Studies in absorption / B.H. Buxton and J.C. Torrey.

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STUDIES IN ABSORPTION

(6)

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STUDIES IN ABSORPTION.

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(*From the Laboratory of Experimental Pathology, Cornell University Medical College.*)

ABSORPTION FROM THE PERITONEAL CAVITY.*

PART I. — *Absorption of particles in suspension.*

B. H. BUXTON AND J. C. TORREY.

The present communication is the first of a series of papers, all of which deal with the subject of the absorption of substances in suspension from the peritoneal cavity. The ultimate object of the investigation concerns the method by which the organism disposes of bacteria injected into the peritoneum; and each of the papers is so intimately connected with the others that no one can be considered complete without the other four. The five papers may, therefore, be regarded as one complete study, and references in one paper may relate to topics discussed in later portions of the study.

The papers are:—

I. Absorption of inert particles.
II. Absorption of bacteria (typhoid).
III. The paths of absorption. Function of the diaphragm.
IV. The paths of absorption. Function of the omentum (inert particles).
V. The paths of absorption. Function of the omentum (typhoid bacilli).

I. ABSORPTION OF INERT PARTICLES. — In the present communication we shall confine ourselves to the question of absorption from the peritoneal cavity of guinea-pigs of insoluble particles, such as lampblack. The subject matter may be divided into two parts: *A.* Examination of the peritoneal fluid; *B.* Examination of the organs.

We may say briefly in advance:

1. Some of the particles remain in the peritoneal cavity and are there taken up first by the polynuclear leucocytes and later by the mononuclear macrophages.

2. Some of the particles leave the peritoneal cavity and

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very rapidly reach the organs where they may be found in immense numbers.

A. The Peritoneal Fluid. — The French authors, with Metchnikoff at their head, tell us that if fluid is withdrawn from the peritoneal cavity of a normal animal — guinea-pigs are generally used — the fluid contains a considerable number both of lymphocytes and polynuclear leucocytes. Kanthack and Hardy further divide the former into the small lymphocytes with deeply-staining nuclei and scarcely any cytoplasm, and the larger mononuclears with vesicular, pale-staining nuclei and abundant non-granular homogeneous cytoplasm — their "hyaline cells." Kanthack and Hardy are also of the opinion that the polynuclear leucocytes of the fluid are all eosinophiles. They speak of the eosinophiles as cells of the body cavities as opposed to the ordinary polynuclears, which are distinctively cells of the blood under normal conditions.

Shortly — in a few minutes — after injection of particles in suspension into the peritoneal cavity (and the same applies to fluids of any kind — serum, bouillon, salt solution, etc.) the polynuclear leucocytes and hyaline cells almost entirely disappear from the fluid, although the number of small lymphocytes is hardly, if at all, affected, and the period of hypoleucocytosis or leucopenia sets in.

Metchnikoff ascribes the leucopenia to destruction of the cells and uses the term "phagolysis" to express it. Pierallini maintains that after injection of particles or fluids large numbers of the leucocytes are deposited on the omentum, many of them showing signs of degeneration. At the same time masses of fibrin are also found on the omentum; clear evidence that considerable numbers of leucocytes have been destroyed. Pierallini considers that there is certainly a partial destruction of leucocytes during the period of leucopenia, and most of the French authors take this view.

It may be mentioned here, in anticipation of what we shall have to describe more fully later on, that we also have frequently found deposits of fibrin on the surface of the

omentum after intraperitoneal injections, but some recent discussions, reported in the "Comptes rendues, *Société de Biologie*, 1903," seem to throw some doubt on the accepted view that fibrin ferment is liberated on the destruction of leucocytes. Arthus, in particular, expressed himself as very strongly of the opinion that the fibrin ferment is an excretion of leucocytes excited to special activity, and is a product of the living cell only. Dastre, Stassano, and others agreed with him in the main. However this may be, it seems in view of these opinions hardly safe to assume that because there is deposition of fibrin on the omentum, therefore there has been phagolysis.

That there is phagolysis at all has been strenuously denied by some. Durham convinced himself that the leucopenia is due solely to clumping or balling of the leucocytes, in which condition they are swept by peristalsis towards the omentum and other peritoneal surfaces on which they are deposited. Consequently they are not brought up by the capillary pipette used for withdrawing the fluid from the peritoneal cavity, and from an examination of the fluid alone one would be led to suppose that the leucocytes had been destroyed. Lambotte and Stiennon maintain that leucocytes are by no means so fragile as is usually supposed, and refuse to believe in the doctrine of phagolysis.

The period of leucopenia lasts from one to three or four hours, varying with the reacting power of the animal and the nature of the substance injected, after which fresh polynuclear cells make their appearance in large numbers and actively take up the particles. The polynuclears now arriving are the ordinary polynuclears of the circulation with finely granular cytoplasm. Ehrlich's description of their granules as "amphophile" or "neutrophile" has been objected to by Kanthack and Hardy, who maintain that the granules are distinctly acidophile, staining readily with eosin, and Durham has proposed to call the cells "microxycytes," a term which seems to us legitimate, and which we shall make use of in these articles.

The microxycytes can be readily distinguished from the

true eosinophile cells, in which the granules are much larger and stain still more strongly with eosin. Durham calls these "megoxycytes."

Our own experiments have been made principally on guinea-pigs with intraperitoneal injections of lampblack. The ordinary moist water color is emulsified in salt solution and the suspension when examined under a high power of the microscope is seen to consist of minute particles with some coarser lumps which can be filtered off. The filtrate is sterilized and used for injection.

We have endeavored always to make up the suspensions to as nearly as possible the same density and have used three cubic centimeters for each injection. From time to time drops of fluid were withdrawn from the peritoneal cavity by means of a capillary pipette and examined both in hanging drops and smears stained with eosinate of methylene blue.

The appearances after such injections have so often been described in detail that we need only say in a general way that we have always found the period of leucopenia well marked, but the question as to whether there is phagolysis or not will be reserved for discussion in a later section.

For about an hour or two there is little evidence of phagocytosis in the fluid, although the few polynuclears which can be found usually contain pigment granules; but in from two to six hours phagocytosis becomes very and increasingly marked, the newly arrived polynuclear leucocytes (microxycytes) taking up the particles very actively, whilst here and there a macrophage can be seen, though as yet not often in active commission (Plate I., 1).

In twenty-four hours the large mononuclear macrophages are present in great numbers, many of them having englobed one or more of the microxycytes with their contents (Plate I., 2).

It is not easy to determine from an examination of the fluid if the macrophages take up pigment directly or only englobe the phagocytes containing the particles. In many of the macrophages may be seen intact polynuclears, whose

cytoplasm is filled with pigment granules, and from these on, all stages of degeneration appear, until the polynuclear cells are dissolved entirely, leaving only the pigment granules scattered about inside the macrophage (Plate I., 3).

