quickly placing a little of the fluid on a small drop of a bouillon culture of anthrax on a warm stage, the initial attack by the eosinophile cells at once takes place. The latter, in the case of the Rat, is pronounced and rapid; while in the Rabbit, which is a more susceptible animal, it also takes place, but is less vigorous. It was impossible, with the method employed, to observe more than the initial stage, on account of the difficulty experienced in keeping the cells alive for longer than fifteen minutes. However, the eosinophile cells deported themselves in exactly the same manner as they do in the Frog, moving towards the chain of bacilli, and fusing along it.

(2.) The following experiments and observations, made on Rabbits, will throw some light on the mode of action of the eosinophile cells:—

LEBER has demonstrated the powerfully solvent action of pus on such substances as copper, gold, and silver, &c. It has been shown that the cellular elements of pure and fresh pus consist of practically nothing else than cells with fine or coarse eosinophile granulation. On repeating some of LEBER's experiments, and placing minute pieces of sterilized copper, steel, and silver wire into the anterior chamber of a Rabbit's eye, under strict aseptic and antiseptic precautions, in all cases a suppuration rapidly ensued, most sudden when copper was used. This pus invariably contained nothing but granular cells. Already, after 24 to 48 hours, the copper was found to be roughened and corroded. We conclude from the observations that the solvent action of pus is due to the eosinophile granulation.

Cellulose, in the shape of sterilized cotton-wool, placed in the anterior chamber under similar precautions, was not affected in the slightest, even after an interval of six weeks. Cellulose is both an extremely indifferent substance and also most resistent.

(3.) Others have already demonstrated the presence of a ferment in pus. Ross-

BACH, before us, succeeded in separating an amylolytic ferment from the leucocytes ('Deutsch. Med. Woch.,' 1890), while LEBER ('Die Entstehung der Entzündung') found that pus digested fibrin and liquefied gelatine.

Summary.

Some of the phenomena described and discussed in §§ II. to VII. may be summarized as follows:—

(1.) The three different kinds of wandering cells, the eosinophile cell, the hyaline or non-granular cell, and the basophile rose-reacting cell, proliferate while free in the body fluids. This may be demonstrated in the Frog, and has, in the case of other animals, been recorded by ourselves and other workers.

(2.) The different kinds of cells multiply independently, so that the numbers of any one kind of cell may vary without a corresponding variation in the numbers of the other cells. There are thus three kinds of leucocytosis, corresponding to the three forms of wandering cells found in lymph or peritoneal fluid.

(3.) The three kinds of cells are differently affected by different substances when introduced into the plasma.

(*a.*) Solid substances of the nature of what are commonly called indifferent substances affect only the hyaline cells which ingest the particle. Coagulated proteid and Indian ink are examples, as are also anthrax spores.*

(*b.*) Anthrax and filamentosus bacilli, when first introduced, attract only the eosinophile cells, which kill or maim them by means of a substance derived from their stored eosinophile granules. After the bacilli have been thus acted on they can become the prey of the ingestive hyaline cells.

(*c.*) Vermilion and yeast cells stand midway between indifferent substances and these bacilli, and attract both hyaline cells and eosinophile cells. Vermilion only slightly attracts the eosinophile cells. Yeast cells attract them strongly, but also to a certain extent are immediately ingested by the hyaline cells.

(*d.*) The rose-reacting cells are increased in number and size by alteration in the chemical composition of the plasma, such as is produced by clotting, or by the introduction of toxic albumose or egg albumen. Hence they are probably chiefly active in maintaining the normal constitution of the plasma so far as dissolved substances are concerned.

(4.) The eosinophile cells are highly specialized bodies endowed with the power of movement, in virtue of the possession of a pseudopodial ectosarc, and with glandular powers directed to the production of a bactericidal, or at least antibiotic, substance.

* *Cf.* note, p. 296.

Section IX.

The facts brought forward in the preceding sections lead to certain conclusions as to the morphological position and physiological attributes to be accorded to the sporadic mesoblast.

Morphology.

In the Frog, the sporadic mesoblast consists of three kinds of cells, the eosinophile cell, the hyaline cell, and the rose-reacting cell, together with the red corpuscles and platelets. Of the two latter, we, personally, can say nothing from our own knowledge; nor have we any reason beyond the general facts of their distribution in the body, for placing them in the same morphological group with the three first-named elements.* Leaving then the red corpuscles and platelets out of account, we find that the sporadic mesoblast of the Frog contains elements precisely similar to those which compose the similar tissue of such a widely divergent animal as *Astacus*. The large phagocyte, derived from the hyaline cell and of temporary existence only, is not only found in both animals, but produced by essentially similar conditions in both. This exact resemblance between animal forms so diverse is helpful to us, since it gives us good reason for supposing that the three cell elements of the Frog have arisen by the differentiation of a primitive homogeneous sporadic mesoblast, that is, one the cell elements of which are all alike.

