THE CELLS FOUND IN INFLAMMATORY EXUDATIONS.

An experimental research as to their function and destiny and also as to the origin of the mononucleated cells.

A THESIS.

presented for the degree of Doctor of Medicine.

by

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I N T R O D U C T I O N.

The objects of the experiments on which this thesis, which I have the honour of presenting for the degree of Doctor of Medicine, is based. was to throw some light on the origin especially of the mononucleated cells of inflammatory exudations, and the special functions of these cells.

The whole of the work has been done in the Pathological Laboratory, and I am very greatly indebted to Professor Greenfield not only for suggesting the line of work, and for very valuable help during the investigations, but also for placing at my disposal much material which has proved of very great value in enabling me to arrive at definite conclusions.

A considerable amount of work especially by means of intraperitoneal injections of Tubercle Bacilli was done in the hope that I might be able to discuss the very much disputed subject as to whether these mononucleated cells are transformed into endothelial cells and fixed connective tissue cells.

During my investigations I saw no evidence of this/

this transformation, but I am convinced that very little importance can be attached to any statements made on this subject which are founded purely on the method of intraperitoneal injection.

The animals either die so early or recover so quickly that there can be very little of this transformation seen.

Other methods of investigation are necessary; and as I have confined myself in this thesis purely to the results of intraperitoneal injection, I do not propose discussing the subject.

The literature is absolutely contradictory, and much of this contradiction appears to me to be due to a too rigid adherence to certain distinctions between cells - distinctions which are seen after fixing and staining, but which do not exist in the living cell.

I have confined myself largely to a study of the cells, as they exist in the fluid withdrawn from the peritoneal sac, and in the tissues from which they arise, after a bacterial infection.

Most of the preparations of the fluid have been illustrated by coloured drawings. This seemed the most satisfactory method, but I have in addition illustrated a number of points where

colour/

colour effects were not so important with Photomicrographs. These latter have the advantage of proving the accuracy of many of the points shewn in the coloured drawings.

For the great care and trouble he has taken with some excellent coloured drawings Figs. IV to XII and with the photomicrographs, my thanks are due to Mr Richard Muir. To him I am also indebted for many hints on preparation of films etc.

I have also to thank the pathologists at the Royal Infirmary, Dr Welsh and Dr Fleming, for the facilities they gave me for obtaining the omentum etc. in various cases of peritonitis.

April, 1901.

Figs. I to. XXXVI. will be found in special volume of illustrations. These were done for me by Mr Richard Muir

Figs. 1 to. 34. in volume with text. These drawings were done by myself.

I desire to express a wish that the microscopic specimens accompanying this thesis may be Retained in the Pathology Department of the University, for the purpose of demonstrating the various changes to the class of Pathology.

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The Cells found in Inflammatory Exudations. -- An experimental research as to their origin, their function and their destiny.

by

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The experimental work on which the main part of this thesis is based, was undertaken with the special object of examining the large phagocytic cells found in so many morbid conditions -- their origin, and the special part they play in these conditions.

During the course of the work, however, one naturally examined closely various other cells found, and these observations will also be recorded.

Methods of investigation:- I have confined myself almost exclusively to the results obtained by setting up a peritonitis. Guinea pigs were almost exclusively used for the experiments. I proposed confirming my results by using rabbits, but one found that the presence of the Bladder worm in the omentum was so common, that it was useless to attempt peritoneal experiments with these animals. Irritation had already been caused by the Bladder worm- and therefore one started with an abnormal peritoneal fluid.

For/

For the purpose of setting up the required reaction, I used the Bacillus Coli in preference to the Staphylococcus Pyogenes Aureus or the Streptococcus Pyogenes used by most observers, mainly because of its motility, but also because it is the organism commonly concerned in cases of Peritonitis in the human subject.

Recent cultures (usually Agar cultures of 24 hours at 37° C.) were used.

A small quantity of the culture was mixed with normal saline solution, or in some cases with sterile peptone broth. Two or three minims of the emulsion (1 small loop of culture to 30 min. of the normal saline solution) was injected into the peritoneal cavity of Guinea Pigs.

By means of sterilised capillary glass-tubes, the peritoneal fluid was withdrawn at varying times (from 5 min. up to 4 to 7 days) after the injection.

The fluid was examined fresh on the warm stage; and also by means of stained films. Both these methods are essential, and the examination on the warm stage, proved of very great importance.

The animals were killed at different periods, for the purpose of examining the serous membranes and the viscera. The serous membranes proved to be of most value, and of these the omentum was generally used.

In/

In a few cases, and generally for special purposes, instead of the B. Coli, cultures of B. Tuberculosis - B. Typhosus and others were used.

Preparation of Films:- The films made in the ordinary way were fixed either by simply drying in the air, or by placing them whilst still wet in a saturated solution of Perchloride of Mercury, for about 5 minutes, washing in water, and then in Methylated Spirit for a few minutes.

I have tried various other fixing reagents for wet films - e.g. Ether and Alcohol; Formalin; Formalin and Alcohol, but the results have never been so satisfactory as those obtained by the Perchloride method.

The dry films are specially of value for demonstrating the Eosinophile granules in the cells.

Staining:- Various stains were used - Eosin (2% watery solution) with Haematein Alum; Eosin with Methyl Blue or Thionin Blue; Saffranin with Light Green; Eosin, Haematein Alum and Light Green - Eosin (saturated alcoholic solution)with Methyl Blue or Thionin Blue.

The same staining solutions were used for the omentum, which was generally most satisfactorily fixed in Methylated Spirit.

Of these stains, for Routine work, the watery solution of Eosin with Haematein Alum or with Methyl Blue/

Blue was mainly used. The results are very satisfactory, and especially so, if the Eosin is fixed in the cells, by a saturated solution of Alum as suggested by Richard Muir.

The Eosinophile granules are brought out by this method, but are especially well seen in dried films which have been exposed to the vapour of Formalin for a few minutes and then stained in an alcoholic solution of Eosin.

For staining the omentum, Haematein-Alum with Light Green (1% alcoholic solution) or Saffranin (a saturated solution in Aniline Oil) with Light Green, gave the best results.

The Light Green is a very good plasma stain, and brings out very clearly the protoplasm of the endothelial cells of the omentum.

In addition to the experimental work, I have had the opportunity of examining, in a number of cases, the omentum, the fluid from the peritoneal sac, and fluid from the pleural sac in acute peritonitis and acute pleurisy in the human subject.

These fluids, etc., have been most valuable, and have enabled me to compare my experimental results in the Guinea Pig, with what occurs in the human subject.

Of the cases of peritonitis (in the human subject) examined, one was after perforation of a typhoid/

hoid ulcer - the others were all due to the B. Coli.

One case of Pleurisy also appeared to be due to the B. Coli.

The Normal Peritoneal Fluid of Guinea Pigs.

Kanthack and Hardy[22] state that the following cells are normally present.

(a) Coarsely granular oxyphile (30 to 50%)
(b) Coarsely granular basophile. These are non-amoeboid, large cells with a rounded nucleus which is centrally placed. The nucleus stains with difficulty. These cells disappear from the fluid very quickly, and are not seen unless the film be fixed at once by heat or by Mercuric Perchloride.
(c) Lymphocytes.
(d) Hyaline cells: these are large amoeboid cells, with a spherical or kidney shaped nucleus. The nuclear network has fine meshes. There is a nucleolus. There are no granules in the protoplasm. The cells number from 50 to 65%.

Durham[2] agrees with these authors as to (a), (c) and (d) above, but he did not find the coarsely granular basophile cells in his films.

Wallgren[48] states that in dogs, there are no lymphocytes and no mononucleated cells with granules. He specially mentions as present "mononucleated leucocytes" with a nucleus, round or oval or indented The nucleus is eccentric. The protoplasm is abundant and stains uniformly with Ehrlich's Triacid stain.

My own observations on a number of Guinea pigs, agree with the observations of Durham.

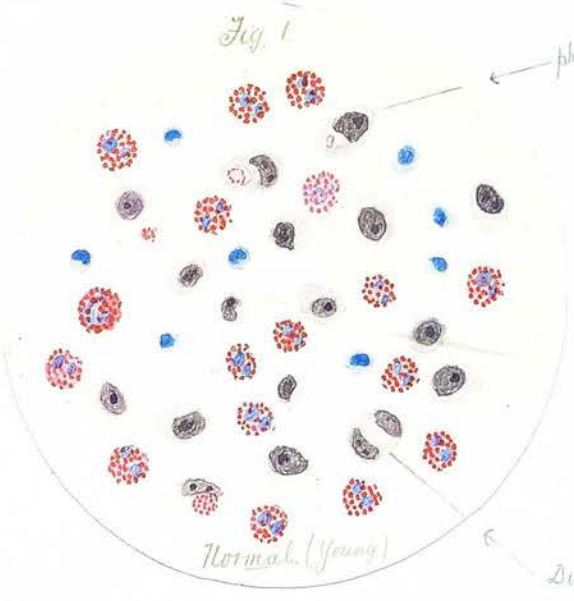
The/

The coarsely granular oxyphile or eosinophile cells are always present, and are usually very numerous. (Fig. III.)

The coarsely granular basophile described by Kanthack and Hardy, I have failed to find in any case, though my films were fixed at once in Mercuric Perchloride.

The Lymphocytes are generally present, but only in very small numbers.

The so-called "Hyaline cells" are the most abundant in the fluid. These cells are of very great importance. They vary much in size. (Fig. II)



The smaller ones resemble lymphocytes. The nucleus may be rounded. More commonly, however, it/

it is somewhat oval and indented. Usually it is centrally placed, but may be eccentric. The staining reactions vary very much. Sometimes the nucleus stains very darkly, and appears almost uniform in structure. At other times, and with different stains, it may be more lightly stained.

In the larger forms, the nucleus is more lightly stained than in the smaller ones. The protoplasm, in the smaller forms, stains well with Eosin, but in the larger ones, the eosin reaction is not so marked though still quite definite.

Careful examination of the larger forms shews an irregular network in the protoplasm. The meshes of this network are of various forms and sizes. This "multiple vacuolation" is of very considerable importance when one is dealing with the cells, after the action of some irritant on them.

Examined on the warm stage, these cells are seen to protrude and retract processes, but I have been able to detect very little movement from place to place. That this amoeboid movement takes place is quite certain, but one cannot expect to see much of it after the fluid has been withdrawn from the peritoneum, seeing that the cells are examined under such abnormal conditions.

These cells are phagocytic, even in the normal peritoneal/

peritoneal fluid and probably act as the principal scavengers of the peritoneal sac. (Fig 1)

Kanthack and Hardy[22] maintain that these cells are derived from lymphocytes, and that they are phagocytic. The phagocytic property is undoubted.

Further one finds in the vessels in the normal peritoneum mononucleated cells of various sizes. A few of these are the same size as the lymphocytes. The nucleus stains very darkly and there is a very small ring of protoplasm. These are no doubt lymphocytes. Besides these, one finds larger cells with an oval or kidney-shaped nucleus, which is less rich in chromatin and usually eccentrically placed. The protoplasm is relatively abundant. These cells vary somewhat in size. They are identical in structure with the cells found free in the peritoneal fluid. I believe them to be the large mononucleated cells of Ehrlich which have migrated from the vessels.

The Results of Intraperitoneal Injection.

Experiments were made with Bacillus Coli, Bacillus Typhosus, Cholera Spirillum, B. of Dysentery (Flexner), Staphylococcus Pyogenes Aureus, B. Tuberculosis and also with foreign particles e.g. Vermilion and Carmine.

The important results were for all practical purposes the same, whatever organism or foreign substance/

substance was introduced. There were certainly differences as to time and intensity of reaction, but except in the case of B, Tuberculosis, these were not of great importance.

The greater part of the work was done with B. Coli, and the description of the effects will, in the main, be confined to the reaction produced by this organism.

In all cases, even with very minute doses, a definite reaction was obtained. In some cases this was slight, while in others, especially with large doses, it was very severe, in some cases the animal dying in from 12 to 18 hours. In one case the animal died 96 hours after the injection, and at the post mortem an acute pericarditis was discovered.

The fluid withdrawn from the peritoneal cavity from 5 minutes to 2 hours after the injection was clear, watery and to appearance exactly like the normal peritoneal fluid. Microscopic examination shewed a slight increase of the polymorpho-nuclear leucocytes from 1 to 2 hours after injection.

At 3 hours the polymorpho-nuclear leucocytes were very definitely increased.

From 4 to 8 hours the fluid is still abundant, watery or slightly turbid. It is still easily obtained by means of the capillary tube. The polymorpho-nuclear/

morpho-nuclear leucocytes are now greatly increased, and there is also an apparent increase of some of the forms of mononucleated cells.

8 to 12 hours. Fluid still easily obtained and fairly abundant. It is, however, much more turbid. Both varieties of cells are greatly increased.

12 to 16 or 18 hours. Fluid in smaller quantity, and viscid. The cells are still greatly increased.

18 to 24 hours. Fluid small in quantity, viscid and turbid. The quantity of fluid diminishes, and the viscosity increases. from 24 to 48 hours. At 48 hours, the fluid is distinctly purulent, and in fatal cases remained so till the death of the animal.

In cases where the animal recovered, the fluid became less purulent, and more abundant from 54 to 72 hours after the injection.

At 84 hours, it was watery and fairly clear; and on the fourth or the fifth day, had resumed practically its appearance before the injection.

Microscopic examination shewed three principal types of cells in the exudation.

1. The Polymorphonuclear.
2. The coarsely granular eosinophile.
3. The mononucleated cells of various varieties.

Before dealing in detail with these cells, it will be well to note the differences after an intra-

peritoneal injection of a pure culture of tubercle bacilli in sterile normal saline solution. The fluid in the peritoneal sac is always small in quantity and generally even in 3 or 4 days only slightly turbid.

Broden(3) states that 6 hours after an intraperitoneal injection of a culture of tubercle bacilli the polymorphonuclear cells appear and remain in great numbers during the whole course of the malady. His experiments were performed on dogs.

Borrel(2) notes the disappearance of the polymorphonuclear leucocytes by the fifth day.

"Kostenitsch and Wolkow"(23) found the polymorpho-nuclear leucocytes abundant in the vessels, and a few in the tissues from 3 to 6 hours after an injection into the substance of the kidney.

Dembinski(6) agrees with these authors as to the early appearance of the polymorpho-nuclear leucocytes. In his experiments in pigeons with B. Tuberculosis of man, he found these leucocytes half an hour after the inoculation.

My observations have not shewn this early appearance of the polymorpho-nuclear leucocytes.

When pure cultures in normal saline solution were injected into the peritoneal cavity, the increase in these cells was hardly seen till 24 hours after the injection. They then appeared in considerable numbers/

numbers and remained fairly numerous till the fifth or the seventh day. It is true that one may see a few of these cells in the fluid at an earlier period, but the number is extremely small.

Summary of the Results of the examination of the peritoneal fluid from 44 guinea pigs. inoculated with various organisms but especially with B. Coli, at various periods after the intra-peritoneal inoculation.

In this summary, I have as far as possible taken my results from cases where examinations were made at various intervals over an extended period in the same animal. For various reasons, such examinations were not possible in all cases. The results, however, have been very uniform throughout. In experiments where examinations were made, say only at 12 and 24 hours, the films were compared with the films of other experiments made at earlier periods and again at 12 and 24 hours. Thus a fairly uniform standard was established. On this standard a careful comparison of the films from all the experiments was made, and on these results the summary is based.

10 min. after inoculation of B. Coli in sterile broth. (Fig. 2.)

1. Polymorphonuclear leucocytes. Only a very few of these cells were present.

2. Coarsely-Granular eosinophile cells. These are present and are fairly numerous. The nucleus is darkly stained with methylene blue.

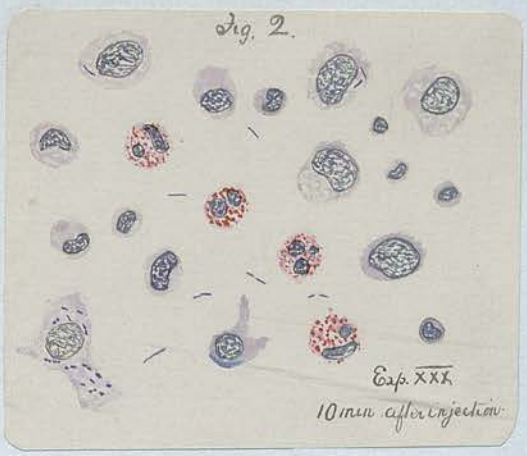
3. Mononucleated cells. (a) Small cells resembling lymphocytes. The nucleus of these is round or slightly indented at one side, so as to give a Kidney shaped appearance to it. It is very rich in chromatin and stains with Eosin and Methylene blue of a lilac tint. The protoplasm is scanty. In some cells it appears granular. It stains more lightly/

ly than the nucleus.

(b) Larger cells. (3, 4 or 5 times the size of the former). In these the nucleus is large. It is oval or Kidney-shaped. It is fairly rich in chromatin, and has more darkly stained chromatin nodes in its network. It does not stain so deeply as the nucleus of the former cells. The protoplasm is more abundant, and in most cells shews a very fine multiple vacuolation. In other cells, the vacuoles are larger and very definite. A few of these contain bacilli.

(c) Between these two varieties there are various intermediate forms.

There are numerous free bacilli. None, however, were found in the polymorphonuclear leucocytes.



At this stage, therefore, one gets a few polymorphonuclear leucocytes.

The coarsely granular eosinophile cells are those normally present.

The mononuclear cells correspond with the so called/

ed "Hyaline cells" of the normal fluid. Some of these shew amoeboid movement and also that they can act as phagocytes to bacteria. Further, some of these cells are irregular in outline, have a faintly staining nucleus, and in every way resemble the endothelial cells of the peritoneum. These, however, shew a very marked phagocytic action to bacteria. The nucleus in some of the cells is eccentric, but in others it is more or less central.

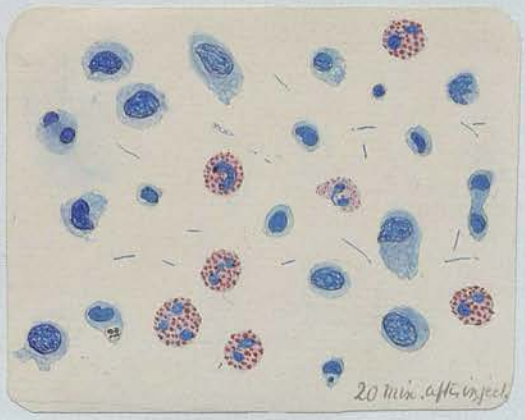
20 min. after inoculation. (B. Coli.) (Fig. 3)

1. Polymorphonuclear leucocytes. Still only a few present. There is no apparent increase in number.

2. Coarsely Granular eosinophile cells. No change in these.

3. Mononucleated cells. These are not much altered from what was seen in films at 10 min. There are, however, a greater number of the small "a" forms.
No bacilli were found in any of the cells, though there were numerous free bacilli in the fluid.
Some of the mononucleated cells - those of medium size - shew a phagocytosis to other cells. Some of these cell inclusions are certainly coarsely granular eosinophile cells, of which the granules alone remain in a "digestive" vacuole.
Division of these mononucleated cells is seen going on. In one cell, in drawing, this division is by mitosis. In others the nucleus is found divided - one part at each pole of the cell. The protoplasm in some cases also shews signs of dividing. Whether this is a direct or a later stage of mitotic division it is difficult to say.

