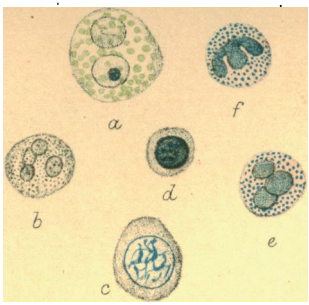
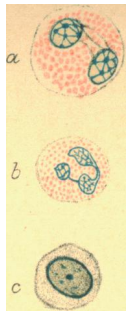
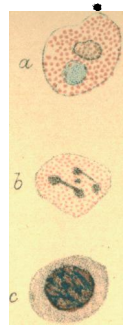


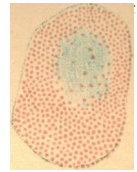
1



2

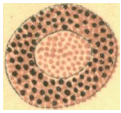


3

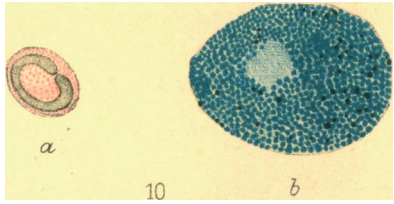


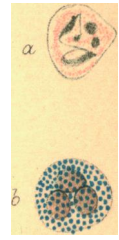
4

5

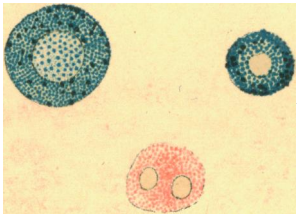


9

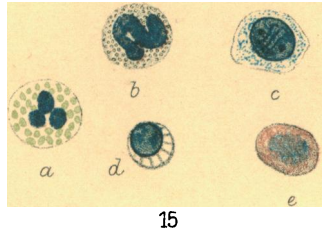




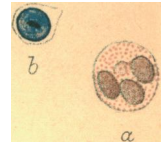
11



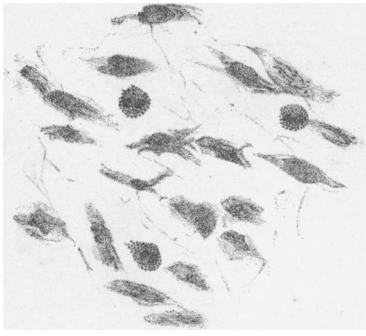
14



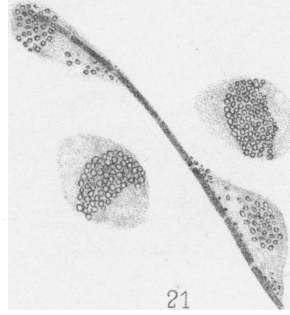
15

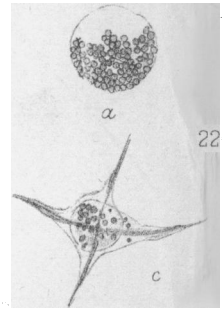


16

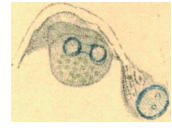


20

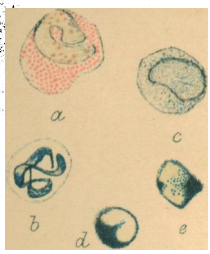




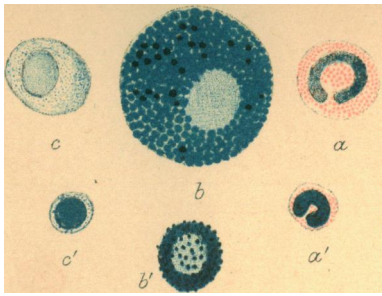
26



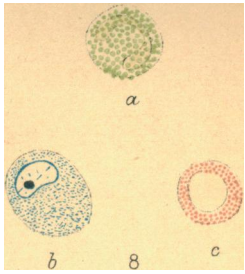
27

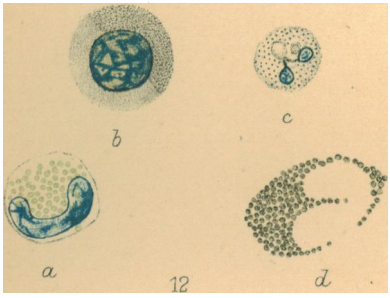


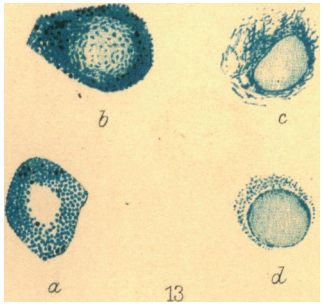
6

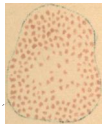


7



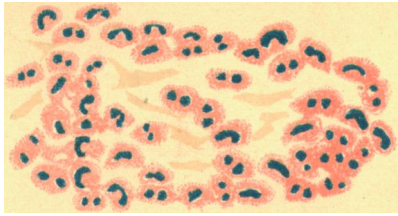




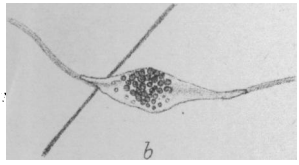


17

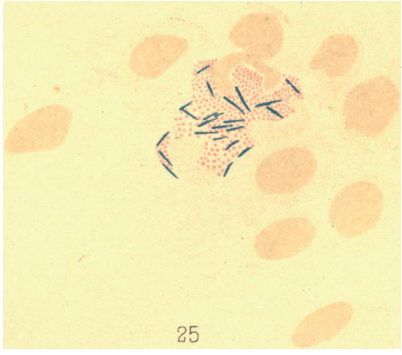
18

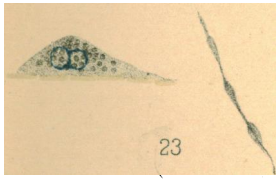


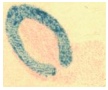
19



24







28

Edwin Wilson Cambridge.

THE MORPHOLOGY AND DISTRIBUTION OF THE WANDERING CELLS OF MAMMALIA[1].

By A. A. KANTHACK, M.D., M.R.C.P., *Lecturer on Pathology to St Bartholomew's Hospital,* AND W. B. HARDY, M.A., *Fellow of Gonville and Caius College, Cambridge.* (Plate II.)

(*From the Physiological Laboratory of Cambridge University.*)

PART I. INTRODUCTORY. CLASSIFICATION OF CELLS.

A FOUNDATION for our knowledge of the morphology of the wandering cells, or sporadic mesoblast, was made by Wharton Jones in 1846[2]. In the first of three memoirs published in the Philosophical Transactions for that year, he described the cells of the blood and lymph in cartilaginous and osseous fishes, in amphibia, in birds and in mammals. The second memoir included the cells of the "lymph" of worms, insects, crustaceans, and molluscs. In the third memoir the author compared the cells found in vertebrates with those found in invertebrates. Throughout these memoirs the white corpuscles are regarded as the young forms of the red corpuscles. Putting this aside, a great advance was made in the clear recognition of the fact that the white cells of blood and lymph are not all of one kind. Two main classes of cells were described (i) the "granule cells," and (ii) the "nucleated cells." The granule cells were again divided into a coarsely granular variety, and a finely granular variety. It is interesting, in connection with the

[1] The research has been carried out on Man and on Rodents (Rat, Mouse, Rabbit and Guinea-Pig). Since, however, excursions to other mammals (Dog and Ox) have revealed similarity of structure and function, and not differences, we feel that we may extend our results to the higher Mammals. Our thanks are due to Prof. M. Foster, Sec. R.S., who has aided us in all departments of this work, and to Dr Ruhemann who has advised us in all that relates to the chemistry of the dyes employed. Part of the expense of this research has been paid by the Scientific Grants Committee of the Brit. Med. Assoc.

[2] Wharton Jones. *Phil. Trans.* 1846.

purport of the second part of our paper, to note that the author describes himself as having borrowed the term "granule cell" from Professor Vogel, who first applied it to the form of cell "developed in inflammatory exudations." Streaming movements of the substance of both granule and nucleated cells were noticed, though the author does not appear to have recognised in these the manifestation of independent locomotor activity.

In 1863 Rindfleisch[1] confirmed the existence of granular (Körnchenzellen) and non-granular forms of colourless corpuscles in the frog.

In 1865 Max Schultze[2] carried the knowledge of the wandering cells to the furthest point attainable with the methods then in use: namely, the study of fresh living cells. By observing fresh blood on the warm stage, this author was able to identify and describe the following forms:—

(i) a small round cell not larger than a red corpuscle, composed of a round nucleus enclosed by a thin film of clear protoplasm which did not exhibit amoeboid movement. The diameter of the smallest of these cells was found to be 0·005 mm.

(ii) Cells like these but larger, the increased size being due to the possession of more abundant protoplasm which showed amoeboid movements.

(iii) Cells whose protoplasm contains fine granules and imbeds one, two, or more nuclei. Diameter 0·009—0·012 mm. These cells are amoeboid and from their abundance are to be regarded as the typical colourless blood cell.

(iv) Cells whose protoplasm contains coarse granules and shows amoeboid movements.

After Max Schultze no further advance was made or indeed was possible in the histological analysis of the sporadic mesoblast until Ehrlich[3], in 1878, furnished a rational basis for the use of staining reagents by his far-reaching discovery that the elective affinity of certain constituents of tissues for particular stains could be referred to two factors, the chemical nature of the staining substance employed and, a point too often neglected by workers who have followed his methods, the nature of the medium in which the stain is dissolved. Ehrlich drew particular attention to the granules, the possession of which characterises

[1] Rindfleisch. *Experimentalstudien über die Histologie des Blutes.* Leipzig, 1863.

[2] Max Schultze. *Arch. für mikr. Anat.* I. 1, 1865.

[3] Ehrlich. *Verhandlungen d. physiol. Gesellschaft zu Berlin*, 1878–79, No. 8. And *Farbenanalytische Untersuchungen.* Berlin, 1891.

various forms of wandering cells. These he divided into five classes differing either in their special affinity for basic, acid, or neutral dyes, or in size. The α or eosinophile granulation colours only with acid dyes, the β granulation colours with both acid and basic dyes (amphophile), the γ granulation colours only with basic dyes and the individual granules are large, the δ granulation colours only with basic dyes; but the individual granules are small and the ϵ granulation colours only in neutral dyes. The nomenclature of the granules was extended to the cells bearing them. Thus the various forms of white cells found by Ehrlich in blood were (i), a small cell free from granules, to which the name *lymphocyte* was given, from the fact that it appears to be developed in lymphoid tissue. This is the small non-amoeboid form of Max Schultze: (ii), a cell characterised by possessing fine granules and one or several nuclei. This is by far the most numerous form of white blood corpuscle in mammalia, and was found by Ehrlich to be neutrophile in man, and amphophile in rabbits and guinea-pigs: (iii) the eosinophile cell, or coarsely granular cell of Wharton Jones and Max Schultze. It occurs only in small numbers in the blood of mammalia, but is abundant in the blood of lower vertebrates: (iv) a basophile cell with fine basophile granules (δ granulation).