However, in earlier stages, about four to six hours, while the macrophages are still few in number, and before they appear to have begun to ingest polynuclears, a certain proportion of them contain pigment granules, so it seems probable that they englobe particles directly as well as indirectly by means of the polynuclears. This observation is confirmed by what we have noticed on the surface of the omentum, as will be described later.

Phagocytosis of animal cells. — Metchnikoff has attempted to show that the chief function of the polynuclears or "microphages" is to take up bacteria and inert particles, whilst for animal cells they show negative chemiotaxis, remaining inactive; the macrophages doing all the work of phagocytosis when foreign animal cells or fragments of cells are introduced into the peritoneal cavity. In some experiments with chicken blood corpuscles we have not found this to be altogether the case. It is true the microxycytes do not actually englobe the chicken corpuscles, except in isolated instances, but this appears to be due simply to the mechanical difficulty of ingesting such large objects. The corpuscles appear to allow of very active positive chemiotaxis, for in one or two hours after injection, before the macrophages have begun to come in to any extent and while the microxycytes themselves are still scanty, each microxycyte that is seen appears to form a center around which a group of corpuscles is formed. Moreover, the cytoplasm of that part of any corpuscle actually in contact with a microxycyte often appears eroded, as if the polynuclear is partially digesting it. Again, the nuclei of chicken corpuscles may often be found in the microxycytes, so that if the corpuscles are reduced in size by loss of their cytoplasm they can be ingested. (Plate I., 4 shows a typical appearance one hour after injection.)

As soon as the macrophages appear they become active

at once and the few which can be found between two and six hours after injection are already stuffed full of chicken corpuscles in various stages of degeneration. The size of the chicken red cells is no hindrance to the phagocytic action of the macrophages, which can distend themselves in a most extraordinary way and take up a dozen or so of corpuscles at a time, although when once taken up, the englobed corpuscles rapidly degenerate and shrink to a fraction of their former size (Plate I., 5). The macrophages certainly appear to attack the chicken corpuscles with much more energy than they attack bacteria or inert particles, and this is readily understood since their natural function is to englobe and carry off worn-out leucocytes or red cells and probably other cells of the organism to which they belong, so that they would be expected to have more affinity for animal cells of any kind than for particles of an altogether different nature. Still, although we can agree with Metchnikoff and other French authors that the macrophages play the chief part in disposing of animal cells, we cannot admit that the microxycytes are an unimportant factor in the methods adopted by the organism for disposing of extraneous particles of animal matter.

B. The Organs. — The second section of this article is concerned with an examination of the organs of the guinea-pigs which have received an injection of a suspension of lampblack particles into the peritoneal cavity according to the method indicated on page 8. The object has been to determine principally how quickly the particles reach the organs, in what amount, and for how long a period they may remain in the tissues.

After injection the animals were killed at various intervals. Some of the organs were then removed, sectioned by the celloidin method and stained lightly with hematoxylin and eosin. The sections were always cut to the same thickness (fifteen microns); and in their examination only those particles which could positively be identified as lampblack were counted. The enumeration, too, was confined to those particles which were manifestly in the interior of the section.

On this account the sections were made of sufficient thickness, so that extraneous particles of dirt lying on either surface might be excluded.

Early in the investigation it was decided to confine the observations to the liver and spleen. At first the lung and kidney were also included. An examination, however, of sections of the normal lung revealed a large number of cells in the alveoli, which contained particles that could not be distinguished from lampblack. These cells have been called by the Germans "staubzellen." In the latter part of this section their function is described. The kidney was finally excluded because it was found after many examinations that exceedingly few particles find their way to this organ. In sections of kidneys from guinea-pigs, killed twenty-four hours after the injection of lampblack, a few particles were occasionally found in the plexus of capillaries within the Malpighian bodies, but at no other place in the organ. These particles were evidently being carried along in the blood stream and were not deposited in the tissue, as is the case in the spleen and liver.

Muscatello, in a similar study, used a suspension of carmine particles in preference to lampblack, for the reason that he found black pigment particles normally present in the lung, lymph nodes, liver, spleen, and kidney. This objection to the use of lampblack certainly holds good for the lung, but an examination of sections of the other organs from normal guinea-pigs has convinced us that these pigment particles occur so infrequently that, for the purposes of our investigation, the possibility of their presence or absence may be disregarded.

In the following table a count is given of the number of aggregations of particles found in twenty fields of a Leitz microscope, using a number 7 objective, a number 3 ocular, and the tube drawn out to one hundred and sixty millimeters. As will be seen, the intervals of time at which the guinea-pigs were killed, after the injection of the lampblack suspension, range from five minutes to twenty-four days. It will also be observed that the first series of pigs was controlled by a second.

TABLE I.

Aggregations of lampblack particles in the liver and spleen.

Time.	Series.	Liver.	Spleen.	Remarks.
5 minutes.	1	0	2	Practically no particles have reached the organs.
15 minutes.	1	9	13	Particles free, scattered. In the liver free particles in the larger veins.
30 minutes.	1	4	0	Fewer particles than in 15 minutes. None to be found in the spleen.
	2	2	0	
1 hour.	1	0	0	A few scattered particles in the larger veins.
	2	0	0	
2 hours.	1	8	36	Granules for the most part free, a few inside leucocytes.
	2	30	21	
4 hours.	1	144	141	The great majority of the black granules inside leucocytes.
	2	154	101	
6 hours.	1	22	57	In both the liver and spleen there is marked drop in the number of particles from that of 4 hours. The aggregations of particles also appear smaller at this stage.
	2	43	45	
24 hours.	1	68	1,000	The spleen is crowded with large aggregations of black particles. A great many macrophages filled with granules in the pulp. The bile ducts of the liver contain a few particles.
	2	183	1,300	
48 hours.	1	66	205	A decrease in the number of particles, similar to that found in 6 hours.
	2	40	164	
3 days.	1	101	690	The black granules have increased in number, especially in the spleen.
4 days.	1	117	800	The conditions are very similar to those at the end of 24 hours. The spleen is again crowded. Some of the granules are free, but the great majority inside cells.
	2	260	1,200	
8 days.	1	50	215	Fewer particles in both the liver and spleen, otherwise about the same as 4 days.
	2	53	456	
16 days.	1	27	60	A further decrease in the number of particles.
24 days.	1	30	142	The spleen of the second series is crowded.
	2	126	2,000	

An examination of the table shows that until two hours after the injection of the lampblack suspension into the peritoneal cavity, the black particles are either entirely lacking in the organs or very few in number.

In the sections of the liver and spleen of the animal killed after five minutes there are practically no particles to be found in the tissues, nor in the veins and lymph spaces is there more than a suggestion of their approach. After fifteen minutes, however, there is no doubt of the presence of free aggregations of lampblack granules in the capillaries of the liver and spleen. In some of the medium-sized veins, too, a large number of free particles may be found. In no instance had they been ingested by leucocytes.