Fig. 3.



Shews. various sizes of cells - division & phagocytosis.
Only a few of the Eosinophile cells are Represented in drawing

40 min. after inoculation (B. Coli.)

There appears to be a considerable diminution in the total number of cells present.

1. Polymorphonuclear Leucocytes. very few of these present.

2. Coarsely granular eosinophile cells. The greater number of the cells present are of this variety. Many of them have a double nucleus, one at each pole of the cell. Others have a single, non-lobed nucleus, and in other cells, the nucleus is indented at both sides, giving it an hour-glass shape.
The nucleus of these cells stains darkly, though at later periods it stains faintly.

3. Mononucleated cells. Very few of these present. Most are of medium or large size, though a few are like lymphocytes.

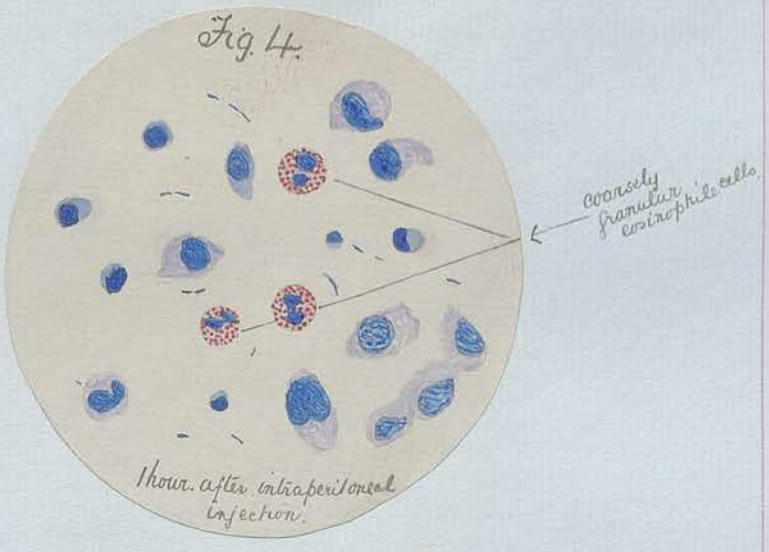
Free bacilli are very numerous. None were found in the cells.

1 hour after inoculation (B. Coli). (Fig. 4)

Still very few cells present. 1 and 2 as in fluid/

fluid at 40 minutes.

3. Mononucleated cells. These are of various sizes.
The nucleus in the larger cells is more faintly stained than that in the smaller ones, and irregular nodes of chromatin are seen in the more faintly stained nucleus. The protoplasm of the larger ones, is more faintly stained than the protoplasm of the smaller ones, and it shews a very distinct multiple vacuolation.



1½ hours after inoculation (B. Coli).

There are still very few cells present. The appearances are practically identical with those described for 1 hour.

The smaller mononucleated forms have, if anything, a more pronounced rim of protoplasm.

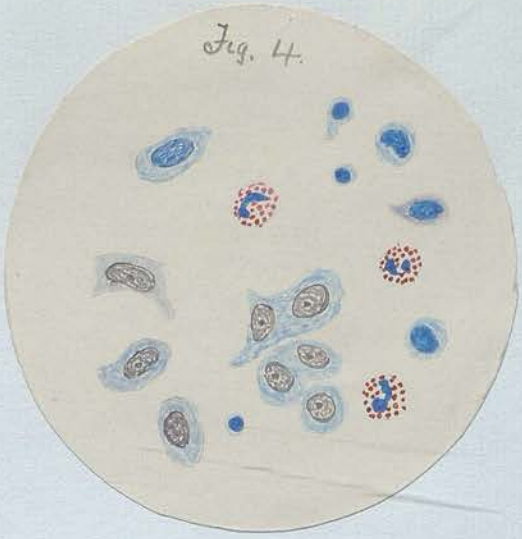
2 hours after inoculation. (B. Coli). (Fig. 5.)

2. The coarsely granular eosinophile cells, are, compared with the other cells, less numerous. In many of them, the nucleus stains faintly with/

with the Methylene Blue. Some are broken up and their granules are scattered.

3. Mononucleated cells. (a) These are few in number, and the smaller ones are indistinguishable from lymphocytes.
(b) These larger cells are more numerous than at earlier periods. They are very irregularly stained. In some the nucleus is blue, in others it has a lilac tint. In many, the protoplasm is very markedly vacuolated. The larger forms of these cells resemble ordinary endothelial cells.

The free bacilli are still fairly numerous. A few bacilli are contained in the large uninucleated cells.



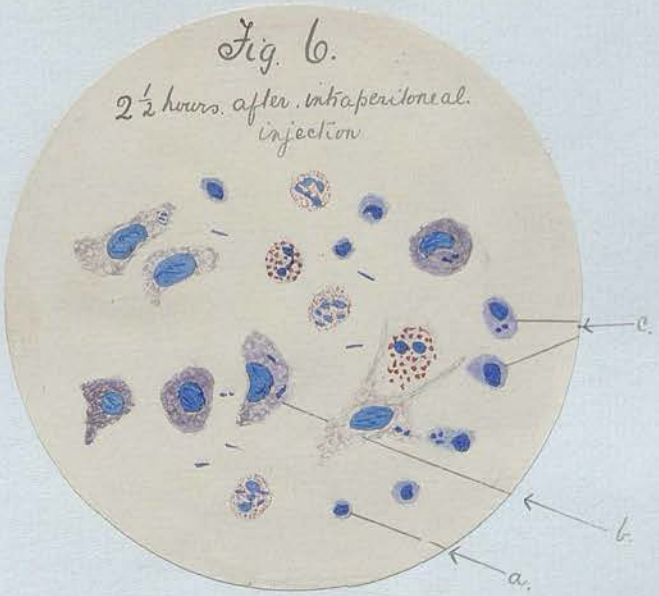
2½ hours after inoculation. (B. Coli) (Fig. 6.)

1. Polymorphonuclear leucocytes. These are now increased considerably. Some of them show bacilli in their interior.

2. Coarsely granular eosinophile cells. These are still present, but apparently much reduced/

duced in number. Bacilli are seen in some of these.

3. Mononucleated cells. (a) Very few of these. The nucleus is rich in chromatin, and stains a dark blue with Methylene Blue. The protoplasm has a distinctly bluish tint, and is small in quantity.
(b) These are fairly numerous and some of them contain bacilli.
(c) The intermediate forms between these small lymphocyte-like cells and the large endothelial-like cells are well seen.



3 hours after inoculation(B. Coli).

As at 2½ hours.

4 hours after inoculation. (B. Coli).

1. Polymorphonuclear leucocytes. These are very greatly increased in number. At parts of the film the whole field was composed of these cells.- 20 to 30 to the field. Many of these cells contain bacilli.

2. Coarsely Granular eosinophile cells. These are still present but now diminished in number.

3. Mononucleated cells. There are very few of these present, and nearly all are the small or/

or medium sized ones.
In the smaller ones, the protoplasm is apparently homogeneous, in the larger ones it appears to contain dark granules, which, however, are not clearly defined.

6 hours after inoculation. (B. Coli)

1. Polymorphonuclear leucocytes. These are very numerous. In some, the pseudopodia are very pronounced.

2. Coarsely granular eosinophile cells, are still present.

3. Mononucleated cells. These cells are fairly numerous and shew all stages from the small forms with very small ring of protoplasm and darkly stained nucleus, up to the large endothelial like cells with abundant protoplasm, an eccentric and often Kidney-shaped nucleus.
The medium sized cells appear to have darkly stained granules in their protoplasm, but an examination of the larger forms shews this apparent granularity to be due to the filaments of a very fine network in the protoplasm.
Some of the smaller forms shew definite pseudopodia. Division by mitosis is seen in some of the cells.
Small rounded masses of protoplasm generally without, but sometimes with a very dark stained nucleus, are seen. These are usually about half the size of a lymphocyte. They are, I believe derived as buds, or possibly as pseudopodia separated from the polymorphonuclear leucocytes.(Fig. IV)
I have never seen any enlargement of these, or any evidence that they increase to form other cells.

A few bacilli are found lying free.

9 hours after inoculation (B. Coli). (Fig. 7)

1. Polymorphonuclear leucocytes. These are very numerous. In a few of them bacilli are found.

2. Coarsely granular eosinophile cells are still present. In the specimens stained with Eosin, Haematein and Light Green the eosin stained granules in these cells have not been completely decolorised by the Light Green, whereas the granules in the polymorphonuclear leucocytes have been decolorised.

3. Mononucleated cells. As at 6 hours, but the larger and medium forms are the more abundant. There are extremely few of the small forms present.
No bacilli are found in these cells at this stage, unless in cases where the bacilli were extremely numerous. A very few of these larger cells shew remains of an included cell in a vacuole in their protoplasm.

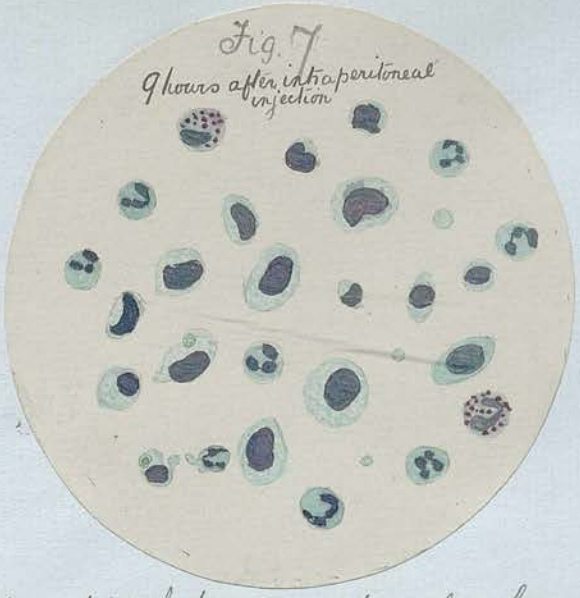


Fig. 7
9 hours after intraperitoneal injection

Only a few of the polymorphonuclear leucocytes are Represented in this and subsequent drawings.

12 hours after inoculation (B. Coli),

The results are practically the same as at 9hours. The large momonucleated cells with eccentric Kidney-

shaped/

shaped nucleus, shew a greater degree of phagocytosis to the polymorphonuclear leucocytes than at 9 hours, but this phagocytosis is still only slight.

18 hours after inoculation (B. Coli). (Fig. 8.)

1. Polymorphonuclear leucocytes. These are still very numerous and in this preparation. (From a fatal case 96 hours after inoculation) they are crowded with bacilli. The bacilli included in the cells stain quite well.

2. Coarsely granular eosinophile cells. Only a few of these are present.

3. Mononucleated cells. The forms a. b. and c. already described are present, but the larger forms (b and c) are the most abundant. In most of these cells the nucleus is placed to one side of the cell and is oval or kidney-shaped. In some, however, the nucleus is more or less rounded and is placed near the centre. These latter are undoubtedly the same cells as the former ones. Their nuclear stain, their protoplasm and their vacuolation being identical. Both act as phagocytes.
This phagocytosis is not very marked at this period, but a few of the cells shew polymorphonuclear leucocytes or remains of them in their "digestive vacuole".

There are also present a few larger cells with protoplasm, vacuolated and faintly stained. The nucleus is rounded or oval and is faintly stained with Methylene Blue. These look like endothelial cells.

There are a considerable number of free bacilli.

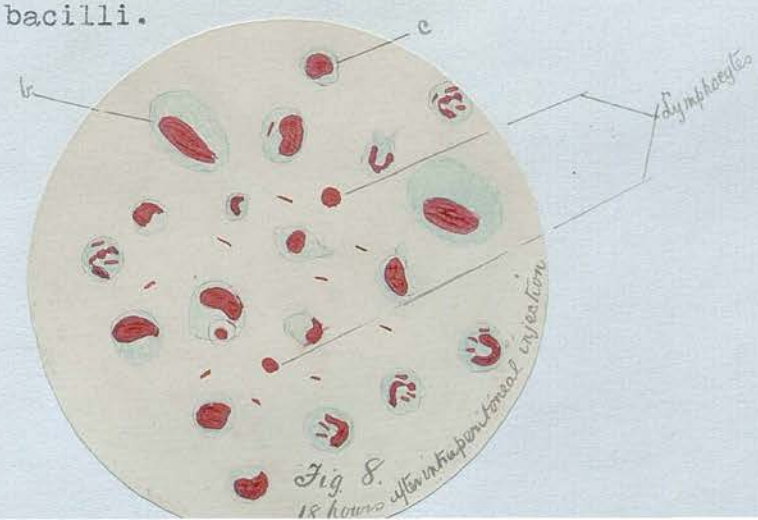


Fig. 8.
18 hours after intraperitoneal injection

24 hours after inoculation. (B. Coli) (Figs. V. VI & 9).

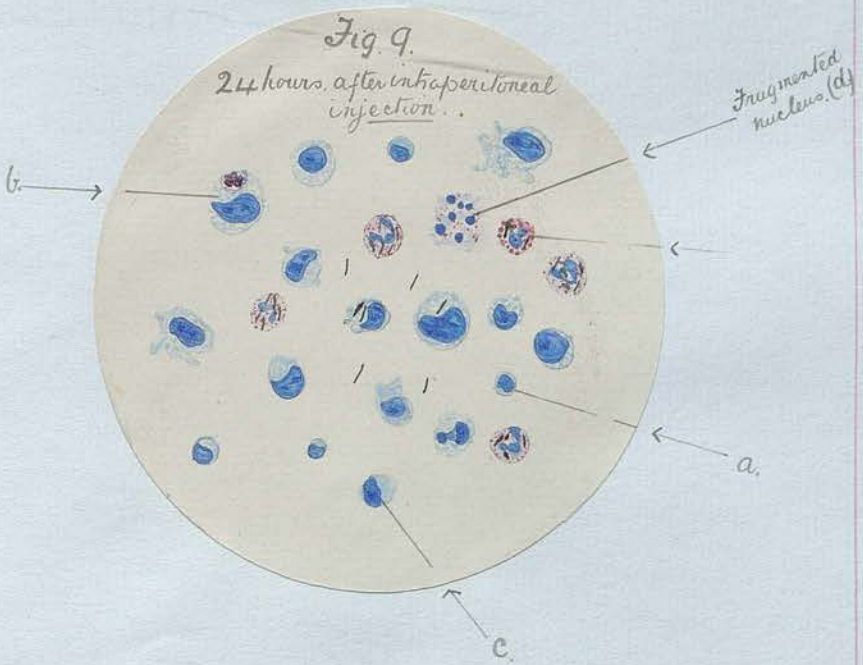
1. Polymorphonuclear leucocytes. These as at 18 hours. They contain a considerable number of bacilli. Some of these cells shew the nucleus divided up into a number of small, rounded parts. (d) Each of these is uniformly and very darkly stained. The protoplasm of these cells stains faintly. The outline is often irregular, and the cell granules scattered.

2. Coarsely granular eosinophile cells. These are few in number. Some of them contain bacilli.

3. Mononucleated cells. The forms a. b. and c. are present. Numerous pseudopodia are seen in many of the cells. The vacuolation of the protoplasm is very marked. A few of the cells contain bacilli in their interior and a few shew a phagocytosis to the polymorphonuclear leycocytes. The nucleus of these included leucocytes in some cases stains darkly with Methylene Blue; but in other cells, it stains a reddish colour with the eosin and does not stain with the Methylene Blue.

Division of the cells by mitosis is seen.

There are very few bacilli lying free.



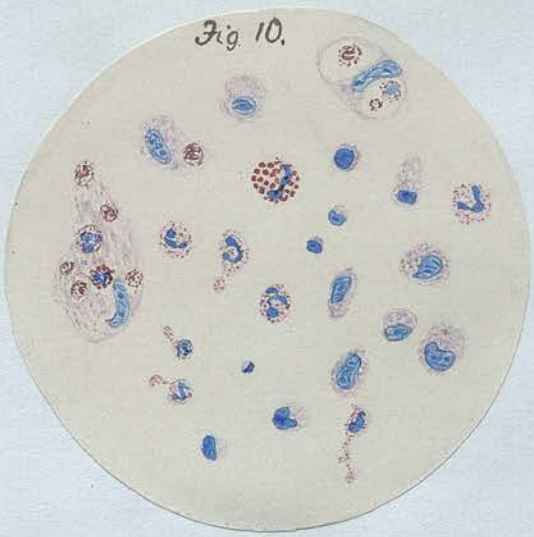
27 hours after inoculation. (B. Coli) (Fig. 10.)

This observation was from an experiment where very few bacilli were injected, and extremely few were found after 12 hours.

The reaction of the cells, however, was practically identical with what was seen in other experiments at the same period.

The cells are like those described for 24 hours, but the mononucleated are now very markedly increased. All forms are seen. Phagocytosis is also more marked. Many of the polymorphonuclear leucocytes that have been ingested have degenerated, or have been digested and are represented in many cases by a few granules only. Many of the polymorphonuclear leucocytes shew budding of their protoplasm (Fig. IV.) These buds may become separated, and appear in the films as small rounded masses of protoplasm sometimes containing bacilli.

Fig. 10.



27 hours after inoculation with B. Coli.

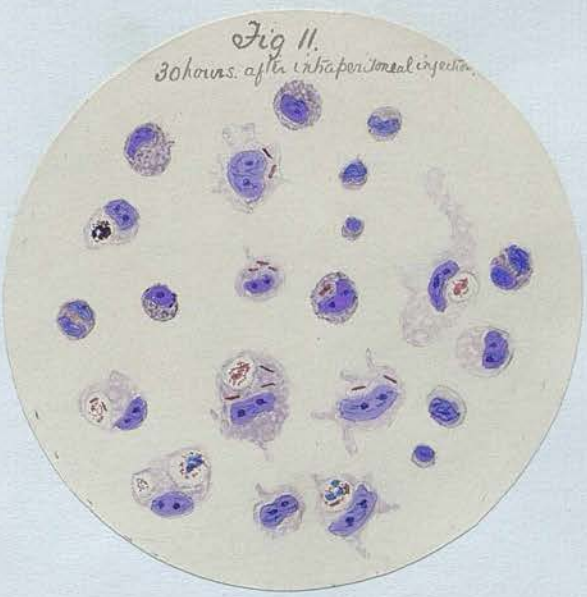
30 hours after inoculation (B. Coli). (Fig. 11.)

The polymorphonuclear leucocytes and the coarsely granular eosinophile cells are as at 24 hours.

The Mononucleated cells. These are very greatly increased and the larger forms especially so. The cells are apparently very active. Their pseudopodia are extremely marked, their vacuolation is greatly in excess of that seen in the earlier stages, and phagocytosis to the polymorphonuclear leucocytes is abundant. Many of the included cells have lost their typical staining properties. There are evidences of proliferation and division of the cells.