The mononuclear amoeboid cells of Max Schultze (ii) are apparently grouped with the neutrophile cells by Ehrlich. In addition to these forms Ehrlich describes a basophile cell with coarse granules (γ granulation), occurring mainly in connective tissues and also in the blood of frogs but not in the blood of mammals. These he calls 'Mastzellen.' Unfortunately neither Ehrlich nor his pupils appear to have attempted the formulation of a connected account of all these forms of wandering cells—in other words, the morphology of the tissues as a whole, the only exception being the attempt to classify the cells as lymphocytes and myelocytes according to whether they have their origin in lymphatic glands or in bone marrow.

From what we have said so far it will be seen that the group of finely granular blood corpuscles described by Max Schultze includes the amphophile and neutrophile, and the finely granular basophile cells of Ehrlich. Since Ehrlich's work no contribution to our knowledge of the morphology of the wandering cells has been made except on points of detail. Mention must, however, be made of the groups of cells recognised by Metschnikoff[1] in his treatise on inflammation (1892). There the term "leucocyte," originally applied by the French school of

[1] Metschnikoff. *L'Inflammation.* Paris, 1892.

physiologists, is used to designate wandering cells, and the following varieties are recognised (i) lymphocytes, (ii) mononuclear leucocytes with abundant protoplasm and a round nucleus, (iii) polynuclear leucocytes or "leucocytes neutrophiles," (iv) eosinophile leucocytes.

Ehrlich's grouping of the granules discussed. Our researches have led us to the conclusion that Ehrlich's grouping of the cells is imperfect in that it has reference solely to the micro-chemical reactions of their specific granules, and none to the relation one to another of the cells which contain these bodies. We have also been led to conclude that granules having a purely neutrophile reaction have not been proved to exist, and that the term "neutrophile cell" is, at any rate when applied to the finely granular and most abundant colourless corpuscle of human blood, a misnomer. For these fine granules have an oxyphile reaction (i.e. they stain with acid dyes) quite similar to, but very much less intense than that shown by the coarse α or eosinophile granules.

The neutrophile granule. The reaction of granules with acid dyes or, as we should perhaps say, the union of the granule substance with the acid pigment, is, as Ehrlich pointed out[1], dependent for its occurrence or non-occurrence on the nature of the solvent of the dye. He found that the staining power of the dye was greater in an aqueous solution, and less in a glycerine solution; and on these lines Schwarze[2] classified the acid dyes as weak or strong, the weak dyes staining only when dissolved in water, the strong dyes staining in both aqueous and glycerine solutions though, of course, very much more strongly in aqueous solution. Orange *G*, for instance, stains only in aqueous solution, eosine stains with overwhelming power in aqueous solution, but has a markedly selective action when dissolved in glycerine colouring those substances only which are strongly oxyphile. We have carried this distinction a stage further by using solutions of eosine in strong alcohol, for these have an even weaker staining action than glycerine solution.

In this way we can distinguish three grades of granules differing in the intensity of their affinity for acid dyes: (i) those which stain with eosine only in aqueous solution or in alcoholic solutions of a percentage below 60: (ii) those which stain with eosine in both aqueous and glycerine solution but are not coloured by a solution of the dye in

[1] *Verhandlung d. physiol. Gesellschaft zu Berlin*, 1878–79, No. 20.

[2] "Ueber eosinophile Zellen"; Inaugural Dissertation, 1880. Reprinted in *Farbenanalytische Untersuchungen* of Ehrlich. Berlin, 1891.

strong alcohol (90 to 95 %): (iii) those which stain with aqueous, glycerine and strong alcoholic solutions[1].

The neutrophile and amphophile granules of Ehrlich constitute the first grade, they are granules which have a minimal attraction for acid dyes or, briefly, a minimal oxyphile reaction. The neutral mixture of Ehrlich is, chemically speaking, not a neutral dye, but an exceedingly powerful though withal an exceedingly differential acid dye, colouring very intensely oxyphile granules of all grades. So far as we can gather, there are no chemical facts which warrant the assumption that a mixture of the two rosaniline derivates acid fuchsine and methyl green with the acid azo-dye Orange *G* will form a neutral dye. In point of fact, the only dyes to which, from their chemical constitution, the term "neutral" can be rightly applied are those coloured salts which are formed by the union of colourless acid and a colourless base, and in which, therefore, the whole salt is a chromophore and not simply the acid or the basic radicle. The fuchsines and methyl green are instances of such truly neutral dyes, and, in neutral solutions, they do not colour the granules in question.

Seeing that the fine granule of that form of white corpuscle which is most abundant in the blood has an affinity for acid dyes which, though it be small as compared with the very great affinity displayed by the large eosinophile or α granule, is quite clear and distinctive, and that it has no affinity to and is not coloured by basic dyes or by truly neutral dyes, we propose to discard the doubtful and possibly misleading term "neutrophile," and in its place to call these granules the fine oxyphile granules, and the cells bearing them the finely granular oxyphile cells, as opposed to the coarse oxyphile granule or eosinophile granule and the coarsely granular oxyphile cells[2].

The other term which was applied to the finely granular oxyphile cells by Metschnikoff and others, namely, "polynuclear leucocyte" is not satisfactory, seeing that, as Fleming, we believe, first pointed out, the different nuclear masses which are dispersed through the body of the cell and appear at first sight to be distinct nuclei are, in point of fact,

[1] It is not our purpose to give any complete account of the factors which modify these micro-chemical tests. We may, however, note that the density of the solution of the stain affects the reaction. In order to eliminate this factor we used in all cases a saturated solution.

Eosine throughout this paper refers solely to Grübler's "wasserlösliches Eosin."

[2] This classification has also been adopted by Sherrington, *Proc. Royal Soc.* LV. p. 186 &c.

joined by threads or bars of nuclear substance so that the cell is really mononuclear with a very much branched nucleus.

The recognition of the fact that the fine granule of the common blood corpuscle of mammalia is oxyphile gives to Ehrlich's classification of the specific granules of wandering cells greater simplicity and homogeneity, since it enables us to arrange them in two main groups, each group being again sub-divided into two. These are:—

I. Oxyphile granules { (*a*) Coarse oxyphile granules (eosinophile of most writers). (*b*) Fine oxyphile granules.

II. Basophile granules { (*a*) Coarse (Ehrlich's γ granulation). (*b*) Fine (Ehrlich's δ granulation).

And similarly the wandering cells of Mammalia fall into three groups:—

I. Oxyphile cells { (*a*) Coarsely granular oxyphile cells. (*b*) Finely granular oxyphile cells.

II. Basophile cells { (*a*) Coarsely granular basophile cell. (*b*) Finely granular basophile cell.

III. Non-granular cells or as we have elsewhere termed them, Hyaline cells.

To these would be added a fourth group:—

IV. Immature cells, or lymphocytes.

Methods Employed.

The reactions of substances to basic and acid dyes are modified by the manner in which the tissues are prepared for staining. Heat, as Ehrlich pointed out, intensifies the oxyphile reaction of the granules[1], fixation with corrosive sublimate, osmic acid, and Flemming's fluid also modifies the histo-chemical reactions. It is therefore necessary to adopt some standard method of preparation for obtaining what may be then called the standard oxyphile or basophile reactions.

Standard method. The films, whether of fixed tissue, of blood or of lymph, were allowed to dry at the temperature of the room. The stain most commonly used was eosine in saturated aqueous and glycerine solutions and in alcoholic solutions in which the alcohol formed 50, 75, 80, 85, 90 and 95 per cent. Orange *G* was also used in saturated aqueous, and in saturated alcoholic solutions. The Ehrlich-Biondi mixture as supplied by Grübler and the "neutral" mixture of Ehrlich which is composed of acid fuchsine, methyl green, and Orange *G* were

[1] *Farbenanalytische Untersuchungen*, p. 14.

also used. The films stained with the aqueous solution of eosine were exposed to it for the shortest possible time and were then washed for 5 minutes in 95 per cent. spirit, dried between filter papers and stained with Loeffler's solution of methylene blue. Films stained in glycerine eosine were dipped in the solution and then washed in 95 per cent. alcohol until all the glycerine was removed. They were then immersed for a moment in fresh alcohol, dried between filter papers and stained with Loeffler's solution. To stain in the alcoholic eosines the films were immersed in the solution for 15 seconds, washed in two changes of 95 per cent. spirit (total time 1 minute), dried between filter paper and stained in Loeffler's solution. The Loeffler's solution was in all cases washed off with water after which the films were finally dried and mounted in balsam.

It will be noticed that the excess of eosine was always washed off with 95 per cent. spirit, and in order to secure trustworthy results, this point must be attended to with scrupulous care. If, for instance, a film which has been stained in alcoholic or glycerine eosine is washed in water, it is exposed in the process to an aqueous solution of the dye which, though dilute, has a staining power considerably above that of the alcoholic solution, and often above that of the glycerine solution.

Orange *G* in saturated aqueous and saturated alcoholic solutions; haematoxyline[1] in neutral saturated aqueous solution, and sodium sulphindigotate in saturated aqueous solution were used as weak acid dyes.

The weak acid dyes (Orange *G*, haematoxyline, and sodium sulphindigotate) were washed out with water. The mixtures (Ehrlich-Biondi and the "neutral" mixture) were washed out with water. Sometimes however in order to secure more differential acid staining the Ehrlich-Biondi mixture was washed out with 95 per cent. spirit. This point will be specially noticed whenever reference is made to this stain.

It is obvious that if eosine in aqueous solution has a very high staining power, and in strong alcohol only a very weak staining power, the staining power of a saturated solution of eosine in a mixture of water and alcohol will become less as the amount of alcohol present becomes greater. In other words, that the staining power varies inversely as the amount of alcohol present. Therefore, by using solutions of eosine in spirit of different strengths very delicate and

[1] Schwarze. *loc. cit.* and *Farbenanalytische Untersuchungen*, p. 90.

exact discrimination may be made between the intensities of the oxyphile reaction of various substances.

In order to preserve very unstable cells, or to fix cells in the position they occupy when attacking micro-organisms, very rapid fixation by heat was resorted to. Some of the fluid, e.g., peritoneal fluid, was brought on to the surface of a carefully cleaned cover-glass which was then at once immersed in the flame of a Bunsen's burner. It is not easy to stain successfully films fixed in this way, because the coagulated plasma colours intensely with methylene blue. We overcame this difficulty by volatilising the excess of methylene blue with the heat of a small spirit flame.