Turning now to the sections from pigs killed in thirty minutes, we find that no particles are to be found in the spleen and very few in the liver. In the larger blood vessels there are also fewer particles than in fifteen minutes. This is still more strikingly the case in one hour. It is, in fact, only after two hours that the lampblack particles can be found in any abundance in the liver and spleen. At this time the black granules are for the most part free, although here and there a leucocyte may be seen which contains a few. This observation agrees in the main with that of Muscatello. He injected a suspension of carmine particles into the peritoneal cavity of dogs and found that they appear in the liver and spleen after an interval of one and a half to two hours, partly free and partly within the leucocytes of the capillaries and venous sinuses. He was not able, however, to discover the presence of the carmine particles in these organs in twenty minutes to one hour after injection. In our sections of the fifteen-minute stage there is no doubt that the lampblack has reached the liver and spleen, but as yet in very small amount. The particles, too, are lying in a free state within the lumen of the capillaries and larger blood vessels.

As will be observed in the table, after four hours the number of the particles has increased considerably. They are apparently somewhat more numerous in the liver than in the spleen. Some of the granules are still free and scattered in

the capillaries, but the great majority have been taken up by leucocytes. In some instances the particles seem to be also inside the liver cells, although it is difficult to be sure of this on account of the thickness of the sections.

As regards the sections from the animals killed after six hours we find a marked decrease in the number and size of the aggregations of particles in the liver. Since our conclusions are based on the results of an examination of only two guinea-pigs at each stage, much stress cannot be laid on this circumstance, for which there is no obvious explanation, but attention may well be called at this point to the remarkable uniformity of the increase and decrease of the number of granules at the various stages in both the series. This seems to be more than an accidental coincidence.

In twenty-four hours the number of granules has increased to some extent in the liver, but enormously in the spleen. This organ is crowded with large aggregations of black particles. The vast majority of these are in the pulp, contained within the macrophages, either in the lymph spaces, the capillaries or the veins. Here and there in the follicles, however, a few particles may be found, generally enclosed in cells. In the liver we find by far the greater number of particles inside wandering cells. Only a few are free, or inside the hepatic cells and the endothelial cells of the larger blood vessels. Some of the black granules, furthermore, may be seen either inside the lumen of the bile ducts or between the duct cells. This observation is of interest in the light of the suggestion of Siebel, that the bile ducts are a possible path of excretion of injected particles.

The most striking feature in the examination of the forty-eight-hour sections is the marked decrease of the number of black granules in both the liver and spleen. This is especially the case in the latter organ. The next stage of four days presents a condition which is practically identical with that at the end of twenty-four hours. The spleen is again crowded, and the aggregation of particles are large and free or contained within cells, probably macrophages for the most part.

From eight days to two months in the first series there is a decrease in the number of granules in the liver, and also to some extent in the spleen. In the second series the spleen was found to be crowded at the end of twenty-four days. This may be accounted for on the ground that this particular pig received a somewhat larger injection of lampblack suspension than the others. In all the later pigs the masses of granules are much larger than in the earlier ones, and the great majority are contained within large wandering cells, the macrophages, although here and there free granules may be found. It will also be observed that the sections of the spleen are far more crowded than those of the liver.

The decided and uniform decrease in the number of the black granules at the end of six hours and again in forty-eight hours is noteworthy. There has apparently been an excretion of the foreign particles at these periods, but by what pathway this has occurred we have not attempted to determine. It should also be borne in mind, however, that particles are continually being transported from the peritoneal cavity to the organs, and the amount of this influx may vary with the different periods.

The question of the fate of particles injected into animals has been the subject of many investigations. As long ago as 1867 Hoffmann and von Recklinghausen injected carmine and vermilion into the circulation. They found that these particles are soon deposited in the various organs, such as the spleen, lymp nodes, and the marrow, thus quickly disappearing from the blood and lymph. Instead of disappearing rapidly from the organs, as is the case with bacteria, these particles remained in the interstitial tissue of the glands for weeks, and were not found to be eliminated by the urine or bile. Similar observations have been made by Ponfick.

Siebel confirmed these observations and discussed the manner by which the particles might ultimately be disposed of, concluding that the principal channels of excretion are the lung, liver, and tonsils, and possibly the follicular structures of the small intestines. In the lung, the particles are taken up by the leucocytes and these in turn wander into the

alveoli, from whence they are thrown off by the sputum. A similar process is carried out under normal circumstances by the "staubzellen" of the Germans. These are wandering cells in the alveoli, which, after picking up particles of dust deposited in the lungs, are carried out of the body in the sputum. In the liver some of the particles, according to Siebel, are carried away by leucocytes and leave the body by way of the tonsils and possibly also through other surface follicular structures, such as the lymphoid patches of the small intestine. On the other hand, other leucocytes containing particles wander out from the capillaries into the surrounding tissue and become connective tissue cells. Thus the particles may remain in the organism for life. Finally, he observed that some particles did not appear to be taken up by the leucocytes at all, but gradually made their way into the bile ducts and were excreted through the gall bladder.

In the experiments which have been mentioned above, the foreign matter was introduced into the circulation. Other investigators have studied the fate of particles injected into the peritoneal cavity. Such were the experiments of Muscatello which are also very similar to our own. As has been stated, he injected a suspension of carmine particles into the peritoneal cavity of dogs and found that the granules quickly absorbed and in one and a half to two hours appeared in the liver and spleen either free or within leucocytes in the blood vessels. As has already been indicated, this is quite in accordance with our own observations. After six hours, according to Muscatello, many particles have appeared in the liver and also in the venous spaces of the spleen. In smaller amount the granules are also found in the lung, the pancreas, and the testicle. Maffucci, after the same period of time, has found the particles only in the liver and the spleen. He concludes that the position of the granules was the same in the liver, whether the injection had been made into the peritoneum or the jugular vein. When the particles are introduced into the circulation they reach the organs with great rapidity. Werigo, after injecting carmine into the ear vein of rabbits found the particles in the

liver and spleen in two to four minutes. The end result, however, is the same whether the injection be made intraperitoneally or intravenously. The difference is merely one of time.

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ABSORPTION FROM THE PERITONEAL CAVITY.*

PART II. — *Absorption of typhoid bacilli.*

B. H. BUXTON.

It was shown in the first of this series of papers that particles of lampblack injected into the peritoneal cavity of guinea-pigs soon find their way to the organs, more particularly the liver and spleen, where they may occur in large quantities. This line of investigation has been followed up with typhoid bacilli, with results analogous to those obtained with lampblack. The bacilli very rapidly reach the organs, where they appear to be deposited in such a way that they are not washed out with the blood on bleeding the animal to death.

Similar experiments have often been carried out after intravenous inoculations, but records of what occurs after injection into the peritoneal cavity are scanty, and there has apparently been no attempt at systematic investigation.

The most important studies which have been made on the fate of bacteria after injection into the circulation are probably those of Werigo and Bail.