This question of the comparative morphology of the sporadic mesoblast may, like other morphological questions, be approached in two ways. We may investigate the phylogenetical position of the tissue or its ontogenetical position. In both directions our knowledge is very incomplete. No conclusions as to lines of development of the tissue within the limits of the Chordata can be based on our present knowledge, for in the lowest member of the Chordata which we have examined, the *Ammocœte* larva, all three types of cells are already present. At the same time, so far as we know, there is no exact description of the histology of the wandering cells of the Tunicata or Cephalocorda. In the Crustacea, the conception of the origin of the different wandering cell types from an archetype finds strong phylogenetic support. The group of the Crustacea is remarkable for including within its limits animals of the very simplest and animals of the most complex organization. The very simple animal *Daphnia* has a sporadic mesoblast composed of only one kind of cell, *Astacus*, on the other hand, representing the most complex members of the group, has three kinds. But the evidence does not rest here. The wandering cells of *Daphnia* are not only of one kind, but also this one cell performs all the functions, and has all the morpho-

* Ziegler, "Die Entstehung des Blutes," 'Ber. d. Naturforsch. Gesellsch. zu Freiburg,' vol. 4, H. 5, as the result of his investigations on the development of the blood, arrives at the conclusion that the red corpuscles are morphologically distinct from the white corpuscles. The former are of intravascular origin, the latter are extravascular at first, but migrate into the blood system.

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logical characters of the three cells of *Astacus*. This fact has already been discussed by one of us in earlier papers,* but we are able to add a further archaic character of the cell to those given there. In the earlier papers, the specific granulation was justly described as rose-reacting, further investigation has revealed the fact that the rose-reacting substance of *Daphnia* is also amphophile. In other words, even in its specific granulation the wandering cell of *Daphnia* is archaic, it is a rose-reacting, amphophile granulation. We may even pursue the search for the cell element of the undifferentiated sporadic mesoblast a stage further back to the wandering cell of the larval *Daphnia*, while still within the broad pouch of the mother. Then we find an amœboid cell with no granules, but the whole cell substance reacts with aniline dyes, giving "a faint but distinct rose-reaction with methylene-blue."

Since Tadpoles are not available in summer or autumn, we have not as yet been able to pursue the ontogenetical study of the corpuscles of the Frog. Fœtal Cats, however, are to be found in all seasons, and in these animals we have so far found only one kind of wandering cell which is without specific granulation, and has a spherical nucleus and scanty cell substance. In the very late fœtus the wandering cells still show no granulation but there are now two kinds of cells present, one with a round nucleus and one with a lobed nucleus. In the adult all these three types are undoubtedly present. Thus we find a certain phylogenetic and ontogenetic support for the statement that the very divergent elements of the sporadic mesoblast have a morphological homogeneity in the fact that they have arisen from a primitive amœboid wandering cell with no specific granulation, by a process of morphological and physiological differentiation akin to that which has, *pari passu*, led to the increasing complexity of the animal generally.

Physiological.

The question of the functional significance to be attributed to the sporadic mesoblast also finds a partial solution in the very incomplete series of facts we possess bearing on the physiology of this structure. Undoubtedly the wandering cells are present largely as a protective mechanism to guard against the intrusion of foreign substances, living or non-living, into the organism. But this is probably only a small part, and not the most primitive of their functions. They are also related to the general processes of the body, notably to the bodily nutrition. In *Daphnia* they may be readily watched engaged in the transportation of fatty particles from the alimentary canal to its place of storage.† Also any abnormal condition of the perivisceral fluid, or blood, leads to the massing of these cells around the special excretory organs of this animal, and this fact suggests that their activities are partly directed to maintaining the normal constitution of the body fluids so far as the dissolved matters are concerned.

* 'Journ. of Physiology,' vol. 13, p. 184, *seq.*, and p. 318.

† 'Journ. of Physiology,' vol. 13, p. 184, *seq.*

Thus, though exact knowledge is wanting, yet it is abundantly clear that the homogeneous sporadic mesoblast of *Daphnia* is intimately related to the processes of general physiological importance taking place in the alimentary canal and the excretory organs; and there is no sufficient reason to lead us to believe that this connection has been entirely, or to any great extent, lost in the course of the divergence and specialization of these cells, which has produced the different wandering cell-types of the higher animals. On the contrary, it is a matter of common physiological knowledge that the wandering cells are profoundly affected by events occurring in the alimentary canal. Thus, though we are not able to point to any particular physiological process carried out by these cells, still we have sufficient grounds for opposing the supposition that they exist only to protect the body from the invasion of foreign particles, and that appears to us to be a valuable step and a most necessary preliminary to a successful study of their functions.