Since film preparations cannot be relied upon to show the true size or shape of cells, measurements and drawings were made from fluid preparations made by quickly adding to fresh blood or other fluid three times its volume of a dilute solution of methylene blue in 40 per cent. alcohol to which a trace of caustic potash and of osmic acid had been added. This solution is especially valuable for the cells of blood; indeed without its aid it would be difficult to study the finely granular basophile cell. In order to make a successful preparation, it is above all things essential that the staining fluid be added to the amount of at least three times the volume of the blood before clotting takes place. It will then be found that the blood is rendered laky and the refractive index of the fluid is so nearly that of the stroma of the red corpuscles that these bodies become quite invisible, so that the wandering cells may be counted or examined with the same ease as in lymph. In preparations of this kind differences in the refractive indices of granules form a striking feature.

In order to determine the relative abundance of the various forms of cells it is very necessary not to use a pipette; the fluid must be allowed to run or drop on to the cover-slip or slide. The percentage of the basophile cells is especially affected by using a pipette since these are very adhesive and remain attached to the sides of the pipette.

Description of the Cells and of their Distribution in the Body.

In this section the cells are dealt with in the order in which they occur in the scheme given on page 86.

The Oxyphile Cells.

1. *The coarsely granular oxyphile cell, or eosinophile cell* (Figs. 1,

2, 6; 7, 8, 10, 12 (*a*), and 14), varies in size in different animals, not only absolutely, but relatively to the dimensions of the other classes of cells. In man it is larger than either the hyaline cell, the finely granular oxyphile cell, or the finely granular basophile cell. In the rat, rabbit, and guinea-pig, on the other hand, it is smaller than the largest hyaline cells, but larger than the finely granular oxyphile and basophile cells[1].

The *nucleus* is typically an elongated body bent to form a horse-shoe. In the rat the arms of the horse-shoe are carried so far round that in film preparations the ends often overlap, giving to the nucleus the appearance of a circle with a large hole in the centre (Figs. 7 and 10 *a*). Sometimes the nucleus is lobed, but we are inclined to regard this appearance as being largely due to the stresses to which the nucleus is subjected when the cell is dying. In the living cell at rest, when it is spherical, the shape of the nucleus, so far as it can be determined by the disposition of the cell-granules, is a simple horse-shoe or crescent. A distinct nuclear network is present.

Cell granules. The cell granules are relatively large, spherical, or slightly ovoid bodies, and are sharply marked off from the cell substance by their very high refractive index, which is so great that in fluid preparations the granules have a brilliant greenish lustre (Figs. 1, 8, and 12 *a*). The cell-substance in which they are imbedded has the appearance of a clear transparent structureless jelly. The intensity of the oxyphile reaction of these granules differs in different animals but is always high. Thus it is very high in the case of the granules of man; these staining with eosine dissolved in 95 per cent. alcohol; it is lowest in the granules of the rat which do not stain with eosine in strong alcohol, but do colour with eosine in glycerine or in alcohol below 85 per cent. The granules also stain with weak acid dyes, such as Orange *G*, haematoxyline and sodium sulphindigotate. Ehrlich-Biondi's mixture (washed out with 95 per cent. spirit) colours these bodies brown-purple, and the "neutral" mixture (washed out with water) stains them a very intense red purple. Corrosive sublimate increases the oxyphile reaction as does also heat when applied to the dried film[2].

2. *The finely granular Oxyphile cell* (Figs. 1, 2, 3, and 6 *b*, 11, 12 *c*, and 15 *b*), is always smaller than the coarsely granular oxyphile

[1] The dimensions of the various cells are given in the table on p. 101.

[2] Cf. Sherrington, *op. cit.* pp. 189 and 190 where a description is given of this cell in the blood of the cat.

cell. On the other hand, it is in man and the guinea-pig rather smaller than the hyaline cell, and in the rat and rabbit very considerably smaller. The *nucleus* is an exceedingly irregular structure branching throughout the cell (Figs. 2 and 6 *b*). The branches swell out here and there into masses of an irregular shape, the intervening or connecting portions being of the nature of slender bars or threads. A fine and close nuclear network can be detected.

The granules are very small spherical bodies which crowd the otherwise clear and optically structureless cell-substance. They have a refractive index only slightly above that of the medium in which they are imbedded so that they are scarcely visible when unstained (Figs. 1 *b* and 12 *c*). Their oxyphile reaction is much feebler than that shewn by the large granules of the coarsely granular oxyphile cells. Thus they do not stain with the weak acid dye Orange *G*, nor do they stain with eosine, except in an aqueous solution or in an alcoholic solution in which the alcohol is less than 60 per cent. On the other hand, they stain with the fuchsine and orange of the "neutral" mixture and with Ehrlich-Biondi's mixture. The dye, however, can be washed out by strong alcohol. Long exposure to haematoxyline stains them and they colour though very feebly with sodium sulphindigotate. In this case they do not retain the colour in presence of water unless it contain a trace of the dye in solution.

The finely granular cells of the rabbit need special mention. The granules of these cells constitute the β granulation of Ehrlich to which he gave the name of "amphophile" granulation. According to the nomenclature of Ehrlich this name can mean only that the granules to which it is applied stain both with acid and with basic dyes. Taken in this sense we cannot see why the fine oxyphile granules of the rabbit were termed amphophile, since so far as we know they fail to stain with any basic dyes. On the contrary, we find them to possess an oxyphile reaction much above that of the similar granules of the other mammals investigated by us, and we are thus led to regard them as especially interesting. Thus the fine oxyphile granules of man have an oxyphile reaction which is slightly less intense than that shown by the red corpuscles, but the fine oxyphile granules of the rabbit possess a reaction considerably above this level, and often verging closely upon that shown by the coarse oxyphile granules of the rat[1]. The red corpuscles in all cases fail to stain with eosine in glycerine solution or in alcoholic solution when the alcohol is above 80 per cent.,

[1] Cf. Sherrington, *op. cit.* pp. 186—189.

or with aqueous haematoxyline or sodium sulphindigotate; but the fine oxyphile granules of the rabbit stain deeply with eosine in glycerine solution, and in alcoholic solution when the alcohol is not above 90 per cent. They also colour with aqueous solutions of sodium sulphindigotate and haematoxyline though very slowly in the last case. The fine oxyphile granules of man, rat, mouse and guinea-pig do not stain with eosine in glycerine solution, nor in alcoholic solution when the percentage of alcohol is above 75.

Corresponding with the higher oxyphile reaction we find that the fine oxyphile granules of the rabbit have a higher refractive index than have the similar granules in the other animals.

The oxyphile reaction and refractive index of the fine oxyphile granules of the rabbit may be very readily raised above the normal by the presence in the blood of minimal amounts of microbic poison, in other words, by what are commonly known as immunising doses. The rabbit is peculiar in the readiness with which this change may be induced. No confusion of the two types of oxyphile cells, however, follows this change, and we only allude to the fact in passing because it might, and possibly has, led to a certain amount of confusion, especially when we bear in mind that so many apparently healthy rabbits have cocci and bacilli present in the cells and fluid of the peritoneal cavity.

Distribution of the Oxyphile cells. In the animals examined the coarsely-granular oxyphile cell is abundant in the fluid occupying the pleural, pericardial and peritoneal cavities—in other words, in the coelomic fluid—in the interstices of areolar connective tissue throughout the body, and in peripheral lymph channels. There are also considerable numbers of these cells in bone marrow and in parts of the spleen and lymphatic glands.

The coelomic fluid of mammals is very richly supplied with wandering cells, indeed it presents from this cause a cloudy turbid appearance. Of the cells present from 30 to 50 per cent. are coarsely granular oxyphile cells (Figs. 7, 8, and 12 *a* and 14). In connective tissue again, the total number of these cells must be very great, for the thinnest film spread out on a slide will frequently show from 5 to 10 in a single field of a Zeiss D, Oc. 4. Thus this cell has an extraordinarily wide distribution outside the vascular system. In sharp contrast to this it occurs in very small numbers only in the blood, forming not more than 2 to 4 per cent. of the white corpuscles there. Wherever found the cells agree in all their histological features—in size and shape, in the features of the nucleus, and in the histochemical reaction of

their granules. We must however except certain of the individuals occurring in bone marrow and in the spleen. Further, under appropriate stimuli the cells of any particular locality may proliferate rapidly. Therefore, we cannot at present point to any part of the body as the head quarters, or special place of origin of these coarsely granular oxyphile cells, and in the present state of our knowledge we can assign no special significance to the accumulation of these cells in the connective tissue of lymphatic glands of the spleen, and of bone marrow.

The relation of the coarsely granular oxyphile cells to the lymphatic system, how far for instance the extra-vascular cells, under normal conditions, find their way by its aid into the blood, is very obscure. For the present we will content ourselves with pointing out that they appear to be always more abundant in the lymph-capillaries and vessels on the far side of lymphatic glands than in the thoracic duct.

The finely granular oxyphile cell has a very limited and precise distribution, for under normal conditions it is entirely absent from extra-vascular spaces and occurs only in the blood, where it is by far the most numerous corpuscle forming from 20 to 70 per cent. of the total number of white corpuscles. The fluctuation in this percentage is probably due in the main to the great periodic variations in the number of lymphocytes present in the blood. Thus the effect of a meal is to cause a considerable increase in the number of lymphocytes in blood and, therefore, a fall in the share of the total white corpuscles due to finely granular cells. If this disturbing factor be eliminated and the percentage of the finely granular oxyphile cells be taken of the adult white corpuscles only, then this is found to be always very high; in man 75 to 90 per cent., and lowest in the rabbit 50 to 70 per cent.

Thus the group of oxyphile cells falls into two sub-groups defined by marked histological peculiarities, and by the fact that the cells of the one sub-group inhabit almost exclusively the extra-vascular body spaces, notably those derived from or in communication with the coelomic spaces; while cells of the other sub-group inhabit solely the vascular channels.

The Basophile Cells.

1. *The coarsely granular Basophile cells* (Figs. 4, 7, 9, 10, 13, and 17) have been described by Ehrlich[1] under the name of 'Mastzellen' and their existence in the coelomic spaces and in the connec-

[1] Ehrlich. *Loc. cit.*

tive tissues was recognised by him and by Ranvier[1]. The cells of the connective tissues and those of the coelomic fluid differ slightly in size and shape, and we will therefore deal with them separately.

The coarsely granular basophile cell found in the coelomic fluid is, so far as we know, under normal conditions not endowed with the power of amoeboid movement. It is a large cell having the form of a very much flattened sphere which might not inaptly be compared with a millstone whose edges had been very much rounded off (Figs. 7 *b*, and 9). The nucleus is rounded, occupies a central position and seems to be singularly devoid of chromatin, staining with difficulty. The cell substance, clear and optically structureless, is charged with a very great number of exceedingly large spherules, the basophile "granules."