Some of the medium sized cells shew a very dark lilac staining of the protoplasm, and a darkly stained nucleus. These cells shew pseudopodia and vacuolation of the their protoplasm as in the larger forms. Examination of a number of these darker cells and forms between these and the larger cells, has convinced me that both cells are of the same nature, and that the more darkly stained ones are simply less actively functioning cells.

Bacilli are found abundantly in the polymorphonuclear leucocytes. There are a few also in the mononucleated cells. None were found free in the fluid.



36 hours after inoculation (B. Coli). (Fig. 12.)

1. Polymorphonuclear leucocytes. These are still very numerous in the films from fatal cases, but not nearly so numerous in the non-fatal ones. A great many of them still contain bacilli. The bacilli often take on a reddish stain with Eosin and Methylene Blue. This is probably on account of degenerative changes.

2. Coarsely granular eosinophile cells. A very few of these are present.

3. Mononucleated cells. The small, medium and large cells are present.
The small lymphocyte-like cells have a dark bluish stained nucleus. The protoplasm is reddish. (Eosin and Methylene Blue).

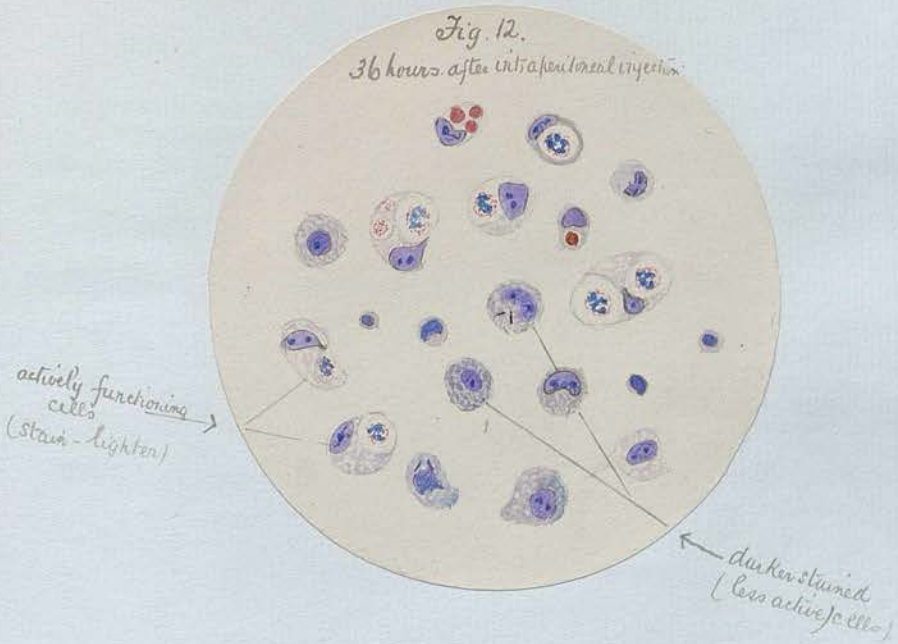
A number of the medium sized cells have a darkly -stained nucleus sometimes placed at the periphery. In other cells it is more or less central. The protoplasm of these is also darkly stained.
Both these darkly stained cells, and the more lightly stained ones are phagocytic, but the phagocytosis is much more abundant in the latter.
These differences in the staining reactions are specially well seen in films stained with Methylene Blue, slightly alkaline.
The lymphocyte-like cells shew a very dark blue nucleus with some even darker points in it. The protoplasm stains a lighter blue.

The medium sized cells, 2 or 3 times the size of the former, also shew a very dark nucleus with darker points in it. The protoplasm also stains very darkly, sometimes as dark or even darker than the nucleus.

Careful examination, however, shews that this is not constant, and that apparently identical cells aften stain more lightly. Further, there are quite definite grades in the intensity of staining in the same kind of cell, apparently quite independent of degenerative changes.
The more actively phagocytic cells are more lightly stained, and it seems that the intensity of staining power varies with the stage/

stage of activity of the cell.

Some of these mononucleated cells have ingested red blood corpuscles.



42 hours after inoculation (B. Coli).

The films at this period shew practically the same as those described for 36 hours. The mononuc-leated cells are now very abundant.

Fewer of the polymorphonuclear leucocytes contain bacilli. Many of the contained bacilli are degenerated, and take on a reddish colour with Eosin and Methylene Blue.

48 hours after inoculation. (B. Coli). (Figs VII, VIII, XVI, XVII, XVIII, XXIII, XXIV, XXV, XXVI and 13.

In non-fatal cases the mononucleated cells are in equal or even greater numbers than the polymorphonuclear/

nuclear leucocytes.

In fatal cases, however, the polymorphonuclear cells still predominate.

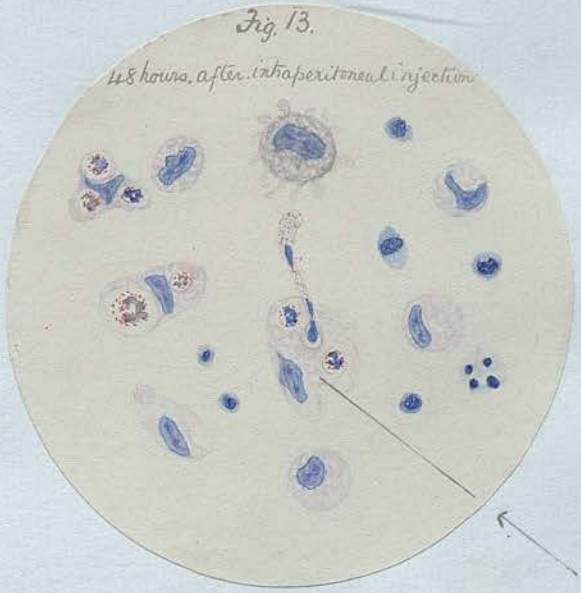
The varieties of cells are those described for 36 hours, but the larger forms predominate, and in appearance many of them are indistinguishable from endothelial plates. They are very actively phagocytic, much more so than at earlier periods. Figs VII & VIII

Some of the polymorphonuclear leucocytes shew a definite fragmentation of their nucleus. (Fig. VII)

A great many of the ingested cells are in various stages of digestion. In many, the nucleus does not take on the blue stain, in others mere debris of the cell is left. (Fig. VII)

Direct division of some of the mononucleated cells is seen, and also mitosis.

Bacilli are still found in some of the cells in fatal cases only.



54 hours after inoculation (B. Coli). (Fig. 14).

In non-fatal cases the mononucleated cells are now in greater numbers than the polymorphonuclear leucocytes.

No bacilli are found, except a few degenerated faintly stained ones in some of the mononucleated cells.

The coarsely granular eosinophile cells. These are again rather more numerous in the non-fatal cases.

The mononucleated cells. The small, the medium and the large are present. The intermediate forms are especially well-shewn.

There are a number of the small cells, and again the nucleus stains more darkly than the nucleus in the medium and the large forms.

Phagocytosis is not so marked as at the earlier periods. Many of the large and medium sized mononucleated cells contain fragments of ingested leucocytes.

Division of the medium sized mononucleated cells by mitosis is going on actively. This division is well seen in the films stained with Picro-Fuchsin and Haematein. (c. d.)

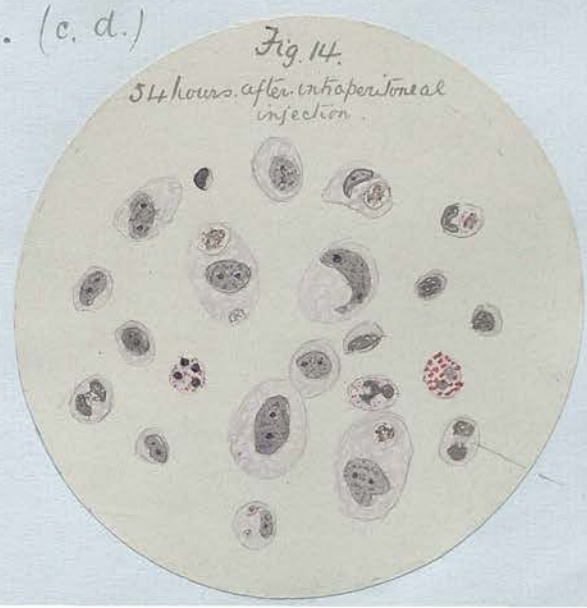


Fig. 14.
54 hours after intraperitoneal injection.

60 hours after inoculation (B. Coli). (Fig. 15.)

As at 54 hours in non-fatal cases, the polymorphonuclear leucocytes are greatly diminished, and the mononucleated cells correspondingly increased.

No bacilli are found either free or in the cells.

The coarsely granular eosinophile cells:- These are present, but still only a few are seen in the films.

The mononucleated cells:- The various forms described are present. The small forms are numerous. In the films stained with Eosin and Methylene Blue the nucleus of some of these small cells is rounded. It stains very dark blue with some darker nodal points in it. These cells resemble in every respect, the ordinary lymphocytes.

Others of these small cells have a Kidney-shaped nucleus which also stains dark blue, and has darker stained nodal points in it, but it does not stain so intensely as the nucleus of the former ones. These latter appear to be identical in every respect except in size with the larger mononucleated cells.

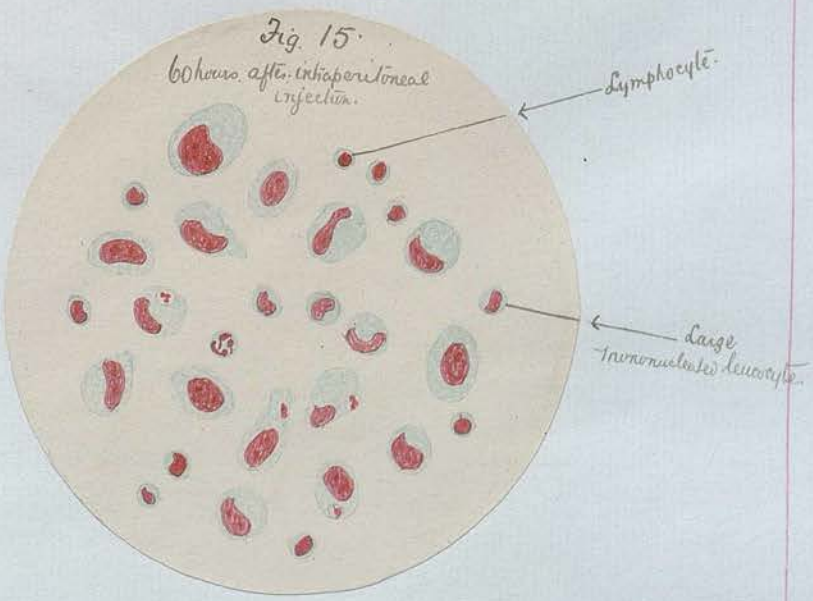
Some of the medium and larger forms shew phagocytosis. The ingested polymorphonuclear leucocytes are much altered, and in most cases mere fragments are left.

This phagocytosis is not nearly so marked as in the earlier stages.

There are also present a few cells of irregular outline. The protoplasm stains very faintly and it is finely vacuolated. The nucleus is poor in chromatin, and usually, two more darkly stained points, apparently nucleoli, stand out. These cells resemble in every respect flattened endothelial cells. They may, however, be degenerated forms of the large mononucleated cells, which they also resemble in all respects except in the intensity of stain. This

point/

point, however, will be discussed later.



66 hours after inoculation (B. Coli).

Taking at this period a fatal case, we find similar cells to those described at 60 hours in a non-fatal case, but with certain important differences.

The polymorphonuclear leucocytes. These are very numerous. In many the nucleus is divided into several rounded fragments, which stain very darkly with Methylene Blue. (Fig. XXII)

The coarsely granular eosinophile cells:- These are very scanty.

The mononucleated cells:- These are as at 60 hours, but the larger forms are more abundant. Phagocytosis to polymorphonuclear leucocytes and to red blood corpuscles is very considerable.

There/

There are also present some small rounded masses of protoplasm with a very darkly stained uniform nucleus. These are half the size of ordinary lymphocytes.

They are probably parts of the other cells separated. This separation has already been referred to and is shewn in Fig. IV.

Free bacilli are present in considerable numbers. The polymorphonuclear leucocytes are crowded with them.

72 hours after inoculation. (B. Coli). (Fig. 16.)

(a) Fatal cases. The appearances are those described for 66 hours.
The polymorphonuclear leucocytes shew more of the forms in which the nucleus is broken up into rounded masses, staining intensely. Some of these cells have broken up and the darkly stained nuclei are seen free.

(b) Non-fatal cases. This shews a very marked contrast to (a).
The polymorphonuclear leucocytes are extremely scanty.
Of the mononucleated cells, the small forms are the most abundant.
Phagocytosis is still seen, and the englobed leucocytes are very much altered, so that mere fragments of their nuclei remain in small vacuoles.

78 hours after inoculation. (B. Coli).

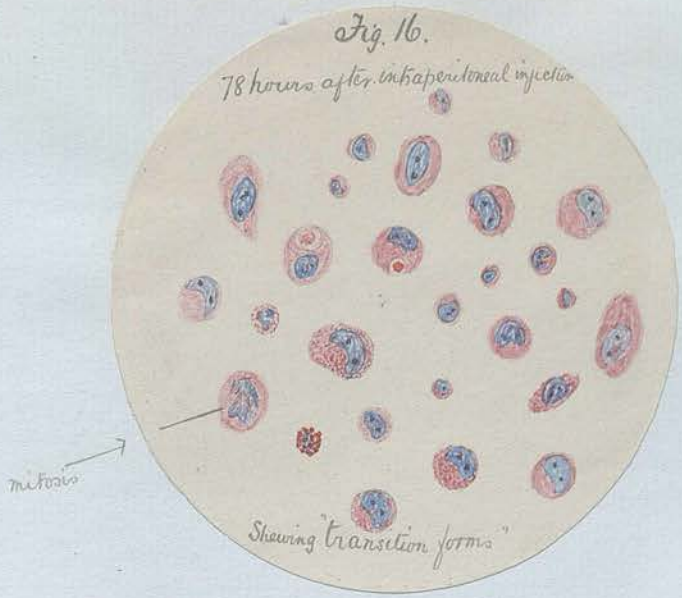
The appearances are the same as at 72 hours. The smaller and the medium forms of the mononucleated cells are abundant, and various transitions between these two are specially well seen at this stage.

The remains of some of the englobed polymorphonuclear leucocytes are still seen in some of the cells.

At this, almost the end of the phagocytic action of/

of the mononucleated cells, it is worthy of note that I have never seen a small mononucleated cell in the interior of a larger one.

Division by mitosis is seen at this period also.



84 hours after inoculation. (B. Coli).

Very few cells now present. Fig. XXI.

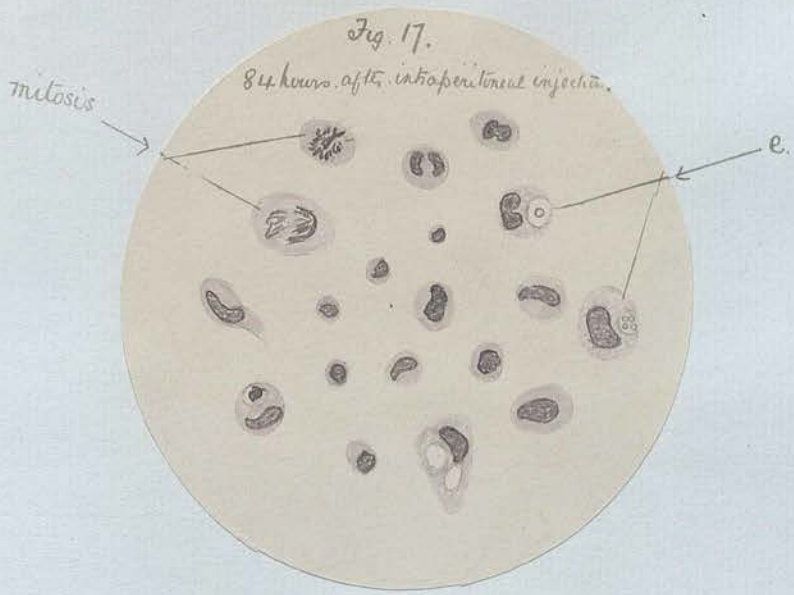
Again in non-fatal cases, the appearances are those seen at 78 hours.

Hardly any polymorphonuclear leucocytes are found. Those ingested by the mononucleated cells are so much altered that what appears to be the nuclear membrane alone remains. (Fig. 17. e)

The small and medium forms of the mononucleated cell cells are abundant, and there is very marked evidence of division by mitosis going on. (Fig. XIXVII).

The/

The multiple vacuolation of the protoplasm of the larger cells is still seen, but it is not so marked as in the earlier periods.



96 hours after inoculation.

(a) Non-fatal cases.

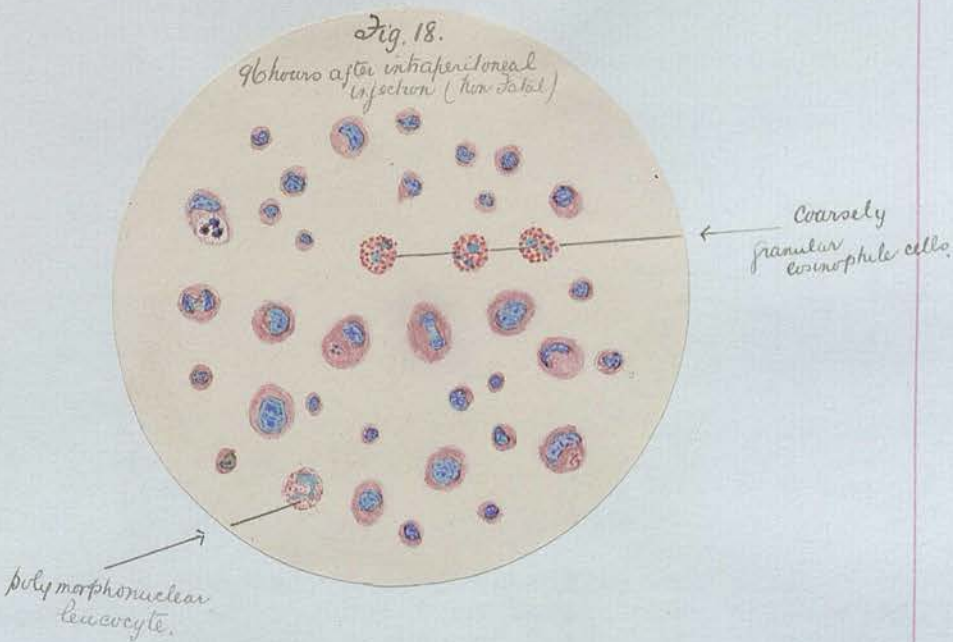
Polymorphonuclear leucocytes: These are present but very scanty. There are not more than one to every 20 or 30 mononucleated cells.