In endeavouring to indicate the lines along which the specialization of the sporadic mesoblast has advanced, probably in most animal groups, we may proceed with perhaps greater sureness. The primitive mobile, ingestive, and glandular cell of *Daphnia* has become in *Astacus* the specially glandular eosinophile cell, the specially mobile and ingestive hyaline cell, and the specially absorptive rose-reacting cell, and what we know of the sporadic mesoblast of the Vertebrates points to its having developed along similar lines.

But both older and later observations* on the part played by wandering cells in the formation of scar tissue, indicate that an even more extended conception of the relations of the sporadic mesoblast may at some future time become necessary, and the convenient fiction, wherein the blood was regarded as a tissue, like cartilage or connective tissue, only with a fluid matrix, may yet be found to embody a morphological truth.

The Relation of the Attack of the Eosinophile Cell to the Ingestive Act of the Hyaline Cell.—A study of the way in which some of the carnivorous Protozoa capture and ingest their prey, throws a clear light on the relations of the peculiar mode in which the eosinophile cell attacks a bacillus to the simple ingestive act of a hyaline cell, or *Amœba*. Further, if all these facts are placed together, they suggest many thoughts on the relation of intra-cellular to extra-cellular digestion.

By comparing the various accounts given by Leidy, M. Greenwood, and other observers, of the manner in which an animal like *Amœba* or *Actinosphærium* captures and ingests its prey, we find that the following processes may be recognized:—(1) Contact is effected with the prey and its movements are arrested; (2) then, after it has thus been maimed or killed, the prey is ingested and digested. But the captured infusorian may resist the benumbing influence of the captor, and may, after being exposed for a long time to the *extra-cellular* attack, as it were, acquire tolerance of it,

* Compare Sherrington and Ballance "On Formation of Scar-tissue." 'Journ. of Physiology,' vol. 10, p. 550.

recover all its powers and escape. The best instances of the killing of prey as a result of mere contact, are furnished by the Suctoria. When a moving animalcule comes into contact with the long stiff, very specialized pseudopodia of these animals, its movements are suddenly arrested. The phenomenon is so striking that GRÜBER, MAUPAS, and others, speak of it as a poisoning of the prey by some substance excreted by the captor.

Expressing these facts in terms of the processes occurring in the cell, we have

(1.) The contact of the prey stimulating the captor to *excrete* a poison;

(2.) The ingestion of the now inert body;

(3.) The *secretion* of a digestive fluid which dissolves the ingested prey.

LEIDY's account of the capture of a *Urocentrum* by an *Amœba*, clearly shows how essentially similar the excretion of the poison is to the secretion of the digestive fluid. His words are, "a second victim of the same kind was included in the fork of a pair of pseudopods, the ends of which were brought into contact so as to imprison the animal in a circle. The latter moved restlessly about within its prison but, after a time, became motionless, and shortly after the ends of the pseudopods, which enclosed it fused together . . . and finally the *Urocentrum* was enclosed."* If we now turn to M. GREENWOOD's† carefully detailed account of the phenomena of ingestion in *Amœba*, and notice the close morphological relation between the space "included in the fork of a pair of pseudopods" and the digestive vacuole, the primitive unity of the two processes cannot fail to be seen. In the very specialized Suctoria, the excretion of the poison and the killing of the prey is a specialized and much more perfect process, as is also the very remarkable manner of its ingestion.

Turning now to the eosinophile and hyaline cells, we see that in the former the *extra-cellular* act has become its special character, and, as in the Suctoria, the perfection of the mechanism for the production of the poison has shortened the latent period of its discharge. The cell is, in short, a unicellular gland, *but* it preserves intact the initial step of the primitive process, it effects contact with its prey; the hyaline cell also effects contact with its prey, but the extra-cellular discharge is insignificant or absent, the ingestive and *intra-cellular* act being, on the other hand, complete and rapid.

We have strictly parallel phenomena in the endodermal cells, and this enables us to advance the line of thought a step further. METSCHNIKOFF‡ has given us good reasons for supposing that the primitive endoderm, or nutritive cell, of the Metazoa, carried out its functions in a manner exactly resembling the *Amœba* described by LEIDY. But in the Cœlenterates we find an endoderm composed of (1) a gland-cell which forms *extra-cellularly* a digestive fluid, (2) an ingestive cell which produces *intra-cellularly* a digestive fluid, and (3) an absorbent cell which is correlated with the gland cell and takes up the fluid products of the extra-cellular digestive process.