The coarsely granular basophile cell of the connective tissue (Figs. 10 *b*, 13 *a*, *c*, *d*, and 17) differs from the other forms of wandering cells in that it appears to be, at any rate in the normal animal, not only non-amoeboid like its coelomic brother but also stationary. It is therefore, except perhaps under certain conditions, not strictly a wandering cell. It is of a rounded or slightly polygonal shape and usually is more flattened, and so rather larger in its extreme dimensions, than the cell of the coelomic fluid. In its other histological features, such as the nature of its granules, nucleus, and cell substance, it exactly resembles the coelomic cells.

The granules in both cases are the same, and in the rat and mouse are large spherules of an average diameter of rather more than 1 μ. Like all forms of basophile granules their refractive index differs only slightly from that of the cell substance, and they are therefore almost undistinguishable when unstained. Their large size enables one to study them in detail, and they are found to present certain features of interest. When microbes or microbic poison or "irritants" are present, these cells are frequently found with their granules so changed that they no longer stain, or stain imperfectly. In place of all the granules in the cell staining, all or some of the granules are now refringent spherules of the same size as the normal granules, but they either completely refuse to stain, or they stain in patches. The phenomenon very strongly suggests that these granules are not entirely composed of the basophile substance, but rather are composed of an unstaining groundwork with which the staining basophile material is associated but from which it may be removed.

The unstable or *explosive nature* of the coarsely granular

[1] Ranvier. *Comptes Rendus*, T. cx. p. 768.

basophile cells in certain animals, is one of their most remarkable characters. In the rat and mouse perfect preparations of these cells may be very easily made, but in the guinea-pig and rabbit they can be preserved only with the most rapid fixation by heat or corrosive sublimate or absolute alcohol. In these animals the mere exposure of the coelomic fluid to the air, or to contact with a coverslip for a few seconds is sufficient to cause their complete disappearance. Cells characterised by great instability have been described elsewhere in Astacus[1] as the "explosive" cell, of that animal, and the basophile cells of the guinea-pig and rabbit might, with equal justice, be designated the explosive cells of those animals. Under the influence of certain chemical stimuli, as will be seen later, the basophile cells of the rat also become explosive. The instability of the coarsely granular cells of the guinea-pig and rabbit is so marked and constant a feature that Ranvier was unable to detect their presence in the coelomic fluid of these animals, and came to the conclusion that they were absent[2]. Examples from the guinea-pig are shown in Fig. 13, (*a*) being from the wall and (*b*) from the fluid of the peritoneal cavity, while (*d*) and (*c*) represent the appearance produced by the bursting of the cells in a film of connective tissue.

The finely granular basophile cell (Figs. 1 *e* and *f*, 6 *d*, 12 *e*, 15 *e*) unlike the last, is a small cell, being usually the smallest of all the wandering cells. It is spherical in shape and possesses a characteristically trilobed nucleus. The cell-substance is clear and optically structureless, and usually contains an immense number of minute granules which often appear as mere points, and are characterised by staining a very opaque blue or purple colour with methylene blue. Another very characteristic feature is the fact that the cell substance colours a purple or pink tinge with the methylene blue solution.

These cells may be readily studied in man and the rabbit, in preparations of blood made with the aid of the methylene blue solution. In the rat, however, these cells appear to be very unstable, and the methylene blue solution fails to preserve them. They may, however, be found in films of blood fixed with the greatest rapidity with absolute alcohol. The blood is allowed to drip from a cut vessel on to a carefully cleaned coverslip—the excess blood is shaken off the coverslip which is then plunged into absolute alcohol. Staining may be best effected by immersing the film in 20 to 30 per cent. spirit to which a trace only of

[1] Hardy. This *Journal*, XIII. Nos. 1 and 2.

[2] Ranvier. *Comptes Rendus*, CXII., footnote p. 923.

methylene blue has been added. In preparations of the blood of the guinea-pig made with the methylene blue solution, cells are found like the one shown in Fig. 12 *e*. The granules are large and stain with the methylene blue, they are therefore, at least under certain conditions, basophile. It is this cell which appears in Table II. as the finely granular basophile cell of the guinea-pig. We are not, however, at all certain that it is really identical with the very characteristic and readily detected cell of man and the rabbit. Our knowledge of the finely granular basophile cell, however, is so deficient that it is perhaps wisest for the present to class it as we have done.

Distribution of the basophile cells. The distribution of the coarsely granular basophile cells in the body resembles that of the coarsely granular oxyphile cells in so far that they occur in the coelomic fluid and in the interstices of the connective tissue. But, unlike the latter, they are not merely rare but completely absent from the blood[1]. Moreover while they form only 10 per cent. (in the rat and mouse) of the cells of the coelomic fluid, they are exceedingly numerous in connective tissue spaces, where they form sometimes an almost complete sheath for the lymph capillaries.

The finely granular basophile cell. These we have found solely in the blood, and even there they are present only in small numbers (1 to 5 per cent.). Their presence and the extent to which they are charged with granules appears to depend very closely on events occurring in the alimentary canal. Thus, in man six to eight hours after a meal, these cells can be detected in the blood only with difficulty, owing largely to the fact that they contain but few granules. About two hours after a meal, however, they can be found with ease and are then seen to form an appreciable percentage of the white corpuscles. Similarly, they form a very conspicuous feature being relatively speaking abundant, and very heavily laden with granules, in the blood of a rabbit which has been kept unfed for ten hours and then given a full meal (cf. Table I.). To say that these cells are found in the body only in very small numbers being confined to the blood and scanty even there, is probably only equivalent to saying that we are at present very ignorant as to their history, distribution and significance. However, since we find this cell in the blood but do not find it either in the coelomic fluid or in the interstitial spaces of the tissues (except perhaps in those of the mucous

[1] Blood obtained by plunging a fine pipette into the heart rarely fails to contain one or two removed from the pericardial space. Blood from a vessel is always entirely free from them.

coat of the alimentary canal), we must, until further facts are forthcoming regard it as the basophile cell of the blood.

Thus our present knowledge of the basophile cells leads us to class them in two great groups—a sub-group with coarse granules occurring only in the extra-vascular spaces, and a sub-group with fine granules occurring only within the vascular system, and these sub-groups correspond closely in structural characteristics and distribution with the sub-groups of the oxyphile cells. At the same time we regard the grouping together of the finely granular basophile cell which is so scarce and the finely granular oxyphile cell which is so abundant in blood as blood cells to be based as much upon ignorance as upon knowledge.

The Hyaline Cell. Figs. 1, 2, 3, 6, 7 *c*, 8 and 12 *b*, and 15 *c*.

This is usually of about the same size as the coarsely granular oxyphile cell and rather larger than the finely granular cell. When at rest it is a spherical cell but, owing to its pseudopodial activity, it mostly appears as a somewhat tenuous body of very irregular shape. The nucleus is usually spherical, it is sometimes kidney-shaped probably as a result of the mode of killing the cell. A very fine nuclear network with large meshes spreads through the nucleus and, in most preparations, a nucleolus is present. The cell substance is always free from discrete granules, it is not, however, very transparent but presents rather the appearance of ground glass.

Distribution in the body fluids. The hyaline cell occurs both in blood and in the extra-vascular spaces and, unlike the granular cells, we are unable to point to any histological differences except perhaps in point of size between the forms found in the blood and those found elsewhere. At the same time, this cell is rare in the blood (2 per cent.), while it is abundant in the coelomic fluid where it forms about 50 to 70 per cent. of the cells, so that its distribution accords closely with that of the coarsely granular oxyphile cell.

We may here point out certain homologues of the hyaline with the other cells. The cell substance of the hyaline cells differs, at first sight, from that of the granule bearing cells, in taking a faint diffuse colouration with basic dyes. The highest powers of the microscope, however, frequently resolve this cloudy and apparently continuous colour into a number of stained points (Figs. 8 and 12 *b*); and the cell substance of the dead hyaline cell is then seen to be composed of an optically structureless substance remarkably indifferent and resistant to all stains, embedding a staining substance dispersed throughout it in the form of a cloud of minute particles. We have had occasion again and again to

define the cell substance of the granule bearing cells as optically structureless, and it also resembles the basis of the cell substance of the hyaline cells in its resistance to stains. Therefore, viewed from the standpoint of the morphology of the cell, the defined and specialized granules of the oxyphile and basophile cells are homologous with the amorphous "points" of staining matter in the hyaline cell. The structural differences are of degree and not of kind.

But the question, What are the homologues of the granules in the less specialized cells? can be carried further towards solution, if we interpret the structure of the cell substance of a dead hyaline cell by what can be seen in living specimens; though, unfortunately, facts of the order of those which we are now considering can only be intelligibly expressed in terms of some hypothesis, the simpler the better.

A striking physical peculiarity of the cell substance of a living hyaline cell is its opacity. The whole thickness of a resting cell is small, and when spread out in amœboid movement, it forms extremely thin films; yet, in spite of this, it hides or blurs to a marked extent the outlines of any object over which it may happen to lie. So great an opacity in so thin a colourless membrane can only be produced in two ways, (i) by the formation of refracting surfaces within the membrane, owing to the presence of substances having different refractive indices, and (ii) by the disposition of these surfaces in such a way that they are concave or convex, or, if plane, inclined at some angle other than a right angle to the rays of light. A simple structure of this order would be composed of two substances having different refractive indices, the one forming a continuous basis in which the other is embedded in the form of spheres or rounded masses. If these spheres or spheroids were sufficiently small and sufficiently near together, a very thin membrane having considerable translucency but slight transparency would be the result.

Following out this hypothesis we may suppose the cell substance of the hyaline cell to be composed of a colourless basis, embedding multitudes of minute spaces or vacuoles filled with a substance, possibly more fluid, of a different refractive power, and we may suppose that the action, for instance of the alcoholic solution of methylene-blue, is to precipitate the contents of these tiny spaces to a minute mass of stained matter. Further, following out this hypothesis, the specific granule may be regarded as the specialized homologue of the contents of one or of a multitude of these tiny vacuoles.

Immature Forms of Wandering Cells present in the Body Fluids.

Three forms of immature cells are readily recognised in the various body fluids of a normal animal. These are as follows:—

(i) A small round cell characterised by possessing a deeply staining horse-shoe shaped nucleus embedded in scanty cell substance. The cell substance is charged with minute granules which give the same reactions as the coarse oxyphile granules (Fig. 7*a'*), that is to say, using the ordinary terminology, they are undoubtedly eosinophile granules. The diameter of the cell in the rat is 6·5 μ. We have found it only in the coelomic fluid, and from this fact and from its histological characters we may unhesitatingly regard it as the immature form of the coarsely granular oxyphile cell.