Coarsely granular eosinophile cells. These are more abundant than at former periods and are more numerous than the polymorphonuclear leucocytes.

Mononucleated cells. These are very numerous compared with the other cells. There is a very great excess of the smaller forms. The two forms of these described in films at 60 hours are well seen here also.

Division by mitosis is going on actively. The transitions between the smaller and the medium sized mononucleated cells are specially well seen in films stained by Benda's method.

No bacilli were present.



(b) Fatal case. Only one of the fatal cases lived to this period.

Post-mortem examination. The intestines were markedly injected and glued together by a recent purulent lymph. The effusion into the abdominal cavity was very scanty, viscid and purulent. There was no ulceration of intestine and certainly no evidence of a perforation.

The liver was covered on its anterior and its under surface with a thick layer of yellowish non-adherent lymph.

This was present to a slight degree over the spleen. The pericardium was also covered with a similar layer of purulent lymph. The other organs were not specially affected.

Examination of fluid from peritoneal sac.

Polymorphonuclear leucocytes. These are very abundant, compared with the other cells. Fig. 19 represents a typical field shewing the proportion of the cells, and is in marked/

marked contrast to Fig. 18. where several fields are combined and only one polymorphonuclear leucocyte was found.
Some of these cells are degenerated.

Coarsely granular eosinophile cells. These are very scanty.

Mononucleated cells. The smaller forms of these are present, but there are very few of them. The larger forms predominate.
Phagocytosis by these cells is seen, but it is certainly not nearly so abundant as in the earlier stages of either non-fatal or fatal cases.

These facts suggest that the mononucleated cells, though present, have lost their activity, as evidenced by active division and by active ingestion of other cells.

Bacilli are very numerous. They are present in some of the cells, but free bacilli are especially numerous. The great number of free bacilli may be accounted for by an active multiplication after the death of the animal, as it was not seen for several hours after death.

Another possibility is that this increase of bacilli may be due to the loss of function of the mononucleated cells. This very interesting point, I have been unable to solve, as I have not succeeded in keeping another animal alive long enough after the injection. I have tried on various occasions but my animals have always died at a much earlier period or have recovered.

120 hours after inoculation. (B. Coli.).

There were only a few cells present in the films All were mononucleated forms, mostly of the small and medium-sized. Some of these, but a very few, shewed remains of ingested polymorphonuclear leucocytes.

144 hours after inoculation (B. Coli).

This is practically the same as film to be described for 150 hours.

150 hours after inoculation (B. Coli).

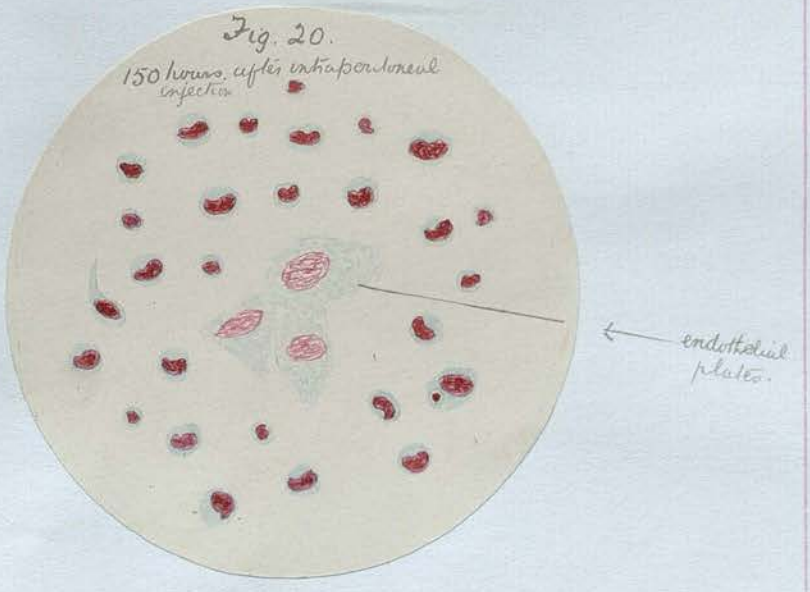
Mononucleated cells. These are very abundant and are mostly the small forms and the transitions between these and the medium forms. (Fig. 20).

A few of the medium sized cells are present and one or two of them shew remains of ingested cells.

Some large cells probably shed endothelial plates, corresponding with those described at 60 hours are seen.

The polymorphonuclear leucocytes. These are again fairly abundant. This is most probably due to some irritation during punctures for withdrawal of fluid. At 144 hours there was a slight increase as compared with 96 hours.

No bacilli were found free or in the cells.



The Polymorpho-nuclear Leucocytes.

These cells in the Guinea Pigs differ somewhat from those found in the human subject. According to Kurloff(25) the granulation is coarser and approaches the coarsely granular eosinophile cells. One principal distinction between the two forms of cells, is that this granulation is very easily dissolved by acid, but remains unchanged in alkaline solutions. The true eosinophile granulation remains, on the other hand, quite unchanged under these conditions.

Kurloff uses the name poly-nuclear pseudoeosinophil cells. Functionally, however, they correspond to the neutrophile of man.

The structural difference between these granules and the granules in those found in the human blood is quite evident. Further the distinction in structure of the granules, and in the staining reaction between these and the coarse granulation of the true eosinophile cells is also clear.

If the cells are stained with a watery solution of eosin and then with Carbol-Thionin - the granules in the finely granular forms stain a very dark blue, while the granules in the coarsely granular cells retain their red colour.

In/

In spite of this difference in the granulations between those in the human blood and those in the blood of the Guinea pig, and especially seeing that the cells have exactly the same function, I think it much better to retain the name polymorpho-nuclear, and in this paper I will speak of these cells, whether found in the Guinea pig or in the human subject as polymorpho-nuclear leucocytes.

These leucocytes are not usually present in the normal peritoneal fluid of Guinea pigs, but they appear very soon after an intraperitoneal injection of B. Coli, or other organism is given.

With B. Coli, they appear even in 10 to 20 minutes after the injection, but no special increase occurs from 2 to 2½ hours. At 3 hours they may be fairly numerous, and at 6 hours the increase becomes marked.

The increase is now continued for an indefinite time. In fatal cases the increase is maintained till the death of the animal in spite of the fact that a very great number is being continually distroyed.

In one of my experiments the animal lived for 96 hours after the injection, and the increase of polymorpho-nuclear leucocytes was maintained till the end.

In cases that are not fatal, the numbers begin /

gin to diminish from 36 to 48 hours after the injection.

From 84 to 96 hours they are very scanty in the fluid. They may, however, persist for several days.

In one experiment (non-fatal) they were fairly numerous 150 hours after the injection, while in another they were entirely absent from the peritoneal fluid 120 hours after the injection.

SUMMARY OF THE RESULTS.

Non-fatal cases - after intraperitoneal injection of B. Coli.

10 to 20 min. Very few polymorphonuclear leucocytes found.

1 Hour. Very slight increase in number.

2 to 2½ hours. Increase now very definite.

3 Hours. A considerable number present.

4½ to 6hours. The increase now very marked.

6 to 30 hours. The increase goes on during these hours, but is most marked from 6 to 12 hours.

30 to 36 or 48 hours. The numbers now begin to diminish.

54 Hours. A very pronounced diminution in numbers.

60 to 72 Hours. Diminution becomes more marked.

78 Hours. Very few polymorphonuclear leucocytes present.

84 to 96 Hours. Still a few present.

They/

They may now persist for a few days, but from the fifth to seventh day, they entirely disappear.

Fatal cases. The cells are found about the same time, but the increase is maintained till the death of the animal.

What is the Role of the Polymorphonuclear Leucocytes?

1. These leucocytes as we have seen are called out early, and they diminish and disappear after a few days in cases where the animal recovers. Usually in 5 to 7 days the exudation is quite free from them.

Their early appearance, and the fact that in a short time (36 to 48 hours after the injection) they begin to diminish in number in animals that recover, has led various authors to the conclusion that they play an extremely important part - in fact that they are essential to the recovery of the animal.

Hankin(11) stated that the presence of large numbers of these cells was associated with increased Bactericidal Power, and he regarded them as the source of his "Alexin" substances.

Hahn(10) showed that if a polymorphonuclear leucocytosis was induced in the pleural cavity, the fluid had a considerably increased Bactericidal Power. He eliminated the Phagocytic action of the leucocytes by killing the cells.

Issaeff/

Issaeff(16) injected a number of substances (urine, serum, saline solution, etc.) which induced a polymorphonuclear leucocytosis. 24 to 48 hours after the injection, he inoculated the animals with various vibrios, and he found that the animals were more resistant to the organism than were normal animals. The resisting power, however, lasted only a few days, and for all practical purposes, it was lost at the same time as the leucocytosis ceased.

Durham(7) confirmed the observations of Issaeff not only with the Cholera Spirillum, but with Bacillus Typhosus and other Bacteria. He states that the protecting power determined by this leucocytosis lasts 4 or 5 days.

Metchnikoff(29) has also noted this increased general resistance after injections with sterile broth. He attributes the recovery of the animal to Phagocytosis. He does not attach much importance to the extracellular destruction of the organisms.

Durham(7) has further shewn that if the Peritoneal fluid is examined at varying times after injection, the motility of the Cholera Spirillum is gradually lost, and that this diminution in activity corresponds with the increase of the polymorphonuclear leucocytes. Further a series of hanging drop experiments shewed, that fluid withdrawn from the peritoneal sac at various times after an injection of sterile broth, produced/

duced an inhibitory action on motile Bacteria (in emulsions of the cultures of various organisms) in proportion as the polymorphonuclear leucocytes increased.

Before seeing Durham's paper, I was specially struck by the sluggish action of the Bacillus Coli shortly after injected in the peritoneal cavity. In consequence of this observation, the peritoneal fluid was examined at varying times in a number of animals after injections of Bacillus Coli, B. Typosus and the Cholera Spirillum.

Invariably it was found that the motility of the organisms was greatly diminished from 8 to 12 hours after the injection, and in cases where the animal recovered, or would probably have recovered, had it not been killed for some special observation, all movement of the organisms practically ceased from 12 to 18 or 24 hours after the injection.

Further experiments were made "in vitro". The fluid withdrawn from the peritoneal cavity at various periods from 12 to 36 hours after injection of B. Coli, was on the warm stage mixed with cultures of the B. Coli in broth. The movement of the Bacilli ceased in a very few minutes. In some cases the fluid withdrawn at 12 hours inhibited the movement - in other cases the movement was not absolutely inhibited by the fluid withdrawn so early, but was inhibited by the fluid withdrawn at a later period.

A/

A drop of the broth culture of B. Coli was placed under the same conditions as to temperature etc. - but without being mixed with the fluid withdrawn from the peritoneal cavity, and in these preparations the Bacilli remained quite active.

Again fluid was withdrawn from the peritoneal cavity of non-infected and healthy Guinea Pigs, and mixed with small quantities of broth cultures of Bacillus Coli. These preparations were placed under exactly similar conditions to the former ones. There was undoubtedly a slight inhibition of movement, but the movement was not stopped even 48 hours after the experiment was started.

It is worthy of note that the period after injection at which one got a fluid having this inhibitory action on the movements of the Bacilli, corresponds almost exactly with the period of increased polymorphonuclear leucocytosis, but it this is not the only new condition. Side by side with this increase in polymorphonuclear leucocytes there is an increase of other cells - and of these the large uninucleated phagocytes to be referred to later, are of special importance.

Further in the two animals of my series that died from the results of the injection, at a later period than 48 hours after the injection, the polymorphonuclear leucocytes were very greatly increased and/

and the large uninucleated phagocytes were present in comparatively small numbers.

Again the fact that one gets an inhibitory action on motile Bacteria when brought in contact with normal peritoneal fluid is against the view that this action results only from the presence of the polymorphonuclear leucocytes.

Metchnikoff[30] in 1899 found that Spermatozoa injected into the peritoneal cavity of Guinea Pigs were after a time rendered immobile. Further the Spermatozoa are rendered immobile in a few minutes in the blood serum of Guinea Pigs, that have been injected several times. It thus appears that after injections of Bacteria etc., there is produced a substance which is antagonistic to the activity and probably injurious to the life of the organism. There does not appear to be sufficient evidence to support the view that this substance is produced by the polymorphonuclear leucocytes.

2. The polymorphonuclear leucocytes act as phagocytes.

Wallgren[48] experimenting with intraperitoneal injections of Streptococcus Pyogenes, gives the following times as those at which this phagocytosis occurs.

(a) With large quantities of Streptococci, the polymorphonuclear cells contained the organism 1 hour after the injection.

(b) Less rapid infection. The micrococci were found in these cells 2 hours after injection.

(c) With less virulent cultures of Streptococci, the organisms were found in the cells 20 min. after the injection.

Borrel[2] using intravenous injections of cultures of tubercle bacillus, found the bacilli in the polymorphonuclear leucocytes a few minutes after the injection.

Metchnikoff[29] agrees with Borrel.

Broden[3] experimenting with intraperitoneal injections of tubercle bacilli found them in the polymorphonuclear cells, though few, 6 hours after the injection.

Kanthack and Hardy[22] state that if you inject bacilli with Indian Ink, the polymorphonuclear leucocytes do not ingest the particles of Indian Ink, but attack the bacilli.

My observations shew that this phagocytosis on the part of these cells is very considerable. The time/

time at which it takes place varies considerably. It is dependent to a great extent on the material injected, on the method of inoculation and on the number and virulence of the organisms injected.

When foreign particles (e.g. Carmine) are injected into the peritoneal cavity of Guinea Pigs, the polymorphonuclear leucocytes are found to have ingested the particles of carmine, 2 to 3 hours after the injection (Fig. 21) Now this is the time at which a leucocytosis, resulting from any irritation in the peritoneal sac, becomes marked. Therefore we must conclude that these cells ingest dead particles almost immediately after they escape from the vessels.

Fig. 21.

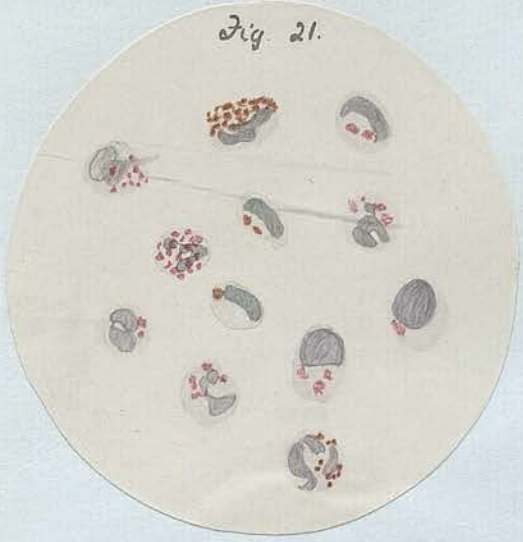


Fig. 21. 3 hours after injection of Carmine into peritoneal sac. Shewing particles of carmine in polymorphonuclear leucocytes.

When/

When cultures of bacilli in small quantities are injected, and especially if the bacilli are actively motile, the ingestion of them by the polymorphonuclear leucocytes is delayed to a certain extent.

Bacilli are found in the cells 4 to 5 hours after the intraperitoneal injection, but only in small numbers, and it is possible that these bacilli which have been ingested so early, were dead, or at any rate that their activity had become much diminished.

It is certainly not till about 9 hours after the injection of a moderate number of the bacilli, that they are found in any considerable numbers in the polymorphonuclear leucocytes, and the numbers ingested now increase till at from 12 to 18 hours, the cells are crowded with the bacilli.

When large quantities of the culture of the bacilli are injected or where the culture is non-virulent, the bacilli are found to be more numerous in the cells, at an earlier period than when small quantities, and especially if the bacilli are very virulent, are injected. This is probably due to the fact that the leucocytosis takes place earlier where large quantities are injected. In these cases, the leucocytosis becomes quite marked 2 hours after the injection.

If the culture injected is a virulent one, though one/

one may find bacilli in the cells early, yet the phagocytosis to bacilli is never marked. The cells rapidly degenerate, so that in a few hours, the bacilli are in enormous quantities, and free in the fluid. The cells are comparatively few in number and many of them degenerated.

Where a non-virulent organism is injected, even if injected in large quantities the leucocytosis may be slight or it may be very marked and occur early. In about 3 hours, very few bacilli are found either free or in the cells, and in 4 to 6 hours, the bacilli have entirely disappeared from the fluid withdrawn from the peritoneal cavity. They are found neither free nor in the cells.

The amount of leucocytosis with the injections of non-virulent organisms is probably to a large extent due not to the organism but to the fluid in which it is suspended.

When injected in sterile peptone broth, the migration of leucocytes is very marked, but when a physiological saline solution is used, the increase of leucocytes is not nearly so pronounced.

Usually, however, the injection of any substance into the peritoneal cavity causes a migration of the polymorphonuclear leucocytes.

Exceptions, however, occur, as shewn by the following three experiments.

1. A/

1. A guinea pig was injected intraperitoneally with a very old and non-virulent culture of Anthrax bacilli. The bacilli were not dead, for a subculture was made from the tube. The bacilli were suspended in normal saline solution. There appeared to be no reaction.

   An attempt was made to withdraw fluid at 3 hours and again at 12 hours after the injection, but none could be got with the capillary tube.

   At 24 hours, the animal was killed. There was no fluid in peritoneal cavity, and absolutely no evidence of any reaction having taken place.

2. A guinea pig was given an intraperitoneal injection of a culture of anthrax bacilli, killed by being kept at a temperature of 90 C for 1 hour. This culture was suspended in normal saline solution.

   At 3 hours after injection, a small quantity of fluid was withdrawn from the peritoneal sac. The fluid simply contained some debris. There was no polymorphonuclear cells present.

   At 12 hours, no fluid could be obtained from the peritoneal cavity.

   At 24 hours the animal was killed. There was no fluid in the peritoneal sac, and again there was absolutely no evidence of any reaction having taken place.

3. An intraperitoneal injection of the Cholera Spirillum in normal saline solution was given to a young guinea pig. The organism was from a subculture of several generations. The spirilla were however actively motile.

   No fluid could be obtained from the peritoneal cavity, though several attempts were made at different intervals.

   The animal seemed perfectly well.

   48 hours after the injection, the animal was killed, and though a very careful examination was made, there was no evidence of any reaction having taken place.

The failure to get a reaction in these cases at once suggested that my injection had been put into the intestine instead of into the peritoneal sac. There certainly was no evidence of this at the post-

mortem/

mortem, Further, these were the only cases out of over 50 experiments, where a reaction was not obtained, and these were the only experiments done with organisms which were known to be non-virulent, and in which the injection was made with physiological saline solution. Therefore one is quite justified in concluding that the absence of reaction was due to the condition of the organism, and not to a fault in the method of injection.

Do the polymorphonuclear leucocytes englobe living and active organisms? Or are the organisms dead, or in a condition in which their activity is greatly impaired?

This is a very difficult question. We are met at the outset with the fact that practically our only method of knowing whether or not a bacterium is dead, is that it will not grow when placed on a suitable medium and at a suitable temperature. This test cannot be applied, in at any rate the early stages, for though many of the bacteria may be dead, there are many still active and still capable of growth.