Werigo[1] injected anthrax bacilli into the jugular vein of rabbits and killed the animals at varying intervals of time after inoculation. By sectioning the organs and counting the bacilli in the sections, he was able to demonstrate that the bacilli were very quickly deposited in the liver, spleen, and lungs. Even in a few minutes after injection the bacilli had largely disappeared from the circulation, being held in the capillaries of the various organs, where they are already contained in the phagocytes. Other investigators have confirmed his observations, but some maintain that during the early stages the bacilli lie free in the capillaries for the most part, being only taken up by the leucocytes later on.

More recently Bail[2] has shown that typhoid and cholera bacilli after intravenous injection into rabbits and guinea-pigs

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undergo a similar fate. In a few minutes they are deposited in the liver, spleen, and marrow, from which they cannot be washed away on bleeding the animal to death. The bacilli may still be found in the organs for several days after inoculation. Bail in some cases also injected into the pleural cavity and from this point the bacilli very quickly found their way into the organs. Bail's methods differed from those of Werigo in that he took portions of the organs and plated them out, counting the colonies on the plates after twenty-four hours' growth, at 37° C.

Since typhoid bacilli do not hold Gram's stain, it is not easy to demonstrate them in sections, so Bail's method of plating out portions of the organs was adopted for the experiments detailed here. An attempt was also made to determine the bactericidal action of the peritoneal fluid itself. For this the usual practice of withdrawing small quantities of fluid from time to time after injection, by means of a capillary pipette, and determining whether the bacilli are dead or not by staining reactions or plating out a few drops, seems open to some objections, and it was considered advisable to attempt the withdrawal of the fluid on a large scale by washing out the peritoneal cavity.

Normal rabbits were used for the experiments, the dose being invariably one-half of an agar culture. The strain of typhoid used was of very moderate virulence, the dose being about the largest which could be used with safety, the object naturally being to inject as many bacilli as possible, yet at the same time to keep below the minimum lethal dose. Briefly the method of procedure for each rabbit was as follows: — A twenty-hour agar culture was emulsified in ten cubic centimeters of salt solution, and one-half or five cubic centimeters injected into the peritoneal cavity. After a stated time — one, two, four or more hours — the rabbit was etherized and bled from the carotid, some of the blood being defibrinated, and some allowed to clot for serum. After bleeding, and just as the rabbit was at the point of death, four hundred cubic centimeters of salt solution were run into the peritoneal cavity and immediately withdrawn again. This

was called "wash water" and was used for plating out. Immediately after washing out, the abdomen was opened and portions of various organs taken out and plated. Having thus given a general survey, it will be necessary to enter more into details in order to understand exactly how the experiments were carried out.

1. The washing out. — As soon as the bleeding is finished the rabbit is covered with a sterile cloth and tied tightly down to the operating table by two broad bands passed over the chest and flanks. Through a slit in the cloth a cut is made in the skin of the abdomen and the tissue cleared down to the muscle. A small slit is now made in the muscle and a glass trochar pushed through the slit into the cavity; the trochar being attached to a rubber hose connected with a flask on a shelf above, containing four hundred cubic centimeters of salt solution. The salt solution is siphoned into the cavity, an operation which only takes about a minute, the rubber hose detached from the trochar, and the operating table, specially designed for this purpose, turned over so that the rabbit is now abdomen downwards, suspended by the broad bands. Below is a flask into which the contents of the peritoneal cavity are run through the trochar. It takes three or four minutes to empty the cavity, and about three hundred and fifty cubic centimeters can be recovered out of the four hundred run in. The wash water is at once put on ice to prevent any increase of the bacilli and returned to later on.

2. The organs. — The operating table is now turned back so that the animal lies in its original position. The abdomen is opened, and if there is any urine in the bladder its surface is seared and one cubic centimeter pipetted out. The organs, or suitable pieces of them, are then taken out, but since they have been bathed in a fluid which may contain a large number of bacilli it is necessary to sterilize the surface. For this purpose, the pieces as they are taken out are plunged for a moment into boiling water and at once put into a cold chamber. Repeated tests have shown that

this is sufficient completely to sterilize the surface, while leaving the bacilli inside the organ unhurt. Of the organs, the spleen, one kidney, a piece of the liver, and a piece of the lung are taken. The bile also is collected by means of a capillary pipette after searing the surface of the gall bladder. About one-quarter to one-half of a cubic centimeter is all that can be obtained as a rule. Finally one of the thigh bones is cut off and put on ice.

3. Preparation of material for plating.—The spleen is taken as a unit, and pieces of the other organs as nearly as possible of the same size. Each piece is dipped once more into boiling water, placed on a square of fine wire gauze (mesh sixty to the inch), and, after cutting up with scissors into small particles, is rubbed with a pestle through the wire gauze into a beaker below, fifteen cubic centimeters of salt solution being gradually poured over the surface (Bail's method). Since the whole operation can be conducted very rapidly the risk of contamination is slight, and has been found by experience to be a negligible quantity. By this method nearly all the organ cells can be washed through, leaving only small particles of connective tissue on the surface of the gauze. The lung, however, can only be partially rubbed through, so that pieces of this about three times the size of the spleen are taken. The content of each beaker is at once emptied into a sterile test-tube and put on ice until all the pulps are ready for plating. The marrow of the thigh bone is scraped out and treated in the same way.

4. Plating out the organs.—As soon as the rubbing down of the organs is completed, a sufficient number of agar tubes, each containing eight cubic centimeters, are melted and cooled to 50° C., in a water bath. By this time the larger particles in the organ pulps have subsided in the test-tubes, and from each tube two cubic centimeters of the supernatant fluid is pipetted out and distributed into two agar tubes, one cubic centimeter in each, for plating. The colonies on each plate, therefore, represent the number of bacilli in one-fifteenth of the spleen, or portion of the other organs. The defibrinated blood, serum, and urine, one cubic centimeter of

each, and the bile are also plated out and the plates all incubated at 37° C., the colonies being always counted after twenty hours.

5. Plating out the wash water. — The flask containing the wash water is well shaken up and one cubic centimeter of the fluid pipetted into nine cubic centimeters of salt solution — dilution of one to ten. Of the dilution of one to ten, one cubic centimeter is pipetted into nine cubic centimeters salt solution — dilution of one to a hundred — and so on up to dilution of one to one million. From each of these dilutions one cubic centimeter is plated out in eight cubic centimeters of agar, and, at the dilutions which are expected to yield the best results for counting, two plates are often made. After twenty hours' incubation the colonies are counted, and an estimation is made of the number of bacilli remaining alive in the peritoneal cavity. As an illustration, we may suppose that at dilution:

1 to 10	colonies	about 1,000
1 to 100	"	121 and 93
1 to 1,000	"	8 and 11
1 to 10,000	"	2 and 0
1 to 100,000	"	0

We may say roughly that we have ten thousand bacilli per cubic centimeter of the wash water, and since four hundred cubic centimeters of salt solution was run in, the total number of bacilli left alive in the cavity may be taken as of the order of $400 \times 10{,}000 = 4{,}000{,}000$. To estimate the number of bacilli which are injected, the remaining five cubic centimeters of the original emulsion — five cubic centimeters have been used for injection — are run into four hundred cubic centimeters of salt solution, from which dilutions are made and the number of bacilli estimated as described for the wash water.