(ii) A round cell, somewhat larger than the last (diameter 8 to 9 μ). It possesses a spherical nucleus and the cell substance is charged with small basophile granules (Figs. 7*b'*, 8*c*). It is readily distinguished from the finely granular basophile cell of the blood by its nucleus, which is a simple sphere instead of being trilobed, and by its granules, which are larger, each being a distinct though small spherule. We have found this cell only in the coelomic fluid and we may without hesitation regard it as the immature form of the coarsely granular basophile cell.

(iii) A small cell which, like the first described, is 6·5 μ in diameter (rat) and possesses a deeply staining nucleus and scanty cell substance (Figs. 1*d*, 5, 7*c'*, 15*d*). The nucleus, however, is spherical and the cell substance is free from granules. These cells have been called *lymphocytes*, from the fact that they are produced in lymphatic glands. They occur in both blood and coelomic fluid, and in the lymph of the thoracic duct. Their number in blood varies in different animals and at different times, and is especially dependent on the digestive activity of the alimentary canal, a meal causing a considerable rise. The blood of the rabbit is characterised by containing them in especial abundance, after a full meal they may in this animal form 70 to 80 per cent. of the wandering cells present[1]. Similarly, in man, about two hours after a full meal they form as many as 30 per cent. of the cells. Thus active digestion produces a condition of the blood which may be called lymphocytosis. On the other hand, starvation decreases the number of these bodies relatively to the other cells. The blood of a rabbit deprived of food for 12 hours was found to contain under 20 per cent. of

[1] Okintschitz. *Arch. f. exp. Path. u. Pharm.* Vol. xxxi., 1893.

these cells. We have reason to think that lymphocytes are produced in lymphoid tissue no matter whether it be found in lymphatic glands, in the spleen, or elsewhere; and from their histological characters we might infer that they are the young forms of the hyaline cells; and we may advance this view with some approach to certainty, for we find in coelomic fluid and in blood, as Max Schultze showed, not only lymphocytes but also all stages of increase in size and relative abundance of cell substance until they merge into undoubted hyaline cells. Beyond this, however, we cannot go, and the further question whether any of the lymphocytes are also the young form of other wandering cells is at present so far from solution that we scarcely have facts at our disposal sufficient to warrant us in discussing the probable answer. Ouskoff[1], as a result of the study of the histology of blood, arrived at the conclusion that these cells developed into the finely granular oxyphile cells. As the original paper is in Russian we are ignorant of the facts on which he bases this conclusion. Certain observations, however, appear at first sight to discredit this view. Briefly they amount to this, if by irritants we produce a local increase in the number of the leucocytes in, for instance, a lymphatic gland, the result always is the development of a very large number of actively amœboid and phagocytic hyaline cells. The finely granular oxyphile cells are invariably absent, unless the lesion is excessive and involves the blood vessels. Muir[2] also points out that in a form of the disease leucocythæmia which is characterised by a large increase in the number of lymphocytes and hyaline cells in the blood there is not only no increase in the number of the "polynuclear leucocytes" (finely granular oxyphile cells) but very frequently an absolute diminution.

Comparative Morphology.

Under this heading but little can be said that can have any pretence to finality, but certain broad conclusions may be pointed out. The great feature of the structure and arrangement of the sporadic mesoblast of the mammalia is the comparatively sharp division of the structure into two portions, one of which mainly inhabits the blood spaces, while the other is gathered into the coelomic spaces, and distributed through the body in the interstitial spaces of the connective

[1] *Le Sang comme tissu.* S. Petersbourg, 1890 (in Russian), quoted by Metschnikoff in *L'Inflammation.*

[2] *Jour. of Pathology*, Vol. I. p. 123. 1892.

tissues. Each of these great divisions comprises three classes of cells, an oxyphile cell, a basophile cell, and a hyaline or granule free cell. This feature, namely, the differentiation of the tissue into two distinct and complete portions is, if the frog may be regarded as typical, absent in the similar tissue of the lowest vertebrates, for in the frog we find that the tissue comprises only three forms of cells, an oxyphile cell, a basophile cell, and a hyaline cell, which are found indifferently inhabiting the vascular and extra-vascular spaces.

The more special question of the origin and morphological relation of the several classes of cells which make up the sporadic mesoblast of the mammalia is still obscure. Here again, however, certain though partial conclusions may be drawn. We may assume that the three cells of the extra-vascular, or, as it might conveniently be called, the coelomic portion, ought to be considered as being entirely distinct from one another in life history and function, until convincing evidence to the contrary is forthcoming. It is difficult to see how the very peculiar and very specialized non-amœboid basophile cell can be derived from either of the other much smaller forms. And the real and permanent distinctness of the coarsely granular oxyphile cells and the hyaline cells is also rendered probable on three grounds: firstly, the presence in the coelomic fluid of the young form of each; secondly, the difficulty of conceiving how the hyaline cell can develop into the oxyphile cell since the former is larger and has different functions from the latter; and, thirdly, the difficulty of conceiving the converse change, namely, the passage from a complex glandular cell endowed with the power of storing up granules definite in shape and in their histochemical characters to a comparatively unspecialized cell which has none of these attributes[1]. Yet, although such evidence points to the independence of each other of the several coelomic cells, we are ignorant of the relations of these cells to those of the blood. We cannot, for instance, determine whether the oxyphile cell of the coelom may or may not change into that of the blood or vice versa, and we are also largely ignorant of the special seat of origin, if they have any, of these several classes of cells, and whether even if they have entirely different life histories they may develop from a common type of young cell.

[1] Cf. Sherrington, *op. cit.* p. 203.

TABLE I.

Showing percentage and size of the various forms of the Wandering Cells of the Blood.

	Oxyphile cells				Basophile cells			Hyaline cells		Lymphocytes	
	Coarsely granular		Finely granular		Coarsely granular	Finely granular					
Man		10 to 11 μ		8 to 9 μ	absent		7 μ		8·5 to 10 μ		6 μ
Rat	2 %	10 μ	45 %	7 to 8 μ	absent			2 %	8 to 10 μ	50 %	6 μ
Rabbit	1 to 2 %	10 to 11·5 μ	20 to 30 %	8 to 9 μ	absent	2 to 5 %	8 μ	2 to 6 %	8 to 11 μ	70 to 80 %	6 μ
Guinea-pig	2 to 3 %	10 μ	62 %	8 μ	absent	0·7 %	8 μ	11 %	9·5 to 10 μ	24 %	6 μ

TABLE II.

Showing percentage and size of the various forms of the Wandering Cells in the Peritoneal fluid.

	Oxyphile cells			Basophile cells			Hyaline cells		Lymphocytes	
	Coarsely granular		Finely granular	Coarsely granular		Finely granular				
Man										
Rat	25 to 40 %	10 μ	absent	5 to 10 %	18 μ	absent	13 μ	65 to 80 %	6·5 μ	
Rabbit		10 μ	absent			absent	12 to 14 μ		6 μ	
Guinea-pig	30 to 50 %	10·5 μ	absent			absent	13 μ	50 to 65 %	6 μ	

Basophile cells in connective tissue of the Rat 23 μ.

The facts of structure and distribution of the cells composing the sporadic mesoblast of the higher mammalia may be expressed in schematic form as follows:

Division I.	I. Oxyphile.	II. Basophile.	III. Hyaline.
Hæmal cells; characterised by being relatively small cells and having fine specific granules.	Nucleus branched; specific granules small, with relatively feeble oxyphile reaction. (The coarsely granular cells also occur to a limited extent in the hæmal system.)	Nucleus lobed; specific granules very small.	Nucleus round; no specific granules.
Division II. Cells of coelomic and interstitial spaces. Characteristic, large size of cells and granules.	Nucleus crescentic; specific granules large with intense oxyphile reaction.	Nucleus round; granules very large.	Nucleus round; no specific granules.

Part II. On Certain Activities of the Coelomic Cells.

In an earlier paper on the phenomena displayed by the wandering cells of the frog when in the presence of microbes and microbic poisons[1], we stated at the outset that our researches demonstrated a disparity of function between the different classes of wandering cells comparable to the disparity in form. In what follows an extreme disparity of function will be shown to exist between the varieties of the coelomic cells of mammalia, and, as in the case of frog, we would again point to these marked differences of function as affording the strongest reasons for believing that the different forms of wandering cells have each a distinct and separate physiological significance.

Leucocytosis and Chemiotaxis.

By the term leucocytosis is meant an increase in the number of wandering cells present in any defined area, or in the body as a whole. We may therefore speak of local leucocytosis or of general leucocytosis. Leucocytosis, whether local or general, may be referred to two possible

[1] Kanthack and Hardy. *Proc. Royal Soc.* Vol. LII. p. 267 and *Phil. Trans.* 1894.

causes—to an excessive proliferation of the wandering cells already present in the area affected, or to an excessive immigration, usually from the blood. It is obvious from what we said in Part I. concerning the distribution of the wandering cells, that a cell accumulation produced by local proliferation must consist solely of coelomic wandering cells, whereas, if the lesion involves the blood capillaries the boundaries between the vascular and extra-vascular cells will be obliterated and a cell accumulation of mixed character will result. On the other hand a leucocytosis which was limited to the blood would affect solely the hæmal system of wandering cells.

The real distinctness of the two forms of leucocytosis, hæmal leucocytosis and coelomic leucocytosis as they might for convenience be called, may be demonstrated either by producing very localised cell accumulations in connective tissue, in chambers or fine tubes placed under the skin, or in a coelomic cavity; or by determining what may be called the order of arrival of the various cells by observing from time to time the character of the cells in any leucocytic focus.

If for instance Ziegler's chambers or capillary tubes which have been filled with bacilli, or their products, or some "irritant" such as nitrate of silver and turpentine, are placed under the skin or in the peritoneal cavity and are allowed to remain there for periods up to 24 hours they will be found to contain a multitude of cells solely of the coelomic type. If however the irritant is situated in such a position that it appeals to the blood vessels of a vascular membrane rather than to the cells of the connective tissue spaces, then the cells will be those of the hæmal system. Even in these cases however we usually find in the earliest stages a preponderance of the coarsely granular oxyphile cells. In cutaneous blistering for instance the irritant appeals immediately to the blood vessels of the dermis; and in over 30 blisters produced on ourselves or on others with the liquor epipasticus we found that the cells present were mainly of the blood type, though the coarsely granular oxyphile cell was always more abundant relatively to the others than in blood.

Experiments of this nature bring to light differences between the different forms of wandering cells as regards their rate of accumulation, or, as it is generally expressed, chemiotaxis. Thus the first cell attracted by microorganism or irritants is always the coarsely granular oxyphile cell. Indeed in the majority of the tube or chamber experiments although many thousands of cells had crowded in they were almost all of this type.