We have already seen that motile bacteria injected into the peritoneal cavity have their movement inhibited, and that this inhibition increases as the polymorphonuclear leucocytes increase. Further, we have noted that the time at which a marked/

ed inhibition takes place, corresponds almost exactly with the time at which a marked ingestion of the bacilli by the leucocytes is taking place, i.e. from 12 to 18 hours after the injection. The fact that the bacilli are much more active than the leucocytes suggests itself as an explanation of this, but with non-motile organisms the results are practically the same.

Again the fact that particles of carmine are taken up as soon as the leucocytes appear, and that with bacteria, the ingestion to any marked extent is somewhat later, also gives support to the view that some impairment of activity is necessary before ingestion can take place.

Also in favour of this view is the fact of the earlier phagocytosis to non-virulent bacteria.

Muir (37) points out that after inoculation of the bacilli of Pseudo tuberculosis into the pectoral muscle of birds, very little phagocytosis to bacilli takes place. If, however, the bacilli are rendered less virulent by passage through white mice phagocytosis becomes well marked.

Kanthack (21) has pointed out that in Frogs, the anthrax bacilli must be weakened before phagocytosis takes place.

Metchnikoff (29) holds that the tissues are resistant to bacilli because of the action of the phagocytes, and/

and that the phagocytes can take up living and active bacteria.

At first it seemed easy to prove this point for motile bacteria, but though numerous observations were made on the warm stage at various periods, when polymorphonuclear leucocytes and bacilli were both active, and when the bacilli were sluggish, and the leucocytes active, I have never seen the englobing of a bacillus by a polymorphonuclear leucocyte. The observations were often continuous for several hours. It is not uncommon to see a bacillus moving round an active leucocyte, and very often the bacillus disappears, but whether it is ingested by the leucocyte is impossible to determine. Sometimes one sees a bacillus anchored to a leucocyte and still active, but even these I have never seen ingested.

Various attempts were made to stain the organisms during life, and thus better to be able to detect them in the leucocytes, but all attempts were unsuccessful.

Further though one has examined on the warm stage fluid in which one knew, from stained films, that the bacteria were in great numbers in the leucocytes, yet no movement of these bacteria was ever seen.

Ingestion of bacilli by, and actual movement of the bacilli in other cells I have observed.

Experiments/

Experiments were made by inducing a polymorphonuclear leucocytosis in the peritoneal cavity by injection of particles of carmine. The fluid was withdrawn 6 hours after injection. It was found to be rich in polymorphonuclear leucocytes, and a great many of these shewed an active phagocytosis to the carmine particles. Now an injection of Staphylococcus Pyogenes Aureus and a recent culture of B. Coli in peptone broth was made into the peritoneal sac. The fluid was withdrawn 15 mins., 45 mins. and 1 hour after the second injection. On examination of the films, it was found that even at 15 min. some of the cells contained the organism and the motile B. Coli was quite as frequently present in the cells as the Staphylococcus Aureus.

Fig. 22.

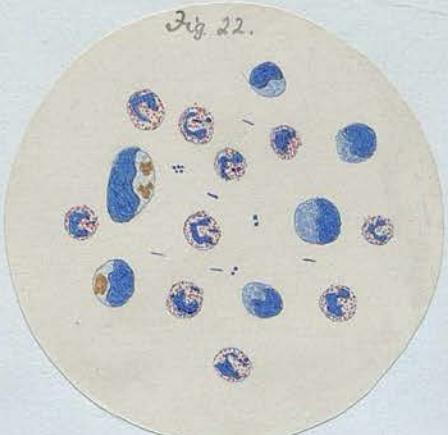


Fig. 23.

Fig. 22. - 6 hours after injection of Carmine. ½ 15 min. " " of B. Coli & Staph. Pyog. Aureus

Fig. 23. - 6½ hrs. after injection of Carmine & 45 min. after injection of B. Coli & Staph. Pyog. Aureus.

~~Examination~~/

Note in Fig. 22. the very dark staining of the less active mononucleated cells.

Examination of the fluid on the warm stage, however, shewed that though many of the B. Coli were actively motile, others were quite motionless 15 min. after they had been introduced into the peritoneal cavity.

Pfeiffer (41) has laid special stress on the extracellular destruction of the bacteria. He experimented with various organisms including the Typhoid bacilli, and maintained that the disappearance from the exudate was due mainly to extracellular degeneration, and that the degenerated organisms were taken up by the phagocytes.

This extracellular degeneration does occur, and in some cases one finds very large numbers of degenerated organisms in the polymorphonuclear leucocytes. On the other hand , it seems from my observations, to be far commoner to get bacilli in the cells quite indistinguishable from the healthy organism.

The evidence on the whole points to some alteration taking place in the organism before it is ingested by the polymorphonuclear leucocytes.

Fate of the Polymorphonuclear Leucocytes.

Borrel[2] in his experiments with tubercle bacilli says the polymorphonuclear cells with bacilli degenerate. Three days after injection the nucleus becomes fragmented.

Kostenitch and Volkow[23] agree with Borrel, but give 4 days as the time at which the fragmentation of the nucleus occurs.

Broden[3] states that the leucocytes containing bacilli disappear from the peritoneal fluid in 3 or 4 days.

Muir[38] experimenting with injections of Staphylococcus Pyogenes Aureus, states that the day after the injection, degeneration of the polymorphonuclear leucocytes is seen in the exudation from the peritoneal cavity. Then, he says, follows an ingestion of these cells by large Phagocytic cells.

My observations confirm this early degeneration of the leucocytes. With small (non-fatal) doses of bacteria, the degeneration is never very marked in the free cells. The ingestion of these cells by the large phagocytes is very marked, and the ingested cells shew very marked evidence of degeneration.

With large and rapidly fatal doses, the degeneration of free cells is much more marked.

The/

The manner in which this degeneration takes place in the free cells is identical with what is seen in the ingested leucocytes. This ingestion and degeneration I propose dealing with fully under "Phagocytosis by the mononucleated cells."

Degeneration certainly does not always take place before the cells are ingested, or at any rate if there is a degeneration always, it is a degeneration not apparent to our methods of demonstration.

One constantly sees polymorphonuclear cells enclosed in large phagocytic cells, and to all appearances these polymorphonuclear cells are perfectly normal, so far as staining can demonstrate a normal structure.

As has been already stated, the polymorphonuclear cells disappear from the peritoneal exudation from the 5th to the 7th day after a non-fatal inoculation.

A great many of them are broken down and destroyed in the peritoneal exudation and on the omentum. A considerable number are ingested and digested by other cells.

Possibly some get back into the blood stream. Possibly some are carried to certain organs and destroyed there, e.g. in the spleen and glands. This subject, however, will be more fully discussed in relation to the other cells.

Have these polymorphonuclear leucocytes any action/

action on the bacteria which they ingest?

Evidence has already been given tending to support the view that injurious agents affected the bacteria before the ingestion took place. Very soon after the ingestion, one finds that the bacteria in the cells have altered in their staining properties. Stained with Eosin and Methyl Blue, numbers of the bacilli in the cells stain blue just as do the normal bacilli, but a considerable number have their outline somewhat obscured and stain a reddish colour, and it is often difficult to distinguish them from granules in the cells.

The bacilli are frequently contained in a clear space in the cell. This so called "digestive vacuole" is, however, by no means always seen.

With regard to tubercle bacilli, I have seldom found bacilli in the polymorphonuclear leucocytes, though in the same cases, they were fairly abundant in the other cells. This may possibly be due to an early degeneration, and a consequent loss of their characteristic staining properties.

Broden says the polymorphonuclear leucocytes containing bacilli completely disappear at the 3rd or 4th day; though the leucocytes without bacilli remain during the whole course of the malady.

I found definite tubercle bacilli in the polymorphonuclear leucocytes 24 hours after inoculation.

In/

In the cells at this early period, the bacilli were "segmented." At later periods I have failed to find the bacilli in these cells.

The Coarsely Granular Eosinphile Cells.

These cells resemble in some ways the polymorphonuclear leucocytes of the Guinea Pig.

The nucleus is often lobed, and the lobes attached by a very delicate strand of chromatin. At times, however, the nucleus is actually divided so that the cells have really two nuclei. The nuclei lie side by side, and the cell shows no evidence of actual division taking place.

The granules are larger, darker in colour (stained with Eosin) and more regularly packed together than are the granules in the polymorphonuclear leucocytes.

The cells, as has already been stated, are found in large numbers in the normal peritoneal fluid/

fluid of Guinea Pigs. In the blood, according to Kurloff, they amount to about 10% of the total number of leucocytes.

I have never seen the slightest evidence that the polymorphonuclear leucocytes are transformed into these cells, as has been stated by various authors.

Do these cells take any share in the process of inflammation? Goldmann,(9) Jadassohn,(17) Ehrlich(8) and others give them a place of equal importance with the polymorphonuclear leucocytes. They maintain that they play exactly the same part under the reaction of certain irritants. They, however, obey only certain specific chemiotactic stimuli. When these special stimuli are present, these cells are called out to do the work ordinarily done by the polymorphonuclear leucocytes, while the latter remain entirely passive. This would explain the observations of Neusser(39) and others, who found exudations composed almost entirely of true eosinophile leucocytes.

After injections of B. Coli and the organisms used during my observations, the coarsely granular eosinophile cells play no important part in the reaction set up.

They were not increased at any period and generally/

generally 36 to 48 hours after the injection there were extremely few found in the films.

This diminution may be more apparent than real, due to their being distributed in a greater mass of fluid, but considering the great number one finds in the normal peritoneal fluid, one is, I think, almost forced to the conclusion that they are actually diminished.

These cells appear to be very fragile. Frequently they are seen in the films broken up, and their granules free. This breaking up and freeing of the granules may be of importance in the life and function in the cells, or it may be simply accidental as a result of manipulation.

Do these cells act as Phagocytes to Bacteria?

Kanthack and Hardy (22) say they are not phagocytic. Mesnil (35) and Durham (7) have both found bacilli in these cells. My observations confirm those of Mesnil and Durham. Bacilli are seen in the cells in several of my films, but this phagocytosis is not usual. Even in cases where the bacilli are in great numbers in the polymorphonuclear leucocytes, none may be found in the coarsely granular eosinophile cells. In the few cases in which I have seen bacilli /

bacilli in these cells, the bacilli were always well stained and shewed no degeneration.

At times, there was some doubt as to whether the bacilli were lying on the cell or were actually in it. In other cases, however, the bacilli were lying in a definite clear space in the cells, just as one so often sees them in the polymorphonuclear leucocytes and in the large mononucleated phagocytic cells. In these cells, there could be no doubt that the bacilli were actually in the cell.

What becomes of these cells?

As already noted they are very fragile. Many of them are no doubt, destroyed in the peritoneal exudation.

Some are ingested by large phagocytic cells, and are digested by them. The granules appear to be more resistant than the rest of the cells, and often these granules alone remain in the "digestive vacuole" in the large phagocytic cells. (Fig VI).

This ingestion and digestion of the coarsely granular eosinophile cells is sometimes seen in films made from the peritoneal fluid of apparently healthy guinea pigs.

The Mononucleated Cells.

In 1891 Unna (49.50) described his "Plasma cells" as occurring in lupus. These cells varied much in size. The protoplasm was finely granular and stained darkly with Unna's alkaline Methylene Blue.

The nucleus was oval, eccentrically placed and stained darkly. In the nucleus and also in the protoplasm, a chromatin network with darker chromatin centres could be made out. The cells multiplied, but Unna did not see any evidence that the division was by mitosis.

He thought they were derived from connective tissue cells.

Jadassohn (18) describes the same cells, and says they may be present in any inflammation. He does not agree with Unna as to the presence of granules in the protoplasm. As to their origin, he does not think they are derived from connective tissue cells.

V. Marschalko (28) also describes these cells as occurring in inflammatory exudations within 24 hours. He says they are usually round, oval or polygonal, but that they may be drawn out into long spindle shaped cells. He lays special stress on the eccentric position of the nucleus. With Jadassohn he says the protoplasm is not granular, but may be arranged/

arranged in irregular masses. The nucleus stains darkly, and at its periphery there are 5 to 8 regular, darkly stained chromatin granules.

Their origin he maintains, is from the lymphoid cells of the blood. They are, he says, normally present in the lymph. glands and in the spleen. During inflammation, they accumulate in the vessels at the periphery of the inflammatory area, and migrate into the tissues.

He has figured the transitions between these lymphoid cells and the larger "Infiltration" or Plasma cells. The fact that they appear in acute purulent inflammations in such numbers in 24 hours is, he maintains, proof that they are not derived from connective tissue cells.

Paltauf (40) agrees in all points with V. Marschalko.

Hodara (44 u 45) found these same cells and called them "pseudoplasmacells." He denies that they occur in normal tissues, and thinks that V. Marschalko has confused the plasma cells with certain kinds of mononuclear leucocytes. He points out that the nucleus of the mononuclear leucocytes varies much both in size and in structure. He derives them from polymorphonuclear leucocytes.

Schottlander (46) describes the "Plasma cells" similar to V. Marschalko, but he says the nucleus is/

is central. Their origin, he says, is from the large mononuclear leucocytes, not from the lymphocytes. They may form epithelioid cells.

Justi [20] notes that in certain parts of wounds, we see in the capillaries a larger or smaller collection of leucocytes with round nuclei (lymphocytes), and also a perivascular infiltration of these cells. Comparing the two, he says their identity cannot be doubted.

The structure of the protoplasm is finely granular, or vacuolated. The margin is nearly, but never uniformly, round. In the larger number of the cells one can, at certain points at the periphery, observe larger or smaller collections of projections. These go to prove the amoeboid character of the cells.

Sometimes you get forms with two nuclei. The nuclei may be close together or at the ends of the cell. The cells certainly divide by direct division, but Justi also found mitotic figures at various parts.

The occurrence in, and the grouping round the vessels, the character of the protoplasm, the size and the structure of the nucleus, speak with almost certainty as to the plasma cells and the lymphocytes being identical. In this, Justi agrees with Marschalko.

He/

He, however, admits that the cells in the vessels are smaller than those round the vessels.

Further, he describes two kinds of lymphocytes - the small ones, and the middle sized ones with round or oval nuclei. The nuclei of these latter may be very rich in chromatin , darkly stained with larger chromatin masses studded through them, or they may be vesicle like and in these the chromatin and the nuclear framework come out more clearly. All stages between these two forms of lymphocytes may be traced.

Justi further distinguished another cell, the "granulation cell" of tubercle. The nucleus of these varies within wide limits. It may be very small and oval, or drawn out ribbon like, or it may be club-like at the ends, with the centre indented slightly, or actually cord-like. The nuclei have a very delicate nuclear framework and a very irregular, notched, darkly stained mass in the centre.

Justi admits that the difference between these granulation (epithelioid) cells, the free lying epithelium and the plasma cells is not only difficult but often impossible to make out.

The peculiar staining of the protoplasm of the plasma cells, and the "leucocytes with round nuclei" has led Justi to the view that these cells are special carriers of some substance. He thinks that substance may be for the nutrition of the rapidly growing granulation/

granulation cells, or perhaps it may be some injurious substance thatthe cells are carrying away or destroying.

Krompecher,(24) working with the infectious granulomata confirmed the view of Marschalko as to the origin of the plasma cells from lymphocytes. He, however, holds that only a part of them is formed in this way. A part of them is derived from polymorphonuclear leucocytes, and another part is formed from the large mononuclear leucocytes. Thus Krompecher agrees with both Hodara and Schottlander.

The protoplasm of the plasma cells often becomes vacuolated. This he takes as an evidence of degeneration. In these cells, he has at times found basophile granules, and also Russell's "Fuchsin bodies."

The epithelioid cells, he maintains, are derived from the plasma cells, and a further transformation then takes place for the epithelioid cells are the progenitors of connective tissue cells. Transitions, he says, can be made out.

Mallory,(26) in the lesions in Typhoid Fever, in Diphtheria, in Pneumonia and in abscesses of the Kidney, has described rounded cells with a small, round, coarsely granular, deeply staining eccentric nucleus. The protoplasm is finely granular and stains/

stains deeply with Unna's Methylene Blue. These he calls "plasma cells" and identifies them with the cells described by Unna, V. Marschalko and others. He says they are non-phagocytic, and he derives them from lymphoid cells.

He also describes large cells with a slightly stained curved or indented nucleus. These are phagocytic and incorporate polymorphonuclear leucocytes, lymphocytes, plasma cells, and red blood corpuscles.

He says they are derived from the proliferation of the endothelial cells of lymph spaces, lymphatics, vessels, lung alveoli, pleura and in fact, any endothelial structure.

He has observed mitosis of the lining endothelium of lymphatics and migration of the endothelial cells into the adjoining connective tissue.

Ribbert (44) has also noted an intense proliferation and desquamation of the endothelial cells lining the lymph-spaces,- and he also notes that these cells are occasionally phagocytic.

Councilman (504) in Acute Keratitis in the rabbit and also in Acute Interstitial, nephritis described the "plasma cells" as derived from lymphoid cells. He and Mallory are in entire agreement as to the characters and the origin of these cells. Both maintain that they are non-phagocytic.

Councilman/

Councilman notes their amoeboid movement. He describes a vacuolation of the protoplasm as a sign of degeneration. He lays special stress on the eccentric position of the nucleus. He found them abundantly in the bone marrow, spleen etc. He says that if stained with Methylene Blue, the ~~nucleus~~ protoplasm of the epithelòid cells stains a lilac tint, while in these cells, it is always a dark blue.

The Epithelioid cells, he also derives from the endothelium of blood vessels, etc. These cells are phagocytic. They are larger than the plasma cells. The protoplasm is finely granular, and stains with eosin. The nucleus is less intensely stained.

In Acute Keratitis, Councilman derives apparently the same cells from progressive changes in the corneal corpuscles. This is brought about as follows:-

The protoplasm becomes more abundant and slightly granular. The nucleus becomes round or oval, and stains more deeply. Then we get division of the nucleus. Protoplasmic processes are now given off from the cell. These, at first, are devoid of a nucleus. Afterwards the young nucleus makes its way into them, and division of the cell takes place.

In these young cells, he adds, we get a great variety of cell inclusions, leucocytes and necrotic corneal corpuscles. Many of these lie in digestive vacuoles/

vacuoles. They may be unchanged or there may be mere fragments of the cell inclusion left.

Councilman also describes as occurring in the Kidney in Acute Interstitial Nephritis, "Lymphoid cells." There are, he says, all transitions between these and the plasma cells.

Borrel (2.) describing the mononucleated cells which ingest tubercle bacilli, and which he identifies with the epithelioid cells, says they are derived from lymphatic cells. He says transitions can be made out.

Broden (3.) describing the same cells, says that no transitions between these and lymphatic cells can be made out. He says they are derived from endothelium. He agrees with Borrel that accumulations of these cells can be seen at the periphery of the vessels. Borrel accepts this as supporting his view. He says they are mononucleated leucocytes which are about to migrate. Broden, on the other hand, suggests that this accumulation at the periphery of the vessel indicates that the cells are produced there by a proliferation of the vascular endothelium.