It may be mentioned that agar tubes of the same size, eleven-sixteenths inch in diameter, were always used for the culture to be injected and a very large number of controls invariably gave nearly the same results, namely, four thousand

millions per half culture. It was not necessary, therefore, to repeat the control experiments in every instance. The number of bacilli injected can always be reckoned as of the order of four thousand millions.

Experimental. — In a series of previous experiments[3] it was found that normal rabbit serum "in vitro" would invariably destroy about the same number — one million per cubic centimeter — of typhoid bacilli, and in view of this regularity it was anticipated that results obtained "in vivo" might show a somewhat similar constancy to those "in vitro." It was anticipated, therefore, that by sacrificing two or three animals at each interval of time chosen some general law might be laid down as to the number of bacilli left alive in the peritoneal cavity, and the numbers occurring in the organs at stated periods after inoculation. It very quickly became apparent, however, that no regularity of the nature of that observed "in vitro" was to be expected, so it was decided to sacrifice six animals at each interval of time and then work up the results into tables to see if any general conclusions could be drawn.

To avoid any necessity for referring back to what has already been said, it may be repeated that rabbits only were used, the inoculations being made intraperitoneally, and the dose one-half of an agar culture of typhoid bacilli of very moderate virulence. The number of bacilli inoculated was of the order of four thousand millions.

As a rule, the rabbits suffered little from the inoculations, although there was frequently a drop in temperature with some dyspnea and somnolence between two and four or five hours after injection, after which there was generally complete recovery with a slight rise of temperature over the normal.

Some rabbits, however, died at intervals of from two to twenty-four hours after inoculation. With these there was a decided drop in temperature, which remained low until death ensued; in other words, these rabbits showed no power of reaction. Animals of varying size were used from two and

a half to four and a half pounds, but the majority were three to four pounds in weight. The actual size of the rabbits seems to have little influence on the results. Sometimes a large rabbit would die, and sometimes a small one, although, on the whole, the smaller ones are slightly more susceptible than the larger animals. The tables give in each case the weight of the rabbit; and from a study of them it will become obvious that the irregularities in the results are not due to irregularity in the size of the animals used. The results depend upon the reacting power of the individual rabbits, and this varies so much that it is impossible to say that there is any minimum lethal dose of living typhoid bacilli. Bail came to the same conclusion in the course of his experiments with cholera and typhoid bacilli.

The intervals of time at which the rabbits were killed after inoculation were at first taken at two, four, and six hours, and the accompanying tables show the results of this series of experiments.

Under the heading "wash" of column two in each table is given an estimation of the total number of living bacilli which could be washed out of the peritoneal cavity, and are, therefore, either free or contained within cells free in the fluid. This, however, by no means represents the total number which are actually alive in the cavity. There is usually an extensive deposit of fibrin on the surface of the omentum, and in this fibrin there may be entangled enormous masses of bacilli which show no signs of degeneration on staining, and are obviously alive. There is always, however, a relation between the number of living bacilli fixed on the omentum and those found free in the fluid. It is a remarkable fact, as will be brought out later on, that a microscopical examination of the omentum enables one to judge roughly of the number of bacilli which are likely to be found in the fluid; and on the other hand, according to the number found alive in the fluid one can say with a fair degree of accuracy what will be the microscopical appearances on the omentum. The results under the heading of "wash," therefore, are fairly comparable one with another. If there are few in the

"wash" there will be few, and if there are many in the "wash" there will be many fixed in the omentum. In other words, the condition of the omentum does not influence the relative amounts under "wash" in the different experiments.

The figures under the organ headings give, in round numbers, the colonies on a single plate. In almost every instance two plates were made from each organ and the average taken. The numbers in the two plates vary so little that the second was practically superfluous.

From the figures we can make a rough estimate of the total number alive in each organ. One cubic centimeter of the fifteen cubic centimeters of pulp was plated out, and since the liver is about one hundred, and one kidney about ten times the size of the spleen, the figures can be multiplied by fifteen for the spleen, one hundred and fifty for one kidney, and fifteen hundred for the liver, to arrive at an idea of the order of the total numbers alive in each of these organs.

TABLE I.

Two hours.

	Weight.	Wash.	Liver.	Spleen.	Lung.	Marrow.	Kidney.	1 cc. Blood.	About ¼ cc. Bile.	Temp.
	lbs.									
1.	4	350,000,000	1,050	525	20	25	20	12	0	Constant.
2.	3	8,000,000	1,500	200	1,500	500	20	200	10?	Drop 1½.
3.	3½	800,000	100	15	3	60	0	3	0	
4.	4	550,000	100	50	10	20	2	0	0	Drop 1¼.
5.	4½	40,000	25	15	0	10	1	0	0	Drop 5.
6.	4¼	0	0	0	0	0	0	0	0	Constant.

TABLE II.

Four hours.

	Weight.	Wash.	Liver.	Spleen.	Lung.	Marrow.	Kidney.	1 cc. Blood.	About ½ cc. Bile.	Temp.
1.	3¾	85,000,000	1,000	150	200	250	.....	11	0	Constant.
2.	3½	70,000,000	20,000	2,000	125	2,500	25	125	1,200?	Drop 3.
3.	4½	60,000,000	100	10	5	15	0	0	0	Drop 2.
4.	4½	12,000,000	5,000	3,000	2,500	800	.....	3	2,500?	
5.	3	3,000,000	150	25	50	125	2	25	.....	Rise 1.
6.	3½	4,000	0	0	0	0	0	0	0	Constant.

TABLE III.

Six hours.

	Weight.	Wash.	Liver.	Spleen.	Lung.	Marrow.	Kidney.	Blood.	Bile.	Temp.
1.	3¾	80,000,000	15,000	1,000	700	700	10	200	65	Drop 3.
2.	4	65,000,000	25,000	200	500	250	.....	1	0	
3.	4½	65,000,000	1,000	0?	.....	100	0			
4.	3¾	10,000,000	6,000	4,000	450	200	5	60	1?	Drop 2.
5.	3½	450,000	75	450	35	150	0	0	0	Drop 2 and rise 2.
6.	3½	0	0	0	0	0	0	0	0	Constant.

A glance at the first three tables shows at once the striking irregularity which has already been commented upon, and it is obviously impossible to say that, at a given period of time after inoculation, a normal rabbit will have disposed of so many bacilli, and that there will be about so many left alive. It is evident, however, that the bacilli which are left alive have not by any means been confined to the peritoneal cavity. There are in most cases large numbers in the various organs; most as a rule in the liver, and after this, in the spleen and marrow. The blood contains comparatively very few.

Another point to be noted is that the numbers of bacilli

still alive in the peritoneal cavity, on the one hand, and in the organs, on the other, bear some relation to each other, for if there are many alive in the cavity, there are also many in the organs, though there are two or three exceptions to this rule.