If we ask ourselves the question where do these cells come from we are able to answer positively, in the case of chambers placed under the skin, that they have their immediate origin in the surrounding connective tissue. Fig. 19 shows the striking appearance presented by a film of connective tissue taken from the immediate neighbourhood of a Ziegler's chamber filled with a dilute broth culture of the cholera vibrio after 7½ hours' sojourn under the ventral skin of a guinea-pig. The chamber was found to contain multitudes of the coarsely granular oxyphile cells, and a comparison of figure 19 with figure 20 will show how strikingly abundant were these cells in this leucocytic focus as compared with the normal connective tissue.

The experiments which we have performed bearing on the subject-matter of this section may be divided into four sets. These are (1) the introduction of chambers or tubes containing irritants or cultures of microorganisms into the peritoneal cavity; (2) the introduction of similarly prepared chambers under the skin; (3) the introduction of copper into the anterior chamber of the eye; and (4) the production of skin blisters by an irritant (liquor epipasticus). The operations necessary in the first three cases were always performed under anæsthetics, and with antiseptic precautions.

1. *Tubes or Chambers in the Peritoneal Cavity.* Fine capillary tubes were used and were filled with very dilute nitrate of silver, or turpentine, or with the diluted filtrate from a broth culture of pyocyanine which had been passed through a Chamberland's filter. The tubes were placed in the peritoneal cavity of rabbits and in 6 to 18 hours the animals were killed and the contents of the tubes converted into films. In all cases the cells were almost without exception of the coarsely granular oxyphile type and were present in great numbers.

The chambers used were those known as Ziegler's chambers. They were made in the usual way by cementing together two coverslips separated by a circular strip of tin foil. Two small openings situated at opposite points were made and through these, by means of capillary attraction, the chambers were filled with broth cultures of bacilli which were not more than one to two days old and had been considerably diluted with fresh sterile broth.

The chambers were inserted through a slit about ¾ inch long made in the linea alba; the wound was then sewn up and dressings applied. They were allowed to remain in the peritoneal cavity for 2, 2½ or 7 hours. The animal was then killed and the chambers were at once removed and the cells on the external surface and those in the interior were examined

at once and without the addition of any reagent. In this way the cells were seen to perform amœboid movements, and to be attacking the bacilli (Fig. 21). The chambers were then split open and the cells adherent to the external surface and those in the interior were examined with the aid of the methylene blue solution, and as film preparations.

The statements made as to the relative abundance of the different forms of corpuscles are founded upon countings made with fresh unopened chambers and with stained preparations.

Cultures of Bacillus ramosus, Bac. anthracis, and of the comma bacillus were used and the experiments were performed on rabbits and guinea-pigs.

The perforation of the abdominal wall led in all cases to a certain amount of bleeding into the peritoneal cavity, and therefore to the presence in the peritoneal fluid of red corpuscles and of a variable number of the finely granular oxyphile cells, in addition to the normal cells of the peritoneal fluid, namely, the coarsely granular oxyphile cell, the coarsely granular basophile cell, the hyaline cell, and lymphocyte.

After their sojourn in the animal the chambers were in all cases found to contain red corpuscles and in some cases also a few flat epithelial cells with large round flattened nuclei were present. Doubtless these had been detached from the peritoneal membrane by the friction of the edges of the chambers. There were present, in addition to these accidentally introduced bodies, intrusive wandering cells. These, unlike the red corpuscles and epithelial scales which were distributed irregularly over the interior of the chambers, were massed at the openings and spread thence fanwise through the interior; both as isolated cells and as masses aggregated round chains of bacilli.

The cells adherent to the external surface of the chambers included the normal cells of the peritoneal cavity together with often a striking number of the finely granular oxyphile cells.

So far we have set down the phenomena common to all the experiments; differences however appear according to the nature of the bacillus employed when we turn to the number and character of the wandering cells present in the interior of the chambers.

As in the case of the tube experiments the different forms of wandering cells do not invade the chambers indifferently in numbers corresponding to their relative abundance in the external fluid, and if we consider simply the order of arrival of the different forms we find, again as in the tube experiments, that in all cases the coarsely-granular oxy-

phile cells are the first to make their way in and the first to attack the bacilli.

If however we consider the rate of arrival of the different forms then a striking difference is found between chambers filled with cultures of the virulent forms Bac. anthracis, and the comma bacillus, as compared with those occupied by culture of the non-pathogenic Bac. ramosus, for in the former case the wandering cells other than the coarsely granular oxyphile cells do not succeed in invading the chamber to any marked extent even in 7 hours, the longest period over which our experiments extended, in the latter case the hyaline cells are found to have invaded the chamber in enormous numbers in as short a period as $2\frac{1}{2}$ hours.

A further difference between virulent and non-virulent cultures appears when we consider the total number of cells present in the chamber. For instance comparing the experiments in which the chambers were allowed to remain in the peritoneal cavity for two hours we find that in the case of Bac. ramosus the number of cells present can only be expressed by the word "countless"; whereas in the case of the virulent forms the total number though very large might have been counted with the aid of extreme patience.

The examination of the contents of the chambers occupied by cultures of virulent and non-virulent bacilli convinced us of the very unsatisfactory nature of the evidence which has been adduced to prove in migratory cells what is known as "negative chemiotaxis."

In our experiments we compare the influence on the cells of cultures of a non-pathogenic bacillus [1] with that of cultures of bacilli which are rapidly fatal to the animals employed, and yet we find that the phenomena observed differ only in degree, for in both cases the cells are attracted to and invade the area occupied by the bacillus, and in both cases the order in which the different cells make their way in is the same. In both cases therefore positive chemiotaxis is seen.

Further, though, as we have pointed out, a very marked difference is found in the rate of accumulation of the cells in the chambers yet it is by no means certain that this can be referred to an equally marked difference in the rate of arrival or rate of attraction of the cells. An examination, especially in the fresh condition of chambers which have been filled with cultures of virulent bacilli, shows that very rapid

[1] The injection of the whole of a large and thick broth culture of Bac. ramosus into the peritoneal cavity of a rabbit does not appear to cause the animal any serious inconvenience.

destruction and disintegration of cells is in process in the interior; all conditions from cells with imperfect granulation to those which have become reduced to a thin bladder-like vesicle surrounding the swollen and distorted remains of the nucleus are to be seen in especial abundance in the neighbourhood of heaps of bacilli. Until we know something of the rate of destruction of the cells, a certain amount of caution must be exercised in referring the paucity of cells present to the action of negative chemiotaxis.

2. *Ziegler's Chambers placed under the Skin.* In order to insert the chambers a small slit was made in the loose skin on the ventral surface and then the skin was separated from the subjacent muscle on either side of the slit with a blunt instrument. In this way two spaces were hollowed out of the loose connective tissue and a chamber was placed in each. A slight amount of bleeding into the spaces always occurred. Dilute broth cultures of Bac. ramosus and the comma bacillus were used to fill the chambers, which were then allowed to remain under the skin for from $7\frac{1}{2}$ to $9\frac{1}{2}$ hours.

It is unnecessary to describe in detail the contents of the chambers when removed since what we have said of similar chambers placed in the peritoneal cavity holds for these experiments. We will therefore briefly state that after 7 to 9 hours' sojourn the chambers were found to contain in the case of the comma bacillus considerable numbers, in the case of Bac. ramosus enormous numbers of the coarsely-granular oxyphile cells. On the external surface of the chambers and in the serous exudation which distended the cavities in which they lay were free coarsely-granular oxyphile cells and hyaline cells together with a number of cells from the blood—but the total number of cells free in the fluid was not great. The most striking change was found in the connective tissue which formed the walls of the space in which the chambers were placed, for this was packed with an immense number of the coarsely-granular oxyphile cells together with a smaller number of the coarsely-granular basophile cells. A teased-out film of areolar connective tissue (Fig. 20) taken from a normal animal is seen to consist, so far as bulk is concerned, mainly of the fibrous matrix. Fixed in spaces in this matrix are the connective tissue corpuscles and a limited number of the coarsely-granular basophile cells, and a few coarsely-granular oxyphile cells are also always present. In a similarly prepared film taken from the wall of the space which has lodged a Ziegler's chamber for 7 to 9 hours, the matrix and its connective corpuscles are seen as before, and no evidence of change in either element is observable in these

preparations. But these now form only a small proportion of the bulk of the tissue, for adherent to the lamellæ or bands of connective tissue are masses of wandering cells in such countless numbers as to render the thinnest film that can be teased out almost opaque when stained. It would be difficult to exaggerate the physiological significance or the extent of the change produced. In 7 to 9 hours after the chambers have been inserted the coarsely-granular oxyphile cells and the coarsely-granular basophile cells have accumulated to such an extraordinary extent that there are thousands where there was previously but a single cell, and the walls of the cavity are now no longer mainly inert, dead matrix, but are formed by a continuous mass of packed living cells which constitute a barrier between the microorganisms and their poisons and the rest of the body possessed of the profound chemical potentialities of protoplasm, which we must therefore look upon as being endowed with powers of destruction and powers of construction—of the destruction of the poisons of the bacillus, and of the construction of bactericidal substances.

The first worker to suggest the connection of the oxyphile granulation with the conflict with microbes was Hankin. He succeeded in isolating *in vitro* a bactericidal substance from the lymphatic glands of cats and dogs[1], and this discovery led him to the view that the resistance of animals to the growth of microbes is due to the production in their bodies of substances possessing bactericidal properties to which he gave the name of defensive proteids[2]. To these bodies Buchner, who was working on the same lines, gave the general name of "alexines[3]." In seeking the origin of alexines in the body Hankin was led to the conclusion that they are formed by the wandering cells. He found support of this view (1) by showing in conjunction with Kanthack that outside the animal body during fever a rise in the bactericidal power of the blood occurs *pari passu* with the increase in the number of leucocytes present[4], and (2) by showing that this increased bactericidal power is apparently correlated with a discharge of oxyphile granules, coarse or fine, into the plasma[5]. In this way Hankin asso-

[1] "A Bacteria-killing Globulin." *Proc. R. S.* Vol. XLVIII. 1890.

[2] "The Conflict between the Organism and the Microbe." *Brit. Med. Journal*, July, 1890.

[3] *Münchener med. Woch.* No. 25, 1891.

[4] Hankin and Kanthack. "On the Fever produced by the Injection of sterilized Vibrio Metschnikovi cultures into Rabbits." *Proc. Camb. Philos. Soc.* Vol. VII. Pt. VI.

[5] *Centralblatt f. Bakt. u. Par.* 1892, Vol. XII. 22 and 23, and also 1893, Vol. XIV. 25.

ciates the bactericidal powers of the body in the first place with the wandering cells in general, and in the second place with the fine and coarse oxyphile granulation in particular. Our observations on the accumulation of coarsely granular oxyphile cells at a centre of infection, and on the discharge of the oxyphile granules during the conflict of particular cells with the microbes may be said to give further support to Hankin's view.