Broden also states that these cells do not shew any evidence of amoeboid movement. They frequently contain bacilli and later may englobe leucocytes.

Durham (7.) describes what Metchnikoff called the "Macrophages."/

"Macrophages." The nucleus of these is large, oval or round and faintly stained. The protoplasm has a coarse spongy texture. These cells pick up bacteria and foreign particles readily. The young forms, he adds, are like lymphocytes. They are derived from endothelial cells. These evidently correspond with the epithelioid cells described by Councilman and Mallory.

Sherrington and Ballance (47) described these same cells, as "plasma cells."

The "hyaline cells" of Kanthack and Hardy, also described by Durham apparently correspond with the "plasma cells" of Mallory, Councilman, V. Marchalko and others.

Durham (7) adds, "During recovery after inoculation, about the fifth or sixth day, various intermediate forms between cells of lymphocyte size and hyaline cells are to be seen. These lymphocytes differ from those which occur in the normal peritoneal fluid, in that their nuclei stain faintly, and their thin rim of protoplasm stains deeply with Methylene Blue, often with the appearance of granulation, which becomes indistinct in the larger cells.

In these respects, they simulate the young macrophages, but on a small scale."

Hinsberg (13) says that the epithelial cells become detached from seven hours after an injection. They lie free in the exudation without the slightest appearance/

appearance of enlargement or multiplication. They shew a slightly stained nucleus. He states definitely that the round cells with the vesicular nucleus are not derived from epithelial cells. The epithelial cells are not wandering cells - the others are.

(42)

Pratt in a paper on the History of Acute Lobar Pneumonia describes the following kinds of cells as found in the exudation.

(a) The "non-granular" cells found in the early stages, rarely after the sixth day. These are larger than the polymorphonuclear leucocytes. They have a relatively large vesicular nucleus (round or oval, usually indented and often horse-shoe shaped.). There is a rim of but slightly granular protoplasm. He says they are similar in appearance to the non-granular leucocytes described by Councilman in his paper on Acute Keratitis in the Rabbit. These cells, he says, are phagocytic and resemble the desquamated epithelial cells with phagocytic properties. They also resemble the transitional leucocytes of Ehrlich. Pratt is uncertain of their origin. They are found in the capillaries and probably migrate from these into the tissues. He, however, says they may possibly be derived from the alveolar epithelium.

(b) Large phagocytic cells. These are round or oval, with oval or crescentic shaped vesicular nucleus placed at the periphery. There are rarely more than 2 or 3 cell inclusions. These cells are derived from the cells lining the alveoli.

(c) Desquamated epithelial cells.

(d) Lymphocytes are present in small numbers.

(e) Plasma cells. These occur late in the disease.

(f) Polymorphonuclear leucocytes: These predominate, and in fact, they are almost the only ones present in cases of death after the third day.

Robert Muir (38) in his recent paper on "The Bone Marrow and Leucocyte Production" incidentally mentions that many of the phagocytic cells in the peritoneal effusion after injections of Staphylococcus Pyogenes Aureus are hyaline leucocytes which increase in size. Others, he adds, are probably as Durham pointed out endothelial cells.

This view of Muir is strictly in accord with my own observations.

Conclusions from Author's Observations.

(a) The endothelial cells as progenitors of phagocytic cells. In the peritoneal cavity the main source of these cells is the endothelium of the omentum. No doubt the rest of the endothelial surface also shares in this work, but one constantly finds the endothelium covering the various viscera in the abdomen quite intact after the endothelium of the omentum has been shed.

The endothelial cells of other serous cavities, of blood vessels, of lymph spaces, of lymphatics, etc. can become phagocytic.

We have a considerable amount of evidence in support of this view.

Durham has pointed out that during the period at which these large phagocytic cells appear in the fluid withdrawn from the peritoneal cavity, the endothelial cells upon the omentum are seen to be of an/

an amoeboid character, the protoplasm spongy instead of thin and homogeneous and that foci of multi and bi-nucleated cells appear.

Mallory has seen mitosis of the lining endothelium of lymphatics and migration of the endothelial cells into the adjoining connective tissue.

The amoeboid character of the cells is undoubted. The pseudopodic processes which are seen projecting from the cells (Figs. VII, XXVI etc) in all directions is almost sufficient indication of their amoeboid character. Observations on the warm stage absolutely prove that these cells not only project and retract protoplasmic processes, but that they actually move from place to place. This movement is certainly not very active, and certainly much less active than the movement of the polymorphonuclear leucocytes.

As we have seen Broden has denied this amoeboid movement, but it is difficult to understand how he could have overlooked it. On one occasion I observed one of these cells pass right across the field of the microscope, and I have never on any occasion watched the fresh fluid on the warm stage without seeing the movement. Most authors admit the amoeboid character of these cells.

Examination of the omentum at various stages shews clearly that many of the phagocytic cells are derived from this serous endothelium.

In the early stages, e.g. 10 hours after infection/

fection , the cells covering the omentum are found to be swollen and separated from one another. At parts isolated cells are seen, but at other parts masses of the cells still adhere to one another though separated from the surrounding parts. (Microscopic Specimen 78.)

The nucleus of these cells is not very rich in chromatin and is vesicular in character. It is usually oval or rounded and has one or more darkly stained points near the centre.

The protoplasm of the cells is spongy or vacuolated in character, and the cells very often have protoplasmic processes branching but not anastomosing (Fig. XXX[illegible]). These processes are, I believe, pseudopodia and give evidence of the amoeboid character of the cells.

At the later stages, from 24 to 36 hours, the greater part of the superficial endothelium of the omentum has been shed. Now we see that the cells lining the meshes of the omental network are in many cases swollen, though still attached, (Fig. 25. C) whereas lying free in the meshes are many of the same cells which have been shed. (Figs XXXIII & XXVIII) & 24 a). Further, both in the early and in the later stages, many of the cells lying on the omentum, cells which are undoubtedly the covering endothelium, shew an active proliferation of their nuclei. (Figs XXXII & 24 d)

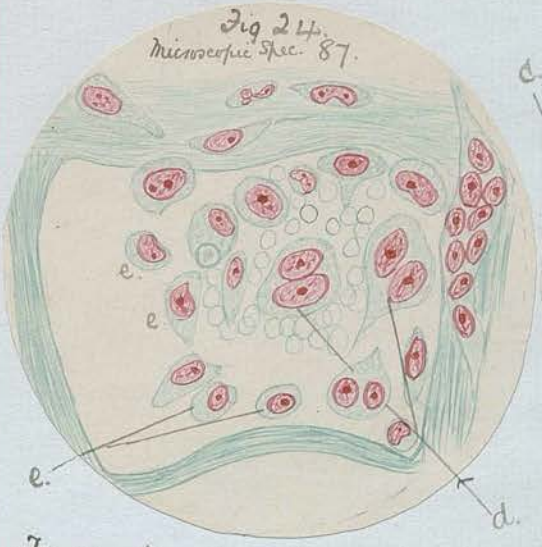


Fig.24: Omentum - Peritonitis following Rupture of Liver.
Shews proliferation & phagocytosis of endothelial cells.

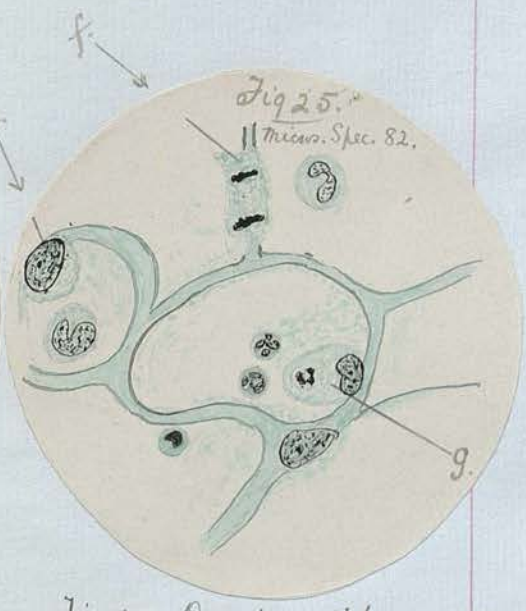


Fig 25. Omentum 36 hours after injection of B. Coli.
Shews. swelling of attached cells - phagocytosis & mitosis.

But not only do we see cells with two or more nuclei - and this is specially well seen in the human omentum - we also see mitotic figures fairly abundantly. This mitosis is seen as early as 10 hours after an injection, but it is much more abundant in the cells found about 36 hours after inoculation. (Fig. 25. f.)

This mitosis appears to take place in the free as well as in the attached cells.

<u>The Phagocytic properties of these cells to other cells.</u>

The ingestion of other cells by these mononucleated ones does not occur to any extent till about 24 hours after inoculation. It is most marked as we have seen from 30 to 48 hours after the intraperitoneal injection.

Therefore/

Therefore we do not expect to find any marked degree of this phagocytosis in the attached cells. This, however, does ~~not~~ occur and examination of the omentum shews, not at all infrequently, the ingestion of other cells by the attached endothelium. (Fig. 25. 9) In one experiment, one of the cells covering the capsule of the mesenteric glands shewing an included cell.

Examination of the fluid withdrawn from the peritoneal sac gives further evidence of this. We see masses of shed cells which are identical in every respect with the cells found on the surface of the omentum. The masses shew not only an active mitosis going on (Figs. XVI & XVII), but they also shew in many cases polymorphonuclear leucocytes or fragments of these and/

and also red-blood corpuscles which have been ingested by them. These cell inclusions are surrounded by a clear space, and thus appear to lie in a vacuole -"the digestive vacùole." (Fig. XXIII v XXV).

Examination of the fluid from the pleural sac in cases of Acute Pleurisy in the human subject gives the same results.

Masses of adherent cells which are undoubtedly masses of shed endothelial cells are found in the fluid. These cells stain perfectly and shew no evidence of degenerative changes. (Figs. XI . XII v XIII)

Many of the cells have two or more nuclei.

These cells shew quite a marked phagocytosis to other cells but especially to polymorphonuclear leucocytes. (Figs XI . v XIII

Further in a number of these cells dark particles of what is apparently carbon pigment is seen. (Fig. XI v XII).

These cells are actively amoeboid, again evidenced not merely by the presence of protoplasmic processes, but by actual movement when watched on the warm stage.

Ribbert (44) has described a proliferation of the endothelial lining of the lymph spaces.

Mallory has noted this in the blood vessels and/

and he has also observed migration of these cells into the surrounding tissues.

In the blood vessels of the omentum it is very common to find a considerable number of mononucleated cells of various sizes. The cells are larger than the lymphocytes. The nucleus is usually less rich in chromatin, and it is oval or kidney shaped and very generally placed to one side of the cell. The protoplasm also is more abundant than the protoplasm of the lymphocyte. The cells are very commonly massed at the periphery of the vessel, but at other times they may be central. Many of these cells are without doubt, the large mononucleated leucocytes of Ehrlich, and these will be dealt with later. Others, however, are derived from the lining endothelium of the vessels. I have never seen mitotic figures in these cells, but Mallory has seen mitosis in the endothelium of lymphatics. The cells lining the vessel become swollen and then become detached from the wall. Stages in this process were not very frequently seen. The arrangement of the cells in a single row along the wall of the vessel suggested very strongly that they had become separated from the wall, and this suggestion was verified by seeing the cells, in the process of separation. (Fig. 27, XXVII)

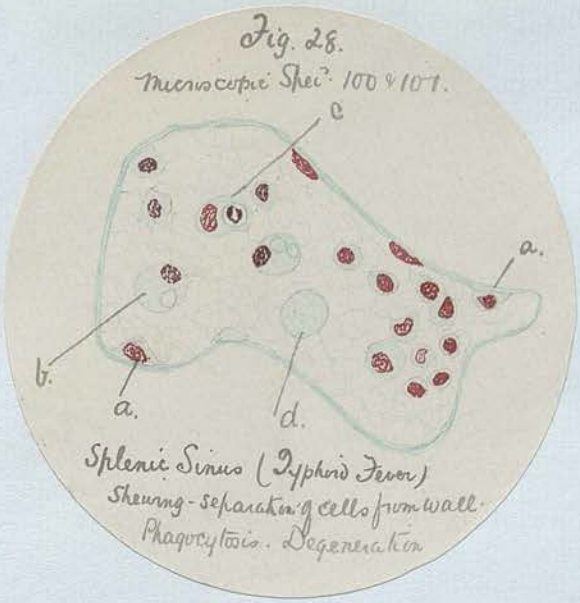
Further proof of this is got by examination of the spleen and the glands in cases of bacterial infection.

Examination of the spleen in cases of Typhoid Fever, shew, as Mallory has pointed out, an active proliferation of the endothelial cells lining the sinuses of the splenic pulp. - (Fig 28.)

The cells lining the sinuses swell, and become two or three or more times the size of the original cells. (a) They then become separated, and many of the sinuses simply become filled with these large cells. Soon after the enlargement vacuolation of the protoplasm is seen. (b)

The cells act as phagocytes and ingest polymorphonuclear leucocytes and other cells. (c) This phagocytosis may take place while the cell is still attached to the wall of the sinus.

Later what is apparently a degeneration of the cell takes place. The nucleus stains less intensely and gradually its staining power is quite lost. Thus we very commonly see in the sinuses masses of protoplasm usually somewhat irregularly stained, vacuolated, and without any trace of the nucleus. (d)



Examination of the spleen and the liver from a case of leprosy shewed a similar condition. (Fig. 29)

The splenic sinuses were filled with these large, mononucleated and vacuolated cells. In the larger forms the nucleus could not be made out. The cells were crowded with the Bacillus of Leprosy. Here again stages can quite well be traced between the cells lining the sinuses and these large cells.

Many/

Many of the smaller desquamated cells contained a few bacilli. (a) Bacilli could also be seen in the cells still attached to the wall of the sinus. (b)

In the liver, cells of apparently the same nature and crowded with bacilli were seen in the capillaries between the liver cells, and no bacilli were found in the liver cells themselves. Unfortunately the liver had not been well preserved. There were other organisms present, and the cells do not stain at all satisfactorily.

Examination of the mesenteric glands after a peritonitis shews a similar condition with more marked phagocytosis however. (Figs. XXXV & XXXVI).

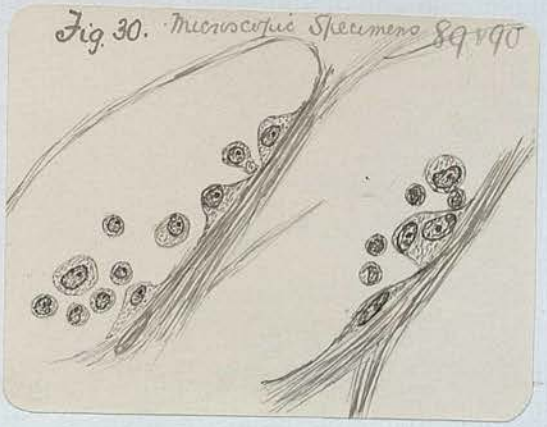


Fig. 30. Omentum - Peritonitis following Malignant Ulcer. Shews swelling and proliferation of attached endothelial cells.

Do these separated cells migrate from the vessels?

This is a very difficult question to answer with any certainty.

We find the cells in the lumen of the vessels and also accumulated outside the vessel. This suggests migration, but we are met at the outset with various difficulties. The first is the difficulty of distinguishing the mononucleated leucocytes from this proliferated endothelium. This will be more fully dealt with later.

Again, the endothelial cells outside the vessel are in a state of active proliferation, and the cells resulting from this proliferation are small and resemble very closely the endothelium of the blood vessels. Of course this occurs generally and is not confined/

confined to the neighbourhood of the vessels.

Accompanying the vessels we have more or less abundant lynphoid tissue. Sometimes this is in considerable amount forming definite lymph nodes. Examination of these shews a proliferation of these cells. These cells resemble the endothelial cells and the mononucleated leucocytes. Even seeing a cell in the act of migration does not settle the matter, for during migration the nucleus, on which the principal distinction is based, is so altered that it is impossible to distinguish the cells clearly.

Councilman and Mallory have both satisfied themselves that migration does take place. The fact that other endothelial cells can wander about is presumptive evidence that the cells of the vessels can migrate; and I think one must ~~accept to~~ agree with Councilman and Mallory.

The cells outside the vessels whether derived from proliferated endothelium of the omentum, or endothelium of vessels grow gradually larger.

The protoplasm increases, the nucleus usually stains more lightly and the darker nodes of chromatin in the nucleus become more evident. The protoplasm becomes vacuolated and we get the cell transformed into the regular phagocytic cell.

(b). <u>The Phagocytic cells derived from mononucleated leucocytes</u>.

In/

In dealing with the so-called "hyaline cells" of the normal peritoneal fluid we have stated that these cells correspond in structure with the large mononucleated leucocytes of Ehrlich, and that accumulations of these can be seen in the vessels of the normal omentum. Ehrlich has pointed out that these cells are not lymphocytes and are not derived from lymphocytes. The nucleus is different in shape and in the intensity of its staining reaction. The cells are larger and the protoplasm more abundant. No transitions between the two are found.

My observations are entirely in accord with the views of Ehrlich, as regards the cells in the vessels.

After an intraperitoneal injection, say of B. Coli these cells are found in considerable numbers in the vessels of the omentum from 18 hours onwards. The vessels may almost be filled with them, but they are usually not so numerous. They are fairly uniformly distributed in the vessel, but they tend to accumulate at the periphery in certain parts. (Fig. XXVII)

The cells are usually rounded and the nucleus is not so rich in chromatin as the nucleus of the lymphocytes. It is usually oval or kidney shaped and lies to one side of the cell. The nucleus , in at any rate, the smaller forms, often stains a bluish tint/

tint, whereas the nucleus of the endothelial cells has a lilac tint when stained with Eosin and Methylene Blue. The cells vary in size, and even in the blood vessels an active proliferation appears to take place. (Fig. 31).

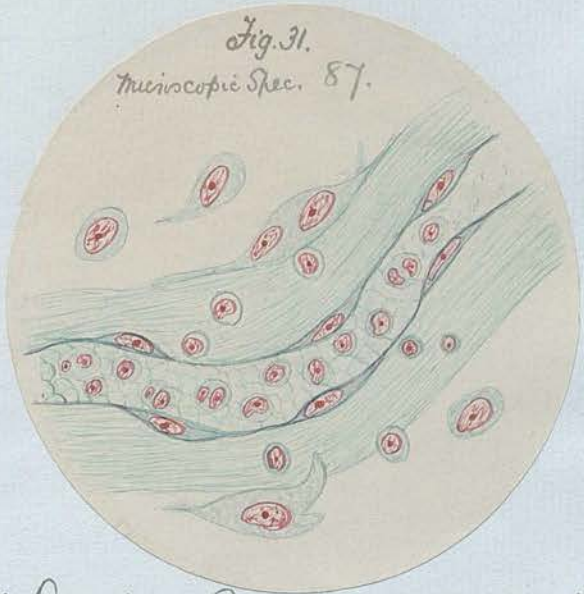


Fig. 31. Omentum - Peritonitis foll. Rupture of Liver.
Shewing - mononucleated leucocytes in vessel & a few outside
The cells in the vessel are proliferating

On the surface of the omentum, as well as free in the fluid from the peritoneum numbers of cells identical in structure, and in staining reaction with these cells but usually somewhat larger are found. They are best seen in preparations stained with Saffranin and Light Green according to Benda's method.