The power of destroying the bacilli varies very much with the different animals. This is most marked in two hours, the numbers still alive in the peritoneal cavity varying from three hundred and fifty millions to zero. Rabbits (Table I., 6, and Table III., 6) appear to have destroyed everything.

We have noted that there are always more in the liver than in the spleen. Bail observed this also after intravenous and intrapleural inoculation, and remarks that "The number of colonies in the plates makes it seem probable that the first organ to be invaded is the liver."

TABLE IV.

Sixteen hours.

	Weight.	Wash.	Liver.	Spleen.	Lung.	Marrow.	Kidney.	Blood.	Bile.	Temp.
1.	4	8,000,000	600	1,200	250	300	4	0	0	Drop 3.
2.	4½	1,000,000	25	30	0	0	.....	0	110	
3.	4	350,000	150	1,500	40	300	5	0	60	Constant.
4.	4½	60,000	15	100	12	00?	0	2?	0	Rise 1½.
5.	3½	.....	3	25	2	5	0	0	0	Rise 1½.
6.	3½	6,000	0	0	0	0	0	0	0	Constant.

TABLE V.

Twenty-four hours.

	Weight.	Wash.	Liver.	Spleen.	Lung.	Marrow.	Kidney.	Blood.	Bile.	Temp.
1.	3	2,000,000	15	15	25	15	1	20	0	Rise 3.
2.	4½	1,000,000	12	800	120	120	0	5	1	Drop 3½.
3.	4	400,000	3	150	10	15	1	6	.....	Drop 3.
4.	3¼	200,000	4,000	400	.....	50	0	1	0	Rise 4.
5.	4	60,000	0	0	6	0	0	1?	10?	Drop 3.
6.	4	10,000	0	30	8	00?	0	0	0	Constant.

TABLE VI.

Forty-eight hours.

	Weight.	Wash.	Liver.	Spleen.	Lung.	Marrow.	Kidney.	Blood.	Bile.	Temp.
1.	3¾	1,000,000	3	200	?	30	0	0	.....	Drop 1, sick.
2.	3¾	350,000	1	650	10	5	3	1	0	Rise 2.
3.	3½	300,000	2	10	30	1	2	0	0	Rise 1.
4.	4	25,000	0	20	1	0	0	0	0	Drop 1½ & rise 3.
5.	3½	8,000	0	0	0	0	0	0	0	Rise 2.
6.	3¼	3,000	0	0	0	0	0	0	.....	Rise 3½ & N.

Tables IV., V., and VI. show the conditions in sixteen, twenty-four, and forty-eight hours after inoculation. The irregularities here are not quite so pronounced as in the first three tables, although sufficiently so to prevent any general conclusions being drawn as to the number of bacilli which one might expect to find alive at each interval of time. The main feature is that the relative position of the liver and spleen have changed. With one or two exceptions the spleen contains relatively more than the liver. This is somewhat analogous to the results obtained with lampblack (Part I.), so does not necessarily indicate that the liver possesses a greater bactericidal power than the spleen. There is probably a gradual shifting of the bacilli toward the spleen, and it is quite possible that the chief part of the destruction during the later periods actually occurs in the spleen, although the numbers of bacilli found alive there may be relatively greater than in the other organs.

These tables also show that the numbers of bacilli left alive in the peritoneal cavity and in the organs have decreased considerably over the earlier periods of time. How long the bacilli persist is a question which has not yet been followed up. Two days is the limit thus far. It is understood that all the animals referred to in the tables are alive at the time of each experiment.

Having shown, then, that so early as two hours after inoculation there may be a large number of bacilli already present in the various organs, it was natural to reduce the time in order to determine how quickly the organs might become invaded.

Tables VII. and VIII. show the condition of rabbits sacrificed in one hour and half an hour after inoculation. It is found that the organs may already be swarming with bacilli; the numbers being even in excess of those found in two hours, and the irregularities more pronounced.

TABLE VII.

One hour.

	Weight.	Wash.	Liver.	Spleen.	Lung.	Marrow.	Kidney.	Blood.	Bile.
1...	2¾	450,000,000	2,500	425	30	500	10	1,000	0
2...	2½	6,000,000	2,000	500	150	900	2	200	
3...	?	800,000	500	?	40	500	.....	3	0
4...	3¾	200,000	3,000	1,500	150	250	5	5	
5...	3¼	12,000	0	0	0	0	0	0	0
6...	2¾	4,000	225	20	0	70	.....	0	

TABLE VIII.

Half hour.

	Weight.	Wash.	Liver.	Spleen.	Lung.	Marrow.	Kidney.	Blood.	Bile.
1...	2¾	500,000,000	5,000	3,500	700	1,500	200	5,000	
2...	3¼	160,000,000	12,000	8,000	150	500	130	150	0
3...	2½	150,000,000	25,000	15,000	5,000	4,000	2,000	25,000	
4...	2¼	6,000,000	70	15	130	10	0	5	
5...	2½	2,500,000	7,000	4,000	40	500	10	20	45?
6...	2½	40,000	5	2	2	1	0	2	0

Finally the whole operation was conducted as quickly as possible. Immediately after inoculation the rabbit was etherized, and bled from the carotid, the time between the inoculation and the stoppage of the circulation not exceeding ten

minutes. Table IX. gives the results with seven rabbits tested in this way.

TABLE IX.

At once.

	Weight.	Wash.	Liver.	Spleen.	Lung.	Marrow.	Kidney.	Blood.
1	2¾	2,500,000,000	25,000	3,500	8,000	2,000	3,000	25,000
2	3½	2,500,000,000	25,000	2,000	3,000	8,000	1,200	15,000
3	2½	1,500,000,000	7,000	3,000	2,000	1,000	100	10,000
4	2½	1,200,000,000	15,000	1,000	3,000	400	300	10,000
5	4	350,000	0	0	0	0	0	0
6	2¾	300,000	0	0	2	0	0	50
7	3	1,000	0	0	0	0	0	0

The findings appear somewhat astonishing. In four cases the animals are literally swarming the bacilli, whereas in three cases almost all of the bacilli have already disappeared, none having apparently found their way into the organs. It may be mentioned here that in these three cases the disappearance of the bacilli is real and not simply apparent on account of errors in the manipulation. The results on the plates are confirmed by microscopical examination of the omentum. Everything in the three cases points to a sudden, one might say, explosive destruction of the bacilli, a destruction which must almost certainly be due to the bactericidal action of the body fluids, and not to the comparatively slow process of phagocytosis. Here we have something akin to the rapid destruction of typhoid bacilli by normal serum "in vitro," whereas in the first four instances there is apparently little or no immediate destruction by the body fluids. It seems probable that in the last three cases the whole bacteriolytic force of the fluid has been expended and that from now on there would most likely have been an increase in the number of bacilli left alive followed by a period of slower destruction in which probably phagocytosis would play the leading part. Rabbits 5 and 6 in the Tables I., II.,

III., are probably cases analogous to those in Table IX., 5, 6, 7, under discussion; only in two instances, Table I., 6, and Table III., 6, does there appear to have been complete destruction of the bacilli. In the other cases there are more in the wash and in the organs than "at once."