* LEIDY, "Rhizopods of North America." See the figs. 5 and *ss* on Plate 1.

† "Journal of Physiology," vols. 7 and 8. "On the Digestive Process in some Rhizopods."

‡ METSCHNIKOFF, 'Zool. Anz.,' 1878, p. 387.

Viewed in this way the eosinophile cell is analogous, in its activities, to the gland cell of the Cœlenterate endoderm, and, for instance, to the cells lining the fundus of a gastric gland of the Mammalia.*

Other instances of an extra-cellular digestive act are found in :—

(1.) The attack of *Vampyrella spirogyræ* and *V. pendula* on the Algæ which furnish them with food. This furnishes a most interesting parallel.

(2.) The digestion of fibrin by the surface cells of the mesenterial filaments as described by KRUKENBERG.†

(3.) The hollowing-out of spaces in the first bone spicules by the osteoclasts.

Lastly, we are not without some feeling of the bearing of our observations on the most difficult question of immunity. But further knowledge has only increased our perception of the complexity of the problem. We see that it depends upon all the possible permutations and combinations of the activities of the three wandering cell elements engaged in the conflict, and of that yet entirely unknown factor, the general physiological reaction of the organism to the microbic poisons. In the face of these difficulties we have deemed it wise to stifle the temptation to theorise on the subject, until, with wider knowledge, a greater capacity to cope with the difficulties shall have been attained.

DESCRIPTION OF FIGURES.

PLATE 29.

Fig. 1. A group of two hyaline cells and one eosinophile cell fixed with ·25 per cent. iodine as rapidly as possible after removal from a subcutaneous lymph sac of a Frog. The hyaline cells are larger than usual. The vertical line represents the relative length of the major axis of a red corpuscle. Oc. 10, Ob. apochr. $\frac{1}{8}$th POWELL and LEALAND. Camera lucida.

Fig. 2. Eosinophile cell fixed by heat and stained with eosin and methylene-blue. From lymph of healthy Frog. Same magnification as fig. 1. Camera lucida.

Fig. 3. Eosinophile cell and a small hyaline cell. Normal Frog. Fixed and stained with methylene-blue solution. Oc. 4, Ob. E ZEISS. Camera lucida.

Fig. 4. Rose-reacting cell and hyaline cell of normal Frog. Fixed and stained with methylene-blue solution. Viewed with artificial light. Oc. 4, Ob. E ZEISS. Camera lucida.

Fig. 5. Rose-reacting cells (*a*) of normal Frog, (*b*) 12 hours after injection of yeast, from the seat of the inoculation. Stained only with methylene-blue.

* Compare M. GREENWOOD, "Digestion in Hydra," 'Journ. of Physiology,' vol. 9.

† KRUKENBERG, "Ueber d. Verdauungsmodus d. Actinien," 'Vergleichend-Physiol. Studien,' Heidelberg, 1881.

Illuminated with yellow light. Same Oculars and Objectives as in fig. 1. Vertical line = length of major axis of red corpuscle.

Fig. 6. Cells from blood of late fœtus of Cat. Fixed with heat and stained with eosin and methylene-blue. (*a*) and (*b*) different forms of white corpuscle, of which (*b*) is much more abundant, (*c*) nucleated red corpuscle—very few present. The vertical line represents the diameter of the non-nucleated red corpuscle. Oc. 10, Ob. apochr. $\frac{1}{8}$th Powell and Lealand. Camera lucida.

Fig. 7. Hyaline cell budding. From hanging drop inoculated with anthrax.

Fig. 8. Young hyaline cell showing active movements, but unable to effect contact with anthrax chain. From same field of microscope as fig. 7.

Fig. 9. Hyaline cell dividing. Leucocytosis produced by injection of urari. Acidulated solution of methyl-green. Oc. 4, Ob. E Zeiss. Camera lucida.

Fig. 10. Eosinophile cell in neighbourhood of anthrax chain.

Fig. 10B. The same cell shortly after it has effected contact with the chain.

Figs. 11, 11A, 11B, 11C, 11D, 11E. Successive stages in the attack of the cells on a chain of anthrax bacilli in a hanging drop of lymph. 11E drawn with camera lucida. Oc. 4, Ob. D Zeiss. Illustrating Experiment I. See p. 300.

Fig. 12. A hyaline cell in the same field as fig. 11E. Not drawn to the same scale. The two sketches illustrate successive phases of this cell's movements.

Fig. 13. An eosinophile cell just about to attack the end of a chain of *B. filamentosus*.