Very commonly one sees a considerable number of them in the neighbourhood of the vessels especially the venules and the capillaries. Most authors are agreed that the cells migrate from the vessels into/

into the surrounding tissues.

The origin of these cells however, is disputed. Borrel,[2] working with injections of Tubercle Bacilli points out the accumulation at the periphery of the vessels and maintains that they are mononucleated leucocytes that migrate.

Broden[3] admits all the facts given by Borrel but he comes to a different conclusion. As we have seen he derives them from the endothelium of the vessels. As we have already stated, it is extremely difficult to be certain on this point. The cells lining the vessels, as we have seen, do proliferate and do migrate into the surrounding tissues. By active proliferation of these cells we get cells of various sizes, but careful staining and examination shew the nucleus of the endothelial cells to be less rich in chromatin than the nucleus of the mononucleated leucocytes. The difference is certainly not very marked and is usually well seen in the smaller cells only. The nucleus of the mononucleated leucocytes is usually of a darker blue than the nucleus of the endothelial cells, when stained with Eosin and Alkaline Methylene Blue. Stained with Saffranin and Light Green the chromatin network is more delicate in the endothelial cells and the nodal points(nucleoli) more definite than in the mononucleated leucocytes.

We/

We find accumulations of these cells in vessels where the endothelium is intact, and shewing no evidence of proliferation throughout the extent of the vessel in the preparations examined. (Fig. 31.)

On these grounds therefore, one must accept the view that a part, at any rate, of these cells accumulated in the vessels are really the large mononucleated leucocytes.

After migration these cells increase in size by an accumulation of protoplasm round the nucleus. The nucleus also enlarges, its chromatin becomes more diffuse, and in consequence, the staining reaction becomes less intense.

The protoplasm becomes vacuolated.

The increase in size continues, and as the cells increase the resemblance between these cells and the cells derived from the endothelium becomes so pronounced that it is almost impossible to distinguish the one from the other.

These cells act as phagocytes to other cells and even in some of the medium sized ones cell inclusions or the remains of these cell inclusions are found. (Figs. XIV. XV. XXVI.)

These cells are evidently the same as those described by Pratt as non-granular leucocytes. The resemblance between them and the endothelial cells has been emphasised by Pratt.

A careful study of the cells at all periods has convinced me that they are derived from the large mononucleated leucocytes.

The origin of these latter is still a matter of great uncertainty. The resemblance between them and the cells derived from the endothelium by active proliferation suggests that they may be derived from some endothelial structure. This is further supported by the fact that both kinds of cells can function in the same manner.

The Plasma Cells.

V. Marschalko, Justi, Councilman, Mallory and others derive these cells from the lymphoid cells of the blood. I take it that by these lymphoid cells, these authors mean the ordinary lymphocytes.

Councilman and Mallory accept in full V. Marschalko's views and Schottlander writing after V. Marschalko evidently accepts him as meaning the lymphocytes, for he denies the origin of these plasma cells from lymphocytes. He says they are derived from the large mononucleated leucocytes.

Justi evidently does not distinguish the lymphocytes from the other mononucleated cells of the blood.

He/

He describes two kinds of lymphocytes - the small and the middle sized ones.

Are these plasma cells and the phagocytic cells derived from the large mononucleated leucocytes, the same cells?

We have seen that both forms originate from the mononucleated leucocytes. It matters little whether we accept the view that their origin is from the lymphocytes or from the large mononucleated cells. My observations, as already stated, are in agreement with Ehrlich's view that there are no transitions between the lymphocytes and the larger mononucleated cells, in the blood.

The structure, position and staining reactions of the nucleus and the staining reaction of the protoplasm have been put forward as features distinguishing these cells from other cells.

It is said, as we have seen, by V. Marschalko, Councilman and Mallory that the nucleus is rounded, rich in Chromatin and therefore staining darkly, and eccentrically placed, and that the protoplasm also stains darkly with Unna's Alkaline Methylene Blue.

Justi, who accepts V. Marschalko's statements, however, admits that in the larger forms the nucleus may be vesicular and the chromatin network may become more evident.

We have seen that in phagocytic cells derived from/

from the large mononucleated leucocytes the nucleus varies in its staining reactions. Sometimes it stains a dark blue, and at other times the stain is much less intense.

The eccentric position of the nucleus is seen in both forms.

As to the staining of the protoplasm this varies much in different specimens of the same cell. This is seen not merely in the films made from the peritoneal fluid, but also in the cells still attached to the surface of the omentum.

If these cells are secreting cells, a subject to be dealt with later, then it is easy to understand why at one time they should stain more darkly than at another.

Besides as the cells enlarge the staining of both protoplasm and nucleus becomes less intense.

Though there are differences in intensity of staining in various cells, and though this difference may at times be of considerable importance in distinguishing one cell from another, still it seems to me that far too much has been made of this difference. One sees constantly in masses of cells (Fig. X) where the indentity of individual cells cannot be doubted a very marked difference in intensity of staining reaction and especially of the nucleus.

Besides as mentioned by Adami, various physiologists/

ogists agree that the nucleus plays an important and in fact a controlling part, not only in the process of cell division but in the function of the cell.

With activity the chromatin of the nucleus becomes used up and discharged into the body of the cell, to combine with certain bodies and thus form the specific secretion of the cell.

Thus we would expect that the smaller cells, which are not functioning very actively would have a nucleus richer in chromatin than the larger more active cell.

My observations bear this out. (Fig. 22.)

Pratt found the Plasma cells in his investigations in Acute Pneumonia in the later stages, of the disease, and apparently not in fatal cases for he mentions that in cases fatal after the third day almost the only cells found are the polymorphonuclear leucocytes.

In Acute Keratitis in the rabbit, Councilman found these plasma cells only after the fifth day from the injury to the cornea.

In his investigations on Typhoid, Mallory describes these cells, but here again several days had necessarily elapsed from the time of infection to the time of examination. The periods given by them are 10 days to 4 weeks. V. Marschalko certainly mentions them as being abundant 24 hours after an infection.

In/

In my own specimens the difference in the plasma stain pointed out by Councilman - the dark blue of the so-called plasma cells and the lilac tint of the endothelial cells and especially the difference in the nuclear stain is quite well seen in the films made from the pleural fluid in a case of acute pleurisy. Here the pleurisy had existed for several days, and it was further noted that at later periods the cells with dark blue nuclei were more abundant.

Stages between these and the cells with lilac nuclei are seen, and further the protoplasm in both contains fine eosinophile granules (Fig. XII).

These granules are not well seen with the ordinary fixing reagents, but are well brought out in films fixed with Muir's fixing reagent. (36) (Fig. XII)

The amoeboid character of the cells and the vacuolation of the protoplasm are admitted for both kinds of cells.

On the question of phagocytosis to other cells V. Marschalko and Justi say nothing.

Krompecher, who agrees with V. Marschalko that part at any rate of the plasma cells are derived from the lymphoid cells of the blood describes in these cells what are apparently cell inclusions. He takes them to be Russell's "Fuchsin bodies." Further he maintains that these plasma cells may be transformed into epithelioid cells.

Borrel/

Borrel[2] notes the ingestion of tubercle bacilli by cells derived from the lymphoid cells of the blood.

Metchnikoff (31) maintains that the mononucleated leucocytes are phagocytic to various bacteria.

Councilman and Mallory on the other hand maintain that these plasma cells are not phagocytic.

I am convinced that the cells are phagocytic not merely to bacteria but also to other cells, and indeed that the cells are identical with the phagocytes derived from the large mononucleated leucocytes. The darker blue colour of the nucleus and the darker colour of the protoplasm after staining with Methylene Blue found in certain of the cells, and as I have shewn especially in the later periods is simply due to the fact that these cells are less active than the others. If some irritant is introduced, these cells are stimulated to greater activity and then staining becomes less intense.

The Lymphoid Tissue round the vessels as a possible site of origin of Phagocytes.

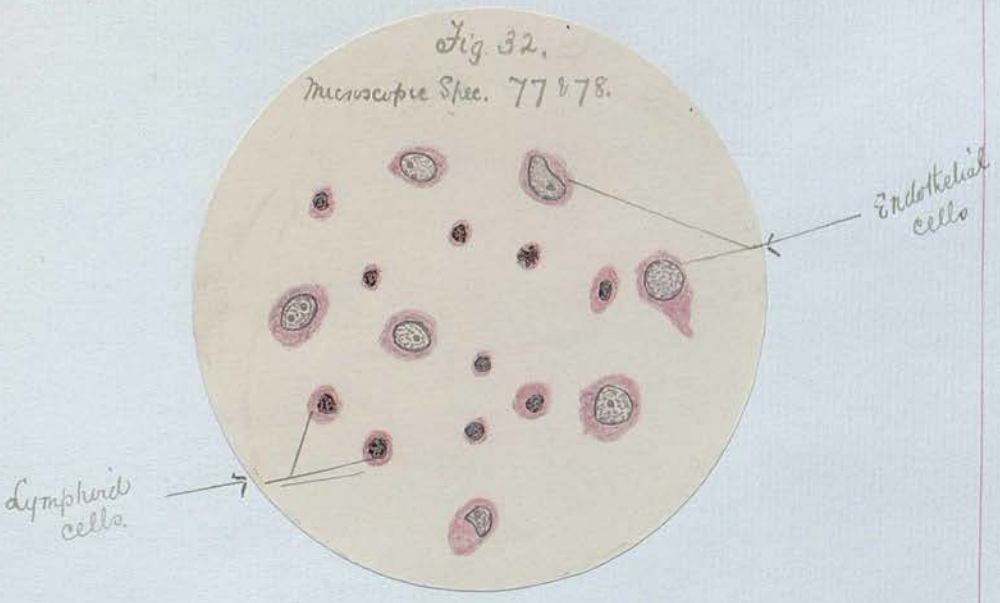
Surrounding the vessels, especially the larger ones, in the omentum of the Guinea Pig and of the Rabbit there is a sheath of lymphoid tissue. At parts this tissue is accumulated into masses, sometimes comparatively large. Round the smaller vessels this lymphoid sheath may be represented by merely a few lymphoid cells. The same condition is seen in the human omentum, but here the condensation into masses is more marked.

The cells of this lymphoid tissue are like the cells of lymphatic glands. They are small. The nucleus is rich in chromatin. Usually it is round and central. The staining is so dark that very little structure can be made out in the nucleus. The protoplasm is generally very scanty and takes on a reddish stain with eosin.

Usually at the periphery of these sheaths or masses some of the cells may be seen quite separated from the rest. Some of these may even be seen at some distance from the vessels in the normal omentum. These separated cells usually have a much more definite rim of protoplasm. The nucleus in most cases remains very dark, though it usually appears somewhat lighter as the cell enlarges.

In the early condition however, the difference between/

between these cells and the endothelial cells is extremely well marked. The nucleus is much darker, and the protoplasm takes a deeper stain with the eosin. (Fig. 32.)



This lymphoid tissue undergoes changes during inflammation. A proliferation takes place. The increase is not very marked in the early stages, but a number of the cells are found at some distance from the vessels and these cells are, as has been already noted, larger than the ones near the vessels.

The increase however, is much better seen where the irritant has been acting for a longer time. Some days after intraperitoneal injection of Tubercle Bacilli the increase of this lymphoid sheath is a striking characteristic of the omentum. It is especially well seen from 12 to 15 days after the injection. (Microscopic Specimen 93.)

After injections of foreign particles (e.g. Vermilion) these masses of lymphoid tissue become very dense and become crowded with the vermilion particles. Thus they appear to act like lymphatic glands.

That proliferation of this tissue takes place, and that cells similar in every way, but usually somewhat larger, may be found at some distance is undoubted. A study of the omentum from my preparations leave me in no doubt on these points.

Whether these cells enlarge further and become phagocytic is almost impossible to demonstrate. By enlargement they come to be very similar to the large mononucleated leucocytes and the young endothelial cells. I have not been able in the later stages to draw a definite line between these cells.

There seems to be no reason why this enlargement should not take place, and it appears to me more likely that the lymphocyte-like cells which have been described as occurring in many of my films are really the cells derived from this lymphoid tissue.

That transitions between lymphocyte-like cells and the phagocytic cells occur in the peritoneal fluid/

fluid has been shewn abundantly in the various films described at an earlier part of this thesis.

That I have not seen transitions between lymphocytes and the large mononucleated leucocytes in the blood has also been stated.

These separate appearances seemed at first difficult of explanation, but with this proliferation of lymphoid tissue outside the vessel under the influence of an irritant these apparent contradictions are explained.

The Origin of the Phagocytic Cells - a Summary.

Briefly stated then the phagocytic cells are derived partly from any endothelial structure, partly from the large mononucleated leucocytes and probably also partly from the lymphoid tissue surrounding the vessels. These structures undergo active proliferation when an irritant acts on them. The cells divide mainly by mitosis. They may increase very considerably in size.

In the early stages the cells are easily distinguished from one another.

The lymphoid tissue cells have a very darkly stained nucleus in which no definite structure can be made out. It is usually centrally placed and more or less rounded. The protoplasm is small in amount/

amount, so that the nucleus occupies almost the whole of the cell.

The mononucleated leucocytes are larger than the lymphoid-tissue cells. The nucleus is oval or often Kidney shaped,and placed to one side of the cell. It is not so rich in chromatin, and a chromatin network with some darkly stained nodal points can usually be made out. By careful staining by Benda's method usually one or two small nucleoli can be demonstrated. The protoplasm usually stains faintly with Eosin.

The endothelial cells vary very much in size. The outline is usually more irregular than that of the others, but this irregularity is not by any means constant.

The nucleus is comparatively poor in chromatin and one or two mucleoli usually stand out prominently even with the ordinary stains.

The protoplasm is fairly abundant, and usually appears somewhat granular and takes on readily the eosin stain.

These distinctions become gradually lost as the cells/

cells enlarge and function more actively, and it may be quite impossible to distinguish the cells at the most actively functioning period.

In the earlier stages of inflammatory reaction the principle phagocytes appear to be those derived from the mononucleated leucocytes or lymphoid cells, but the endothelium very soon becomes active also, so that by 48 hours at any rate and probably earlier both kinds of phagocytes are at work.

The "hyaline cells" of the normal peritoneal fluid are derived principally from the large mononucleated leucocytes.

The desquamated endothelial cells:- We not uncommonly get definite endothelial plates in the fluid from the peritoneum. These apparently are not amoeboid. They shew no evidence of proliferation or of phagocytosis. They are usually very faintly stained.

These are probably some of the covering cells of the peritoneum which have played their part and are cast off. These cells are found in the normal peritoneal fluid and are simply the result of a natural process. They have no special relation to inflammatory processes.

The Lymphocytes:- A few of these are found in the normal peritoneal fluid. They are distinguished from the other cells by their rounded and very darkly stained nucleus which occupies almost the whole of the cell. These cells are found in the fluid

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The Lymphocytes:- A few of these are found in the normal peritoneal fluid. They are distinguished from the other cells by their rounded and very darkly stained nucleus which occupies almost the whole of the cell. These cells are found in the fluid

after intraperitoneal injections, but they do not play any important part in the inflammatory process - at any rate in the acute processes. There are some indications that in more chronic inflammations e.g.that caused by tubercle bacilli their role may be of some importance, but this subject I have as yet not sufficiently investigated to enable any definite statement to be made. I think it is doubtful whether the mononucleated cells which collect round the epithelioid cells in a tubercle nodule are lymphocytes.

The Function of these Mononucleated Phagocytes:-

We have already seen that these cells are present in the normal peritoneal fluid, and that there the protoplasm may be vacuolated and that the cells may act as phagocytes.

This suggests that their function is the dealing with, in some way or other, any injurious substances that may be present in the normal peritoneum.

Very soon after an intraperitoneal injection is given these cells increase in number and in 18 to 24 hours they are very numerous and ingest various cells especially polymorphonuclear leucocytes and red blood corpuscles. Foreign particles are ingested by these cells at a much earlier period. Three hours after an intraperitoneal injection of carmine the uninucleated cells contain numerous particles of the carmine.

These carmine particles are found also, and to an equal degree, in the polymorphonuclear leucocytes./

cocytes. This is not in accord with the view of some authors who state that the polymorphonuclear leucocytes are much less phagocytic to foreign particles than are the mononucleated cells.

Besides the ingestion of foreign particles and of cells, these phagocytes can also take up bacteria, but as Metchnikoff (34) has pointed out they appear to have a selective property.

If a small injection of B. Coli is given intraperitoneally practically all the bacilli are found in the polymorphonuclear leucocytes. None or very few are found in the mononucleated cells.

If, however, the injection is a much larger one the mononucleated cells may contain considerable numbers of the bacilli. In these cases the polymorphonuclear leucocytes are usually crowded with the bacilli.

After injections of pure cultures of tubercle bacilli into the peritoneal sac, we find the bacilli mainly in the mononucleated cells.

A few are found in the polymorphonuclear leucocytes but undoubtedly the greater number are taken up by the mononucleated cells.

This apparent selection may be partly accounted for by the fact that the polymorphonuclear leucocytes do not migrate into the peritoneal cavity so early after injections of pure cultures of tubercle bacilli/

bacilli ~~bacillias~~ as they do after injections of other organisms. The mononucleated cells are present from the beginning and they take up these non-motile organisms just as they take up foreign bodies. Thus before the polymorphonuclear leucocytes have been called out, the mononucleated cells have already ingested the bacilli.

In support of this view, we have the fact that when the tubercle bacilli are injected in association with other organisms e.g. in sputum or in pus. the polymorphonuclear leucocytes are called out early and we find the bacilli in these cells rather than in the mononucleated cells.

The ingestion of bacilli by these mononucleated cells is also seen in the spleen and the liver in Leprosy. This has been already referred to in discussing the phagocytic cells derived from the endothelium of the sinuses in the spleen.

Metchnikoff (31) and Sawtchenko (45) have shewn that the Leprosy bacilli are never englobed by the polymorphonuclear leucocytes, but are readily devoured by the mononuclear cells.

Do these cells ingest living organisms?

On this point there can be no doubt. On the warm stage I have seen living bacilli in vacuoles in the cells. In one case there were two of the colon bacilli in a single vacuole: One of these was quite motionless while the other was actively motile though/

though necessarily the motion was somewhat limited. Brownian movement was going on in the granules of the adjacent polymorphonuclear leucocytes and this movement was quite different from the movement in the vacuole. I have not the least doubt that the movement was that of a living bacillus. Frequently bacilli are found as if anchored to the cells and a wriggling movement then goes on.