Not less remarkable than the explosive destruction in these three cases is the rapidity with which the organs of the other four rabbits have been invaded by the bacilli. Twenty-five thousand colonies on the liver plate indicates that there must be something like thirty to forty millions in the entire liver, and the numbers in the other organs are also astonishingly great. At this stage we find enormous quantities also in the blood, and a comparison of the various tables makes it appear as if the bacilli quickly enter the circulation and are deposited from the blood in the various organs, for shortly afterwards in one, two, or more hours, the bacilli are comparatively few in the blood, but are still found in large numbers in the organs. But even in the organs there is a rapid decrease, there being far fewer in from one to six hours than immediately after inoculation. A reference may be made here to Bail's work. He conducted a number of experiments "in vitro" with serum plus organ cells, and observed that in the presence of such cells — of liver, spleen, etc. — the serum to a great extent was deprived of its bacteriolytic action. These observations of his were perfectly correct. I have made numerous tests on this point, following Bail's methods, with results entirely in accord with his, but it would be superfluous to give the experiments in detail. Hoke [4] and others have also made such experiments and have attributed the loss in bacteriolytic power of the serum to absorption of complement by the organ cells.

Now, Bail, finding that, after intravenous or intrapleural injections, bacilli were deposited in the organs and remained alive there for a considerable time, concluded that in such situations the conditions were analogous to those "in vitro," and that bacilli, once they had reached the organs, found themselves in an environment of blood plasma plus organ

cells, so were to a great extent protected from the bactericidal action of the blood.

It seems to me, however, that this conclusion is erroneous, for, as we have seen by the foregoing tables, the bacilli rapidly decrease in the organs at first. After the primary invasion of the organs there is evidently a rapid initial destruction of bacilli, followed by a later and slower process, but no evidence that the bacilli are better protected in the liver and spleen than in the peritoneal cavity itself. It is probable that in the organs, as well as in the peritoneal cavity, there is usually a rapid extracellular bacteriolysis at first, and that after this has expended its force, the phagocytes continue the process, but more slowly. This initial destruction may be of an explosive character (Table IX., 5, 6, 7) or somewhat slower, though still manifest within an hour after inoculation. There is apparently, however, a period from two to six hours during which the bacilli may show some increase in the organs. If we take from each table the three rabbits which show the largest number of bacilli in the organs, and are, therefore, fairly comparable, by averaging the totals we get the results given in Table X. The averages are only made out in round numbers.

TABLE X.

Averages at different periods of time. Three rabbits from each table (I–IX.).

	IX.	VIII.	VII.	I.	II.	III.	IV.	V.	VI.
	At once.	30 min.	1 hr.	2 hrs.	4 hrs.	6 hrs.	16 hrs.	24 hrs.	48 hrs.
Liver.....	22,000	15,000	2,500	850	8,500	15,000	250	10	2
Spleen.....	4,000	9,000	500	250	1,700	1,800	900	450	300
Lung.....	4,500	2,000	100	10	130	500	100	45	20
Marrow ...	3,700	2,000	630	200	400	400	300	60	12
Kidney....	1,500	700	6	14	9	3	3	1	2
Blood.....	17,000	10,000	400	70	40	20?	0	10	0

It appears from this table that during the first two hours there is a rapid decrease in the liver, followed by a considerable increase during the succeeding four hours. The period

between six and sixteen hours has not been bridged, but probably in six hours the second period of high figures is at its maximum. The spleen shows very similar results, but it seems to take somewhat longer than in the liver for the bacilli to reach the initial maximum amount. The second period of decrease in the spleen is much slower than in the case of the liver, a point which has already been commented upon. The other organs will be dealt with later.

Since the number of bacilli in the organs varies so much from one animal to another, it cannot be maintained that these averages are very reliable, but it is hardly probable that the figures are purely accidental, and so far as it may be considered to have value, Table X. seems to support the argument that there is an initial rapid destruction of bacilli, which may be explosive or may be spread over an hour or so, the body fluids expending the whole of their bactericidal force upon it, after which there is nothing to hinder the remaining bacilli from multiplying for a time until the phagocytes have arrived in full force. We know that in the peritoneal cavity it takes five or six hours before the phagocytes have arrived in any large quantity, and no doubt the same holds good for the organs. The second period of decrease, however, may be due to the body fluids gradually recovering their bactericidal power.

It may, however, be contended that the initial decreases shown in Table X. are not real, but only apparent, since in the earliest stages there are enormous quantities of bacilli in the circulation. The blood remaining in the organs, therefore, might appreciably increase the numbers of bacilli found in the liver and spleen, under "at once," so that a considerable proportion of the colonies on the plates might represent bacilli which were in reality free in the circulation and not deposited in the organs. After thoroughly bleeding an animal, however, surprisingly little blood is left in the vessels or capillaries. The spleen pulps, indeed, are usually tinged with red, but the liver pulp scarcely shows a trace of it. Moreover, on centrifuging the pulps the red blood cells separate out into a distinct layer of their own, and it is then seen

how very few there are, even in the spleen, as compared with the mass of organ cells. It seems unmistakably evident that the immense majority of the bacilli must be almost immediately deposited in the organs in such a way that they are not washed out in bleeding, and those which can be considered as free in the blood current represent an insignificant fraction of the total number on the liver and spleen plates, even in ten minutes after inoculation (Table IX.).

In the foregoing comments on the tables the liver, spleen, and blood have been dealt with, but it remains to consider the other organs and fluids.

Lungs. — In making up the averages for the lung, a very marked exception at two hours and another at four hours have been omitted. It is difficult to determine from Table X. if bacilli are deposited in the lung in the sense in which they appear to be deposited in the liver and in the spleen. In the earlier stages the numbers found in the lung are always considerably less than in the blood, and seem to rise and fall with the numbers found in the blood, although later than two hours there is an increase of bacilli in the lung, notwithstanding that by this time the blood hardly contains any bacilli at all. In all probability, therefore, there is a certain amount of true deposition in the lung, though to a less extent than in the liver or spleen. The lung pulps are usually tinged with hemoglobin to about the same extent as the spleen, and since the numbers of bacilli found "at once" in these two organs are fairly comparable, it may be that in both cases a large proportion are in reality free in the blood at this early stage. Tchistovich,[5] commenting upon the well-known fact that shortly after intravenous injection of streptococci (or other bacteria) into a rabbit there is marked hypoleucocytosis, maintains that this is because the polynuclear leucocytes englobe the cocci and become arrested in the capillaries of the lung. The lung is the only organ in which phagocytosis by the polynuclears is manifest. In the liver it is the cells of Kupffer which act as phagocytes, while in the spleen, marrow, and kidney there is practically no

phagocytosis. If this observation is correct, and holds good for typhoid bacilli, it would account for the large numbers of bacilli found in the lung during the earliest stages.