Nothing is more remarkable than the fixed habit of the cells. As we have already noticed the serous exudation contains comparatively speaking only a very few free corpuscles. The application of a coverslip to the connective tissue fails to dislodge the cells, and even the spreading out of a portion of the fresh tissue on a coverslip to form a film frees only a few. Yet an examination of such teased films with high powers convinces one that the cells are not in the lamellæ of connective tissue but are closely attached in layers two, three or several cells deep to their surfaces.

There is yet another inference which may be drawn from the experiments with Ziegler's chambers. In all cases, both when the chambers were placed in the peritoneal cavity and when they were placed in cavities in the subcutaneous connective tissue, the surrounding fluid contained in addition to the cells of the coelomic portion of the sporadic mesoblast, a very appreciable number of finely granular oxyphile cells derived from the blood which had found their way there together with red corpuscles and other elements owing to slight bleeding. In our experiments we always found that the finely granular oxyphile cells either were not found within the chamber or were present in much smaller relative numbers than in the fluid outside.

3. *The Introduction of Copper into the Anterior Chamber of the Eye of a Rabbit.* A small strip of copper foil carefully sterilised was used for this experiment. The operation was carried out on a deeply anæsthetised animal, the cornea being moreover rendered anæsthetic with sterile cocaine, and with all possible antiseptic precautions. The wound healed rapidly and completely. The aqueous humour however became charged with cells and the copper was dissolved. After 2 or 3 days the animal was killed and films were prepared from the aqueous humour. These showed a large number of cells consisting practically of the coarsely granular type (about 95 per cent.).

4. *Blisters.* These were produced on the forearm on ourselves and on others. In the fully formed blister the fluid contained both forms of oxyphile cells, and hyaline cells. In all cases the majority of the

cells present were of the finely granular oxyphile type, that is to say the leucocytosis is chiefly hæmal in character.

The proportion of coarsely granular oxyphile cells varied between 6 to 45 per cent., some individuals producing blisters with a large number of these cells, while in others they were always scanty.

In certain cases the blister fluid was examined from time to time, and a variation in the proportion of the cells at different periods was detected. Only a few cases were followed in this way but they agreed in showing the presence of a larger relative number of coarsely granular oxyphile cells in the early stages.

On the effect on the cells of the introduction of cultures of bacilli into the peritoneal cavity and into blisters.

In these experiments the bacilli used were either Bac. anthracis, Bac. pyocyaneus, or the comma bacillus, and the animals employed were rats or guinea-pigs. The amount injected was usually small.

After a certain interval the animals were killed, the peritoneal cavity carefully opened and the fluid from various regions examined as fluid preparations or as films stained in various ways. The cells were always counted with a $\frac{1}{12}$th oil immersion and Oc. 4. The cells were entered in different columns according to their nature, the extent to which they were charged with granules and whether they were or were not attacking or ingesting bacilli. No possible means suggested itself to us of measuring the total amount of fluid in the peritoneal cavity, and we therefore judged it useless to measure the total number of cells per cubic millimetre. Thus the percentages obtained convey only a limited information.

The first and instantaneous effect of the introduction of cultures of bacilli into the peritoneal cavity is the disintegration of a considerable number of the cells. This effect is of constant occurrence but varies largely in extent. It may probably be referred largely not to the immediate influence of the bacilli, but to the action of the substances injected with them, for the more carefully the bacilli were freed before injection from these products the less was the destruction produced. Therefore the variations in the extent of the destruction in different cases was not found to depend at any rate entirely upon the species of the bacillus injected, but upon the conditions of growth of the bacillus, the nature of the medium used as the vehicle for their injection, and upon the dose.

As these variations are not germane to the main issues of this paper no attempt will be made to analyse more fully their causes; it is sufficient to mention the fact that an immediate destruction of cells follows the injection of bacilli and their products, and that this destruction involves mainly the basophile and oxyphile cells, so that, if it is extensive free basophile granules and fragments of oxyphile cells may be detected in considerable numbers in the plasma. This disintegration of cells must profoundly alter the chemical constitution of the plasma and therefore may play an important part in the struggle with the bacilli.

In an incredibly short time after the introduction of the bacilli they are attacked by the coarsely granular oxyphile cells. The attack consists in the application of the cells to the bacilli, and it entails the using up of the substances stored as the oxyphile granules.

The rapidity with which the coarsely granular oxyphile cells attack the bacilli may be gathered from the fact that in specimens prepared 5 minutes after the introduction of anthrax bacilli into a rat no less than 40 per cent. of these cells were already applied to bacilli (Tables III. and IV.).

At a slightly later period the hyaline cells begin to ingest the bacilli; thus 10 minutes after the injection of anthrax bacilli into a rat 60 per cent. of the coarsely granular oxyphile cells were attacking the bacilli and 27 per cent. of the hyaline cells had commenced the ingestive act, while in 15 minutes 85 per cent. of the hyaline cells were ingesting, and in 30 minutes 96 per cent.

In an earlier communication on the behaviour of the cells of the frog towards micro-organisms we pointed out the difference between the attack of the oxyphile cell, which we described as the application of the cell to the bacillus and the excretion of the granule substance, and the true ingestive or phagocytic activity of the hyaline cells. Similar differences occur between the mode of action of the coarsely granular oxyphile cells and the hyaline cells of mammals.

We have been able to observe the details of the process by watching living cells at work either in hanging drops of the fluid taken from blisters which had been inoculated with a small quantity of a fresh broth culture of Bac. anthracis or Bac. ramosus and were observed on a warm stage, or by examining on a warm stage samples of the fluid of blisters into which a culture of Bac. ramosus had been injected (Figs. 22, 23 human, 21 guinea-pig, 27 rat). In this way the coarsely-granular oxyphile cells were seen to apply themselves to a chain of bacilli and

to extend along it. The granules then travelled, usually in groups, to those portions of the cell in immediate contact with the bacilli and there they were seen to diminish in size to mere points and ultimately to disappear. The rate of loss of granular substance is very rapid as compared with the rate of loss in the gland cells of salivary glands or of the pancreas during the act of secretion, for an appreciable diminution may be observed in a quarter of an hour.

In two experiments an attempt to obtain a numerical expression of the loss of oxyphile granules was made. In the first case a fully formed blister was tapped and films made from the fluid. These films were labelled normal blister fluid. Then a few drops of the sediment from a fresh (20 hours old) broth culture of Bacillus ramosus was injected into the blister. 20 minutes afterwards film preparations were made of the contents of the blister, and also hanging drops which were kept for 2 hours on the warm stage. In the normal blister fluid the coarsely granular oxyphile cells were found to form 5 per cent., after 20 minutes this proportion was not materially changed, but after 2 hours the percentage had dropped to 3·9. In the second experiment anthrax bacilli were used and control drops were kept on the warm stage and at the temperature of the room. A number of hanging drops were made and divided in four sets, two sets were inoculated with anthrax bacilli and two were kept as controls. A set inoculated with anthrax bacilli and a control set were kept on warm stages for one hour, the rest were kept at the temperature of the room for $2\frac{1}{2}$ hours. It was found (1) that the control drops on the warm stage contained at the close of the hour 13 per cent. of coarsely granular oxyphile cells, while those inoculated with anthrax contained only 7·6 to 8·5 per cent., (2) that the controls kept at the temperature of the room (15° C.) contained at the end of the $2\frac{1}{2}$ hours 12 per cent. of coarsely granular oxyphile cells, while those inoculated with anthrax contained 6 per cent. In both experiments coarsely granular cells were seen under the microscope to attack chains of bacilli and to suffer diminution of granulation[1].

The hyaline cells unlike the coarsely-granular oxyphile cells manifest a true phagocytosis, i.e. the intussusception of discrete particles into their substance, and their solution in digestive vacuoles (Fig. 24 human).

[1] Sherrington (*op. cit.* pp. 201—203) discusses the diminution or loss of granulation at length and has not been able to convince himself that any change in size or number of the oxyphile granules occurs in the coarsely granular hæmic cells of cats in which he set up an acute local inflammation. His method however differs so much from our own that it is impossible to compare the two sets of experiments.

The difference between the activities of these two kinds of cells finds in a certain sense numerical expression in the tabular statement of the results obtained by injecting into the peritoneal cavity, cultures of bacilli to which Indian ink had been added. Table IV. shows that the oxyphile cells neither ingest nor do they attach to themselves the particles of the ink but do swiftly attack the bacilli, while on the other hand the hyaline cells rapidly ingest the ink particles and the bacilli. All observed cases of what appeared as mere contact between hyaline cells and bacilli are included as cases of ingestion, and the table therefore takes no account of the fact that the hyaline cells were more heavily laden with ink particles than with bacilli.

The destruction and removal of the bacilli therefore is due to the different activities of the two kinds of cells, the coarsely granular oxyphile cell and the hyaline cell.

The attack of the oxyphile cells on the bacilli must result very frequently in the destruction of the cell. This occurs sometimes to such a marked extent that in film preparation from $\frac{1}{2}$ to 2 hours' experiments a large number of the bacilli are seen to have attached to themselves heaps of still intact granules or amorphous masses of oxyphile substance, while the body of the cell has either broken away or disintegrated.

In a former paper[1] we described the formation of plasmodial masses of wandering cells in the course of the conflict with the micro-organisms. Such bodies are readily seen in hanging drops of blister fluid, in Ziegler's chambers, or in preparations of peritoneal fluid containing bacilli. Fig. 27 shows a striking case, a chain of anthrax being attacked by a coarsely granular oxyphile cell at one place, while at the same time it is being ingested by a phagocyte at another point. Fig. 25 shows an interesting stage, the oxyphile cells being massed in the centre of a number of hyaline cells.

A point which, although it does not bear on the subject of this portion of the paper, namely, the activities of the coelomic wandering cells, must be mentioned in order to complete the picture of the events occurring in the peritoneal cavity, is the extent to which the bacilli and their products present 'induce' immigration of cells from the vascular system. The experiments with anthrax were all performed on the rat and did not endure above 30 minutes. In none of these was any immigration of hæmal cells detected. In the experiments with pyo-

[1] Kanthack and Hardy. *Loc. cit.*

cyaneus on the rat, however, a large number of finely granular oxyphile cells and of red corpuscles were present in 2 and 2½ hours, and a very few even could be detected in 15 minutes. The contrast is interesting when we remember that the rat is extremely resistant to anthrax and susceptible to infection by the bacillus pyocyaneus. The number of experiments however is much too small and they are not sufficiently contrasted to allow us to draw any conclusions from them.

General Results of Part II.

1. Two kinds of leucocytosis can be recognised. In the one the wandering cells are entirely or mainly of the coelomic type, in the other they are entirely or mainly of the hæmal type.

2. In all cases investigated by us the first cells to accumulate at a leucocytic focus were oxyphile cells.

3. Of the two forms of oxyphile cells the coarsely granular form accumulates more quickly than the finely granular form.

4. When the conflict with the bacilli is watched in hanging drops of blister fluid, or in Ziegler's chambers the coarsely granular oxyphile cells are seen to attack the bacilli and to suffer thereby a diminution of granulation.