Another suggestion is that the organisms are the active agents and the cell passive.

The action of the cells to other cells, and to foreign particles makes it more probably that the cell is the active agent even in the ingestion of bacteria, spermatozoa etc.

Metchnikoff (32) has shewn that bacilli perform active movements although enclosed in vacuoles in the leucocytes of the frog. He has further shewn that anthrax bacilli in the leucocytes of the pigeon when introduced into bouillon grow. They pierce the protoplasm of the cell, and form well developed filaments.

Further in support of this view we have what appears to be an active proliferation of the tubercle bacilli in epithelioid cells - cells which I believe to be produced by an active proliferation of endothelial cells.

Examination/

Examination of the fluid withdrawn from the peritoneal cavity at late periods - in one case 25 days after the injection of tubercle bacilli shew masses of the bacilli grouped together in the cells. Sometimes these masses appear to be in a vacuole, at other times no vacuole is apparent. (Fig. IX).

These bacilli stain quite as well as those got in the early stages.

These appearances at least suggest that active proliferation of the bacilli is going on in the cells.

With the B. Coli nothing suggesting proliferation was seen.

The ingestion of Polymorphonuclear leucocytes by these mononucleated cells.

The polymorphonuclear leucocytes are found englobed by the mononucleated cells 18 to 24 hours after an intraperitoneal injection. This englobing becomes much more marked from 36 to 48 hours after the injection.

Some of the appearances got in my films (Figs. 13a) would suggest that the polymorphonuclear leucocytes were the active agents in this process; that they came in contact with the mononucleated cells, shot the chromatin of their nuclei into the bodies of the mononucleated cells and then themselves/

selves gradually passed into these cells.

Other specimens however, shew the protoplasmic processes of the mononucleated cells being thrown out round the polymorphonuclear leucocytes and the gradual taking up of these cells by the mononucleated ones (Figs. VI & XXIII).

This latter appearance is much more common.

Even in the cells which shew the former condition small processes are seen projecting on each side of the polymorphonuclear leucocyte, and it seems more probable that in these isolated instances the ingestion by the mononucleated cell had just begun, and during the fixing of the cell its pseudopodia were retracted. The polymorphonuclear leucocyte was thus left with a small portion only in the mononucleated cell.

The changes in the polymorphonuclear leucocytes after ingestion by the mononucleated cells.

The chromatin of the nucleus appears to become condensed so that the nuclear stain is very dark and almost uniform. The appearance issoften as if a mononucleated cell had been ingested(Fig. XXIV 13a). Careful examination, however, shews that this darkly stained nucleus still retains some evidence of its previously lobed character.

The nucleus then breaks up into several, usual-

ly/

ly 3 or 4, rounded bodies which stain uniformly and very darkly. (Fig. 33. a.)

These stages in degeneration are frequently seen in the free cells, and probably some in this stage are englobed by the mononucleated cells.

That it is not necessary for this degeneration to take place before ingestion is evidenced by the fact that the great majority of the ingested cells shew characters that are indistinguishable from the healthy polymorphonuclear leucocytes.

Whether these cells are ingested in a living condition I have not been able to determine. Observations were made on the warm stage continuously for hours at the periods when ingestion was known to be most pronounced, but the process of ingestion was never seen.

After the nucleus has broken up into its several parts there begins an apparent digestion of the chromatin.

The centre of the darkly stained bodies becomes lighter until we get an unstained centre and a ring of stained chromatin. (Fig. 33. b.)

The digestive process still extends outwards and in the next stage we get a faintly stained ring which seems to be simply the nuclear membrane.

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This membrane now breaks up into small fragments and we get the nucleus at this stage represented merely by a few faintly stained granules. (Fig. 33. c)

Eventually these granules also disappear. (d)

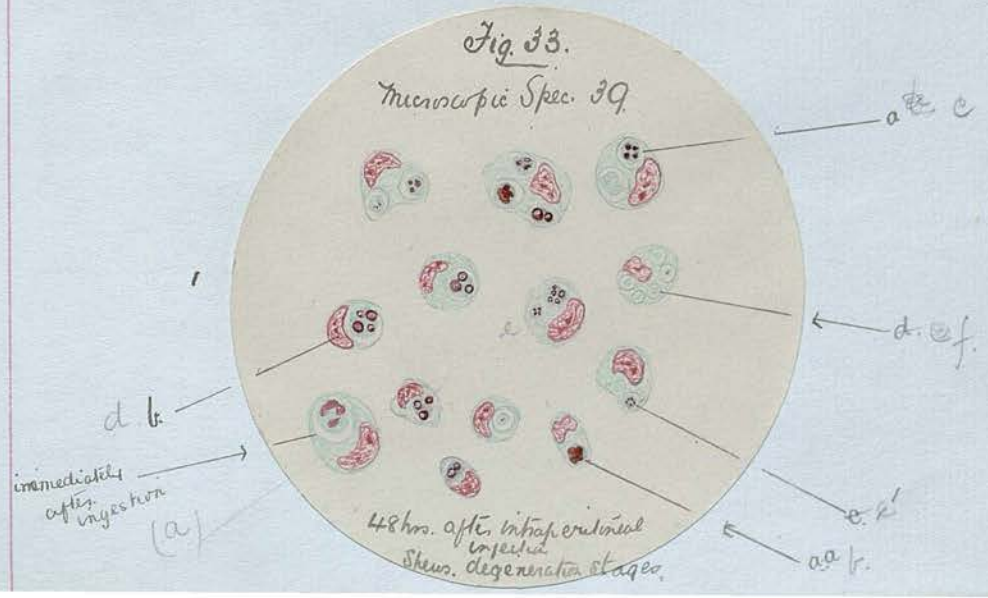
(Figs. VII & 33.)

The protoplasm of the cell does not shew very definite changes. It gradually becomes smaller in amount and stains more faintly until we see mere fragments of it in the phagocytic cell.

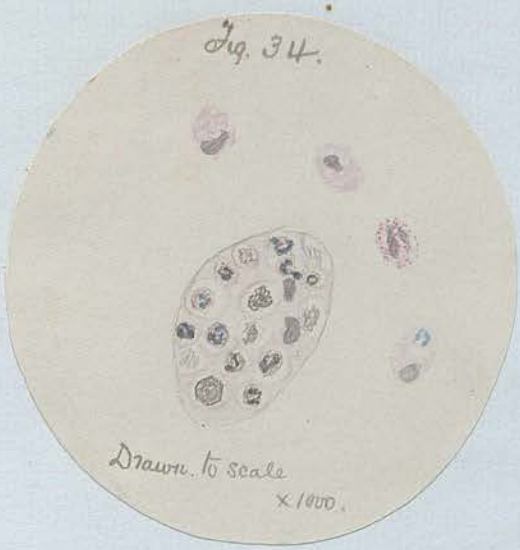
Eventually all trace of the ingested cell disappears. The vacuole in which the ingestion took place may remain, though very much reduced in size.

This, I believe, also may disappear and the cell return to at any rate a fairly normal condition - a condition in which it is able to ingest and to digest other cells.

This digestion of cells can however, only go on for a limited time, for the life of the phagocytes, judging by the great numbers of degenerated forms one sees, is a very short one.



Cells in all stages of digestion may be seen in these phagocytes shewing that the ingestion of one or even several of these cells does not destroy their life or their functioning power. (Fig. 34).



Do these cells secrete a ferment?

Metchnikoff (31.) referring to the vacuolation of these cells regarded it not as a degeneration, a view taken by Councilman, (4.) Krompecher (24.) and others, but rather as an abundant secretion of digestive fluid analagous to that observed in the Protozoa whilst intracellular digestion is going on.

Justi, (20.) as already noted, thinks that the cells may be special carriers of some substance either for nutrition/

nutrition of other cells, or some noxious substance which they are carrying away or destroying.

Ranvier (43) in a recent paper on "The Clasmatocytes" in the Triton says these are like ordinary leucocytes but much larger.

The enlargement, he says, is specially by the addition to the protoplasm of a particular substance the chemical nature of which is unknown. This substance is a secretion of the cells which may play a part in the nutrition of the tissues. The processes of these cells can be enlarged to store up the secreted material, or retracted to very delicate protoplasmic processes.

This is in accord with a former view of Ranvier that the leucocytes are unincellular glands.

Clasmatocytes. from. normal omentum. (Guinea Pig) Micros. Spec. 73.

In the normal omentum fixed with Osmic Acid and stained with Methyl Violet 5B according to the directions of Ranvier these clasmatocytes can be demonstrated. The stain, however, is very unsatisfactory as a distinctive stain, and I have not satisfied myself that with other stains I have seen the same cells.

One certainly finds cells with long protoplasmic/

ic processes (Figs. XXX & XXXI). These processes often shew branching, and the protoplasm may be very markedly vacuolated. The appearances, however, presented by the Clasmatocytes fixed and stained by Ranvier's method, are so different from those presented by the above cells, that one must wait for further light before coming to any definite conclusion about them. They do not appear to have any special function apart from the other cells, for as Ranvier points out, they become transformed into leucocytes during the inflammatory reaction.

The cells with branching processes which have been referred to, are, I am convinced, either endothelial cells or cells derived from the large mononucleated leucocytes in a condition of active amoeboid movement and possibly also special functioning activity.

The multiple vacuolation of these phagocytic cells whether derived from the endothelial or from the mononucleated leucocytes is quite apparent.

It is present in the cells in the peritoneal fluid taken from healthy animals. It becomes much more marked at the period when the activity of the cell is most pronounced e.g. about 36 to 48 hours after an injection into the peritoneal cavity. (Fig. VII & VIII)

It/

It is seen also in the cells in the sinuses of the spleen in typhoid fever, and in the lepra cells in the splenic sinuses.

At the later periods, when the animal has practically recovered - at the period when active phagocytosis has ceased, the vacuolation becomes less pronounced in the cells. (Compare Figs. XI & XII).

It is not uncommon to find some of these mononucleated cells in which the protoplasm stains very darkly, even more darkly than the nucleus and in which the vacuolation is not seen. This suggests the accumulation of some substance in the protoplasm of the cell, and probably a secretion of the cell.

When the cell commences to function actively this secretion is discharged either as a nutritive agent or as some special ferment or possibly antitoxic body. The cell now becomes clearer and its vacuolation more marked. (Fig. 22.)

The fact that very often this vacuolation of the protoplasm precedes degenerative changes in the cell is not evidence against the view that the vacuolation may be the result of a secreting function of the cell.

We/

We have seen that the cells have only a short life. Charged with their secretion they migrate from their original position, carry out their work, and now cut off as it were from their supplies they probably die.

Further evidence of the production of some ferment or other body by the cell is given by the method of gradual digestion of the ingested cells.

The gradual dissolving away of the chromatin of the nucleus, followed by the gradual disappearance of the protoplasm in a clear space in the cell suggests very strongly the pouring out of some digestive ferment round the ingested cell.

If we are to accept this view of Metchnikoff (31) and it seems to me every thing points in that direction , we must regard these mononucleated phagocytes as the most important of the cells of inflammatory exudations.

The possibility of these cells playing an important part in the early stages of infection by the production of a substance which injuriously affects the bacteria has been pointed out.

In the healthy peritoneal fluid and in the later stages of infection where recovery is taking place, they/

they are almost the only cells present.

In all of my experiments where the animal recovered from the infection these mononucleated cells became greatly increased as compared with the polymorphonuclear leucocytes from about 30 hours onwards. In fatal cases the polymorphonuclear leucocytes were always in excess. This agrees with Pratt's observations in cases of Pneumonia. In the case of Acute Pleurisy examined and in a case of Pneumonia with some effusion into the pleural cavity the cells in the fluid were mostly of the mononucleated type. (Figs. XI & XII). They were well stained and shewed an active phagocytosis. Vacuolation was well marked in the fluid from the more recent pleural accumulation, and less marked at a later stage. Both patients recovered.

The presence, therefore, of an excess of these mononucleated cells, especially if actively functioning as shewn by the vacuolation and phagocytosis, in an early inflammatory effusion must be regarded as a favourable sign.

The Destiny of these Mononucleated Phagocytes.

A great many of them are destroyed in the peritoneal cavity. Not only is this the case with those that have ingested other cells, but also with the cells which shew no evidence of having acted as phagocytes.

This degeneration is abundantly seen not merely in the fluid withdrawn from the peritoneal sac, but also in the omentum, in the spleen and in the mesenteric glands.

The stages in the degeneration are apparently not uniform. Sometimes the only evidence we have is a gradual loss of the staining reaction of the nucleus, and a final disappearance of the nucleus.

In other cases we see the chromatin of the nucleus broken up into a great number of minute fragments.

The central fragments are the first to disappear, and we have left rounded or irregular masses of protoplasm which usually shew multiple vacuolation and an irregular staining of the protoplasm.

Are any of these cells carried to and deposited in various viscera?

Metchnikoff (30) found that after the injection of the blood of the goose into the peritoneal sac of Guinea pigs the mononucleated cells which ingested these carried them to the spleen, the liver and the lymphatic glands.

Muir (38) found no constant changes in the spleen after intraperitoneal injections. In one case the spleen was remarkable for the presence in it of large cells containing fragments of disintegrated red blood corpuscles and leucocytes.

During my investigations I have examined the spleen in a number of cases at different periods after injections of B. Coli, of Vermilion and Carmine particles, and of peptonate of iron. With the latter there was also injected a fairly large dose of the B. Coli.

In not a single case except the spleen of the animal injected with Peptonate of Iron and B. Coli have I detected any changes in the spleen except a slight amount of congestion.

In the spleen of the animal injected with Peptonate of Iron and B. Coli, there was a considerable number of the large mononucleated cells in the sinuses. Some of these shewed phagocytosis

to/

to other cells. The cells lining the sinuses showed similar changes to those described as seen in Typhoid Fever and in Leprosy.

A few but very few of the cells gave any iron reaction with Ferrocyanide of Potassium and Hydrochloric Acid.

The animal died of Septicaemia 2 days after the injection was given. Organisms were found in the blood, but unfortunately, several hours had elapsed after death before the animal was seen.

I have not the least doubt that the organisms were circulating in the blood, and that the presence of the phagocytic cells in the sinuses was due to an active proliferation of the cells of the sinuses, and not to cells carried to the spleen from the peritoneal cavity.

The liver, kidneys and lungs were examined in a few cases, but no trace of these phagocytes of the peritoneal sac was found in any of these organs.

Examination of the glands of the abdomen and especially of the mesenteric glands shewedconsiderable changes in some cases but these changes are not constant.

The/

The changes are well seen 3 to 4 days after an intraperitoneal injection. (Figs. XXXV & XXXVI).

We find a large number of mononucleated cells with englobed leucocytes. These polymorphonuclear leucocytes are very markedly degenerated. At parts of the gland we cannot make out any definite cells. It seems to be made up of a mass of broken down and degenerated polymorphonuclear leucocytes and mononucleated cells. At the periphery of these areas however, the mononucleated cells with their ingested leucocytes are well made out. Where foreign particles are injected into the peritoneal cavity these particles are found in these large mononucleated cells.

In the animal already referred to where Peptonate of Iron was injected no changes were seen in the mesenteric glands, but these large phagocytic cells, many of them containing free iron, were found in the glands in the hilum of the spleen.

The great number of the cells present and the marked degeneration of the included cells suggest that the cells are those of the peritoneal sac - that the cells have passed or have been carried to these glands.

This may be so, but we must consider that the glands are really in the inflamed area, and one is not justified in drawing the conclusion that the cells are not produced in the glands. We see abundant evidence that/

that a proliferation of the cells of the Reticulum of the gland takes place, and this may quite well account for all the phenomena one sees in these glands.

As the inflammation subsides the cells found are mainly the mononucleated and usually the medium sized ones. The vacuolation of the protoplasm is less marked, and very little phagocytosis is seen.

On the question of whether these cells may settle down again and give rise to endothelial or connective tissue my present research has not yielded any definite information. I had hoped in my work with the tubercle bacilli to have got some information on this subject, as well as on the formation of giant cells and the part they play.

What I have done on the subject does not justify me in making any definite statements, and therefore, for the present, I think it better to leave the subject practically untouched.

GENERAL CONCLUSIONS.

After an injection of bacteria into the abdominal cavity various cells appear in the exudation.

In the early stages the polymorphonuclear leucocytes migrate from the vessels, and appear in great numbers in the peritoneal effusion.

These are found abundantly from 6 to 48 or 54 hours after the injection.

In non-fatal cases, they diminish from 48 to 60 hours, but in fatal cases the increase persists till the death of the animal.

These leucocytes are the main bacterial phagocytes. The bacilli of Tuberculosis and of Leprosy seem to be exceptional, but this is probably explained by their slower growth, and consequently a less marked and later polymorphonuclear leucocytosis.

During/

During the time they are in excess there appears to be produced a substance injurious to the life or at any rate to the activity of the bacilli, but it is doubtful whether this is produced by these leucocytes or by the mononucleated cells which are also in a state of great activity.

The activity of the bacteria seems to be impaired before they are ingested by the leucocytes.

These leucocytes are largely destroyed in the fluid either as free cells or in the interior of other cells.

The coarsely granular eosinophiles, which are very numerous in the peritoneal fluid in healthy Guinea Pigs, play no important part, at any rate, in relation to the bacteria used during my investigations.

The mononucleated phagocytes are found at all stages. They are most abundant from 36 hours onwards.

The/

The cells are derived partly from the endothelium of serous membranes, of blood vessels, of lymph vessels, of lymph sinuses etc., and partly from the large mononucleated leucocytes, probably also from the lymphoid tissue round the vessels.

They are amoeboid and shew especially during their stages of greatest activity a marked multiple vacuolation of their protoplasm.

The vacuolation of the protoplasm is probably evidence of secreting action on the part of these cells, and not merely a degenerative change.

These cells are specially phagocytic to other cells. They may also ingest bacteria, and certain forms apparently more readily than others. The bacilli of Tuberculosis and of Leprosy are found in great numbers in these cells, and they appear to actually multiply in the cells.

The ingested leucocytes undergo a gradual process of digestion by these mononucleated cells.

These/

These cells are largely destroyed in the peritoneal sac. None of the internal organs, so far as I have been able to ascertain, appear to act as storehouses for or destroyers of these cells with the exception possibly of the lymph glands.

Great numbers of the cells are always found on the omentum.

These mononucleated phagocytes are the most important cells of inflammatory exudations. In cases of peritonitis the endothelium of the omentum furnished a great number of these cells.

The omentum must therefore, be regarded as an important agent in protecting the individual from infection by way of the peritoneum.

The presence of large numbers of these mononucleated cells, if they are actively functioning, in inflammatory exudations must be regarded as a favourable sign.

The Plasma cells, which Mallory suggests may in Typhoid/

Typhoid fever produce the antitoxins, are, I believe, the mononucleated cells which are derived from the large mononucleated leucocytes. They can and do act as phagocytes, but in the later stages of infection this phagocytic action is not seen, simply because there is no need for them to function in th this way.

They are not confined to any special form of inflammation, but are most numerous in cases of bacterial or of toxic infection.

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