Fig. 14. Eosinophile cell which has just attacked an anthrax chain. Fixed and stained with the methyl-blue solution.

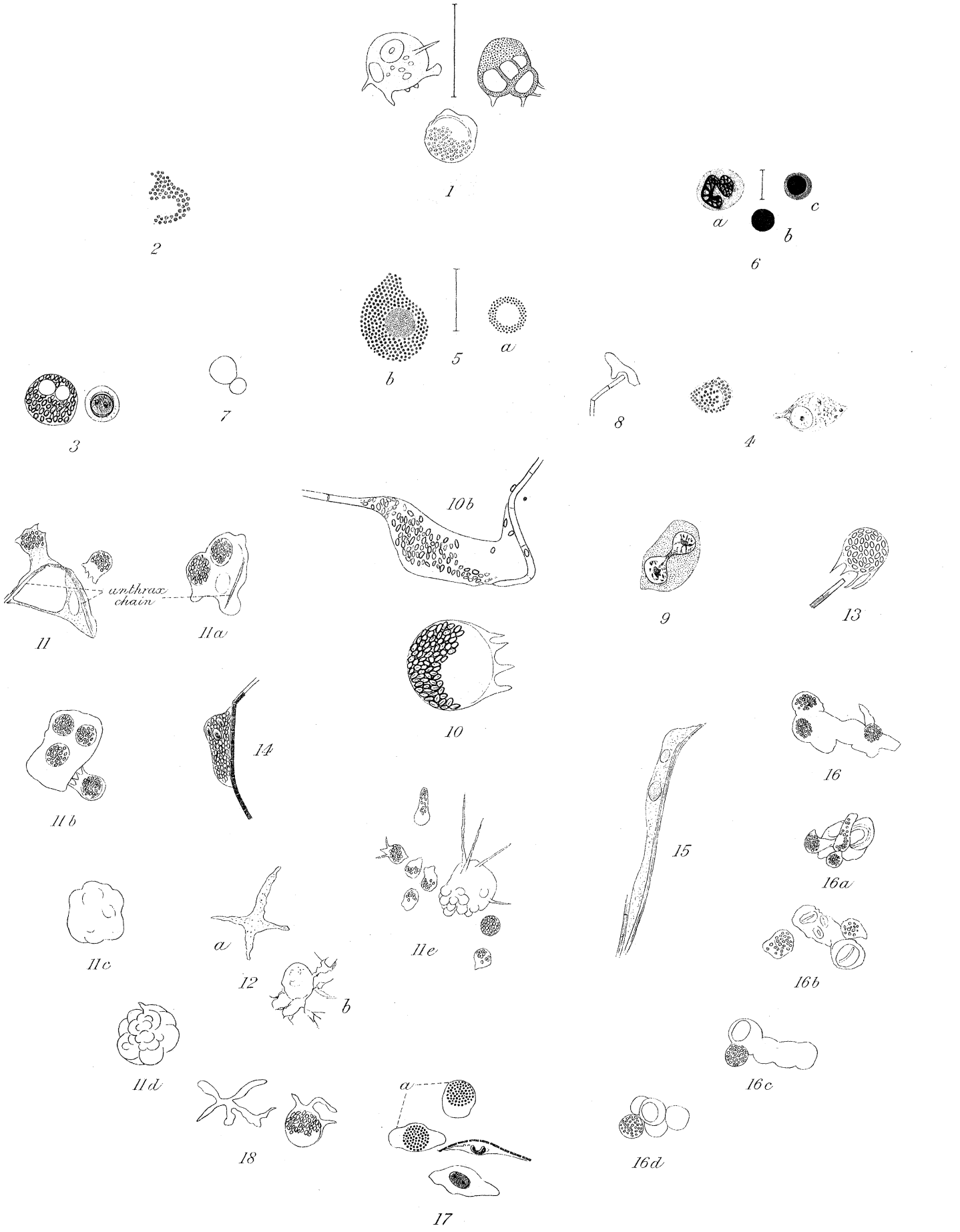
Fig. 15. An eosinophile cell with granules completely discharged, attacking anthrax chains. Fixed and stained with the methylene-blue solution.

Figs. 16, 16A, 16B, 16C, 16D. Successive stages of conflict with a chain of anthrax, which in fig. 16 is already hidden in the cell mass. Illustrating Experiment II. See p. 302.

Fig. 17. The amphophile condition of the eosinophile cells, also an eosinophile cell which has completely lost its granules, and a hyaline cell. See Experiment VI., p. 307.

Fig. 18. A hyaline cell and an eosinophile cell. In the same field of the microscope. See Experiment III., p. 303.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

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