5. The attack is very rapidly carried out, and is quickly followed by phagocytosis. The latter process, which is carried out by the hyaline cells, commences at a much earlier period than is usually supposed and is at its maximum in about 25 minutes after the introduction of the bacilli.

6. Bacilli or their products in the cases examined by us were always found to attract the wandering cells even when the animal employed was not immune to the particular bacillus employed. At the same time there was destruction of cells at the focus of conflict. This destruction was very much greater in the case of pathogenic than in that of non-pathogenic bacilli. We have been led to think that a great deal of what is known as negative chemiotaxis is based upon those cases where the rate of destruction nearly or quite equals the rate of arrival of cells at a leucocytic focus.

The main interest of the facts set forth in the second part of this paper lies we believe in the marked difference which they show to exist between the activities displayed by the coarsely granular oxyphile cell and the hyaline cell. It may be that these cells change into one

another[1]. But for our own part the facts at present known lead us to regard these cells as morphological units as distinct as are the striped and unstriped cells of muscle tissue.

TABLE III.

Showing the percentage of each kind of cell attacking bacilli.

Rat		Coarsely granular Oxyphile cells	Hyaline cells
Anthrax	5 mins.	39	1
„	10 „	60	27
„	15 „	40	85
„	30 „	44	96
Pyocyaneus	15 „	63	65
„	2 hours	70	85
„	$2\frac{1}{2}$ „	27	62
Guinea-pig			
Cholera	30 mins.	93	100

TABLE IV.

Showing the percentage of each form of cell found to be attacking bacilli or ingesting Indian ink where bacilli and ink were injected together.

Rat		Attacking bacilli		Cells containing Indian ink	
		Coarsely gran. Oxyphile cells	Hyaline cells	Coarsely gr. Oxyphile cells	Hyaline cells
Pyocyaneus	15 mins.	63	65	0	65
„	2 hours	70	85	0	80 to 90

[1] Cf. Sherrington, *op. cit.* p. 203.

TABLE V.

Cultures injected into peritoneal cavity. The table shows the percentage of the different forms of cells found in the peritoneal fluid at varying intervals after the introduction of the bacilli. The percentage in the normal animal is given for comparison.

	Oxyphile			Hyaline	Lymphocytes	Basophile
	Coarsely granular		Finely granular			
Rat.		Granules all gone. Nucleus characteristic				
Normal	25 to 40	—	—	65 to 80		5 to 10
Anthrax. 5 mins. Broth culture centrifugalised and 0·2 c.c. of sediment injected	4·5	0·7	0	89		6
,, 15 mins. Same dose	7·2	1·4	0	83		8
,, 10 mins. Agar culture; bacilli washed off in normal salt solution. 0·2 c.c. injected	19·4	0	0	70	(no record)	10
,, 30 mins. Agar culture washed off in broth. 0·2 c.c. injected	3·1	0	0	79	15	2·4
,, 24 hours. 1 c.c. of broth culture	10	45		45		0
Pyocyaneus. 15 mins. Potato culture; bacilli washed off with distilled water. Indian ink added and 0·2 c.c. injected	23	6	0·9	41	29	—
,, 2 hours. ditto.	6	70		19		3·7
,, 2·5 hours. The same but broth used in place of water	1	79		14		1·9
,, 45 mins. Whole potato culture injected in 1·25 c.c. distilled water	Cells all destroyed except a few basophiles					
Guinea-pig.						
Normal	30 to 50	—	—	50 to 65		Doubtful
*Cholera. 5 mins. One-sixth agar culture in 0·2 c.c. distilled water	70	4		22	6	— Slight bleeding into peritoneal cavity
,, 30 mins. ditto	13	1		58	28	—

* The guinea-pigs used had been previously immunised to cholera by Haffkine's method. This may account for the very large number of coarsely granular oxyphile cells present.

PLATE II.

All the figures are to the same scale and drawn with Oc. 4, Obj. $\frac{1}{12}$th hom. imm. with a camera lucida.

Figures 1, 2, 3, 4 and 5 Human.

Fig. 1. Cells of blood of healthy boy. Fluid preparation made with the methylene blue solution. (*a*) coarsely granular oxyphile cell, (*b*) finely granular oxyphile cell, (*c*) hyaline cell, (*d*) lymphocyte, (*e*) and (*f*) finely granular basophile cell.

Fig. 2. Blood from same boy. Film preparation exposed to a cold saturated solution of eosine in 50 per cent. spirit for about 5 seconds, then to Loeffler's methylene blue. (*a*) coarsely granular oxyphile cell, (*b*) finely granular oxyphile cell, (*c*) hyaline cell.

Fig. 3. Blood from healthy adult. Film preparation stained with the "neutral" mixture described on page 84. (*a*) coarsely granular oxyphile cell, (*b*) finely granular oxyphile cell, (*c*) hyaline cell.

Fig. 4. Coarsely granular basophile cell, connective tissue. Human.

Fig. 5. Lymphocyte to show nuclear network. Flemming's fluid. Hæmatoxyline.

Fig. 6. Rat. Cells from blood. Film preparation stained with glycerine eosine and Loeffler's methylene blue. (*a*) coarsely granular oxyphile cell, (*b*) finely granular oxyphile cell (note the complete absence of staining in the granules, cp. with Fig. 11), (*c*) hyaline cell.

Fig. 7. Rat. Cells of peritoneal fluid. Film preparation stained with glycerine eosine and Loeffler's methylene blue. (*a*) adult and (*a'*) young coarsely granular oxyphile cell, (*b*) adult and (*b'*) young coarsely granular basophile cell, (*c*) adult and (*c'*) young hyaline cell.

Fig. 8. Rat. Cells of peritoneal fluid. Fluid preparation made with the methylene blue solution. (*a*) coarsely granular oxyphile cell, (*b*) hyaline cell, (*c*) young form of coarsely granular basophile cell. The adult form of the coarsely granular basophile cell is omitted and—

Fig. 9. The coarsely granular basophile cell of the mouse is given in its place. From a fluid preparation made with the methylene blue solution.

Fig. 10. Rat. Cells from a film of subcutaneous connective tissue, stained with glycerine eosine and Loeffler's methylene blue. (*a*) coarsely granular oxyphile cell, (*b*) coarsely granular basophile cell.

Fig. 11. Rat. Finely granular oxyphile cell from film of blood stained with the "neutral" mixture as described on page 84.

Fig. 12. Guinea-pig.

(*a*) and (*b*) coarsely granular oxyphile cell, and hyaline cell from a fluid preparation of peritoneal fluid made with the methylene blue solution.

(*c*) finely granular oxyphile cell from fluid preparation of blood made

with the methylene blue solution. To show the differences between the oxyphile cell of the peritoneal fluid and the finely granular oxyphile cell of the blood.

(*d*) coarsely granular oxyphile cell from a film of peritoneal fluid stained with an aqueous solution of sodium sulphindigotate. A very much flattened cell was chosen for drawing in order to show clearly the individual granules and the clear unstained space which represents the nucleus.

Fig. 13. Guinea-pig. Coarsely granular basophile cell in various stages of disintegration. Film preparations stained with glycerine eosine and Loeffler's methylene blue.

(*a*) from wall of peritoneal cavity, fixed with absolute alcohol.

(*b*) from film of peritoneal fluid, fixed by heat.

(*c*) and (*d*) from film of subcutaneous tissue, fixed by drying at temperature of room.

Fig. 14. Rabbit. Coarsely granular oxyphile cell from film of peritoneal fluid stained with glycerine eosine and Loeffler's methylene blue.

Fig. 15. Rabbit. Cells of blood shortly after a heavy meal. Fluid preparation made with the methylene blue solution. (*a*) coarsely granular oxyphile cell, (*b*) finely granular oxyphile cell, (*c*) hyaline cell, (*d*) lymphocyte, (*e*) finely granular basophile cell.

Fig. 16. Rabbit. Blood; fluid preparation made with the methylene blue solution. About 10 hours after a meal. (*a*) finely granular basophile cell, (*b*) lymphocyte.

Fig. 17. Rabbit. Coarsely granular basophile cell from subcutaneous connective tissue stained with the methylene blue solution.

Fig. 18. Rabbit. Finely granular basophile cell, inflamed area stained with the methylene blue solution.

Fig. 19. Film of subcutaneous connective tissue taken from the neighbourhood of a Ziegler's chamber filled with diluted broth culture of the comma bacillus seven hours after introduction of the chamber. Guinea-pig. Eosine in 90 per cent. alcohol, Loeffler's methylene blue, Oc. 4, Ob. D, cam. luc. A part of the preparation where the coarsely granular oxyphiles were relatively few in number was chosen for the drawing in order to show the connective tissue elements.

Fig. 20. Film of subcutaneous connective tissue from normal guinea-pig for comparison with fig. 19.

Fig. 21. Living cells attacking a chain of Bacillus ramosus. Ziegler's chamber $2\frac{1}{2}$ hours in peritoneal cavity of guinea-pig. The chamber was removed unopened to warm stage and examined with Oc. 4, Ob. D.

Fig. 22. Hanging drop of blister fluid from forearm inoculated with Bacillus ramosus. (*a*) coarsely granular oxyphile cell at rest, hanging drop at temp. of room. Warm water was then allowed to run into warm stage, cell became active and applied itself to bacilli—(*b*) and (*c*). Human.

Fig. 23. Blister fluid inoculated with Bacillus ramosus. (*a*) chain of bacilli with three cells applied to it, Oc. 2, Ob. A, (*b*) part of the same chain stained with the methylene blue solution, Oc. 4, Ob. $\frac{1}{12}$th, cam. luc. Human.

Fig. 24. Hanging drop of blister fluid. Phagocyte with ingested fragments of Bac. anthracis, Oc. 4, Ob. D, cam. luc. Human.

Fig. 25. Peritoneal fluid, rat. 15 minutes after injection of Bac. pyocyaneus. Plasmodial mass of coarsely granular oxyphile cells with surrounding hyaline cells. Oc. 4, Ob. $\frac{1}{12}$th, cam. luc.

Fig. 26. Same experiment as last. Single cell attacking bacilli. Oc. 4, Ob. $\frac{1}{12}$th, cam. luc.

Fig. 27. Peritoneal fluid, rat. 10 minutes after injection of bac. anthracis. Fluid preparation stained with the methylene blue solution. Part of much distorted anthrax chain attacked by oxyphile cell and also being ingested by phagocyte.

Fig. 28. Peritoneal fluid, rat. 15 minutes after injection of anthrax. Coarsely granular oxyphile cell which has discharged its granules. Fixed by heat. Oc. 10, Ob. $\frac{1}{12}$, apochr. Powell and Lealand.