Marrow. — One exception at four hours has been made in making up the average. That the marrow cells show marked changes in infection is well shown, but there is nothing in the tables to indicate that the marrow plays any specially important part in bacteriolysis. The question of the bacteriolytic action of marrow cells and extracts has been much disputed by various authors without any definite conclusions being reached, and it would be unprofitable to discuss the point here.

The kidney. — Bail, in his series of experiments already alluded to, never found many colonies on his kidney plates, and these observations are fully confirmed by our tables. The bacilli do not appear to be held firmly in the organ, and the comparatively large numbers found initially are probably due to the small quantities of blood in the pulps. Taking this for granted, it affords an indication of the numbers which should be deducted from the liver and spleen plates in order to determine what proportion of the colonies should be reckoned as free in the blood current. It is obvious from a study of the tables that the totals would only be affected to an insignificant extent, except "at once" in the case of the spleen, a further indication that the spleen is not invaded so quickly as the liver.

In connection with the kidney it may be mentioned that whenever the bladder contained urine, one cubic centimeter was plated out. The plates invariably remained sterile, except in one or two instances, where the urine appeared to have been previously infected. No typhoid colonies were ever found on the plates. Living typhoid bacilli, therefore, are not eliminated in the urine under the conditions of these experiments. This fact appears to lend some support to Metchnikoff's argument that bacteria are not eliminated by the kidneys unless there is some definite lesion. He discusses this question at length in "l'Immunité," but space forbids a recapitulation. Canon,[6] in a review of the literature

on the subject, also comes to the conclusion that whenever the bacteria of invasion are found in the urine it always points to a lesion of the kidney.

The bile. — It cannot be said that the experiments afford any support to the contention that the bile is a regular channel for the excretion of bacteria. Only in two instances (Table II., four hours) were any large numbers of colonies found on the plates, and these are so exceptional that some error of manipulation must be suspected. Apart from these and three or four other doubtful cases, marked "?" in the tables, there are only three instances, one at six and two at sixteen hours, where the bacilli seem to occur in the bile.

Doerr[7] inoculated rabbits intravenously with typhoid bacilli, and always found bacilli present in the gall bladder after twenty-four and forty-eight hours. After intraperitoneal injections the bile was always sterile. But our own experiments show that, except in some instances where the bacilli are destroyed explosively intraperitoneally, injection into the peritoneal cavity is practically the same as intravenous injection in its immediate effects. The liver is very rapidly invaded in either case, so that the results obtained by Doerr are probably not reliable.

Serum. — In many instances one cubic centimeter of the serum was collected and plated out between one and two hours after bleeding. The plates were generally sterile, only comparatively few colonies appearing even when the defibrinated blood showed immense numbers.

In all probability the great mass of the bacilli are held in the clot and do not work out with the serum. Moreover, in one or two hours at room temperature the strongly bactericidal action of the drawn blood may have some influence. With the defibrinated blood this factor could hardly have produced any appreciable effect, since the blood was already on ice within five or six minutes after being drawn.

Temperature. — Up to one hour after injection there is practically never any change of temperature, although, as already remarked, there is usually a drop in two or three hours, which is followed later by a rise. But the temperature of

normal rabbits varies so much that a drop or rise of one or two degrees is of little significance. Table I., 5, however, shows a drop of five degrees in two hours, and this rabbit would almost certainly have died. It has killed almost all the bacilli, and one might infer from this that if the bacilli are killed explosively by the rabbit, the rabbit is likely to be killed explosively by the toxins liberated. This may have been so in this instance, but the very next rabbit, Table I., 6, has killed all the bacilli in two hours, yet the temperature remains constant. Similar contradictory results are to be observed in the other tables, and one can only say that the temperature appears to give no clue as to what the result of the experiment will be. A glance forward, however, to Table XI. shows that there is always a marked drop in temperature when the animal succumbs.

Before concluding the article a table may be given showing the results obtained with six rabbits which died inopportunely. Only those are included the moment of whose death was observed. If for various reasons the manipulation could not be carried out at once, the rabbit was immediately packed in ice, so that there could not have been any material increase in the number of bacilli from the moment of death until the organs became thoroughly cooled down.

TABLE XI.

After death.

	Weight.	Wash.	Liver.	Spleen.	Lung.	Marrow.	Kidney.	Died in	Examined after death in	Temp.
1.	4½	4,800,000,000	125	0	300	00	350	17 hrs.	At once	
2.	4½	480,000,000	20,000	150	600	450	150	3 "	At once	Drop 5.
3.	3½	35,000,000	5,000	1,500	750	2,500	25	2½ "	1 hour	Drop 3½.
4.	3	6,000,000	1,500	1,200	40	700	2	2 "	3 hours	
5.	4¼	6,000,000	1,500	250	60	200	3	2½ "	2 "	Drop 3½.
6.	3	2,500,000	100	300	50	3,500	0	23 "	At once	Drop 5.

If we consider the four animals which died very shortly after inoculation, it appears that with the exception of Rabbit

2 the number of bacilli left alive in the peritoneal cavity and in the organs are of much the same order as in the case of those killed in two and four hours, many of which appeared to be none the worse for the inoculation. There is no obvious reason why some should have died and others lived. Of the two rabbits which survived until the day after the inoculation, the most striking point of similarity is the very large number of bacilli in the marrow as compared with the other organs, but it is impossible with only two experiences to say if this has any significance or not. This similarity, too, is offset by the great differences in the number of bacilli found alive in the peritoneal cavity, a proportion of five thousand to six.

Nor does the table appear to afford much insight into the action of the endotoxins. It is generally accepted that if an animal dies within a very short time after inoculation of bacteria death is due to liberation of endotoxins, on account of the organism having destroyed immense numbers of bacteria. We might suppose this to be the case with XI , 4 and 5, but XI., 2, is swarming with bacilli, and one could hardly imagine that in this instance sufficient endotoxins have been liberated to destroy the animal, unless it is assumed that, the whole organism being quickly overrun with bacilli, some may have been killed off at a specially susceptible focus, such as a nerve center, where a comparatively little toxin being absorbed directly might be sufficient to destroy life. In all likelihood, however, it will remain a puzzle for some time to come why some rabbits can kill millions of bacilli and appear none the worse, while others die in consequence of having killed millions, and again why some become flooded with bacilli and live, while others die in consequence of being so flooded.

To sum up, it may be remarked that we have here a number of observations which in many respects appear contradictory and difficult to reconcile with the current theories of natural immunity. A discussion of their bearing upon these theories will have to be postponed until after a parallel series of experiments shall have been made with immunized

animals. It may then be possible, with the help of observations on the paths of absorption from the peritoneal cavity and the part played by the omentum, to bring a certain degree of order out of the somewhat chaotic mass of facts obtained thus far.

CONCLUSIONS.

After intraperitoneal injection of typhoid bacilli into rabbits:

1. There may be an explosive destruction of the vast majority of the bacilli, few or none reaching the organs in such cases.

2. The destruction may take place more slowly, though still strikingly manifest within one hour after inoculation.

3. In the latter case large numbers of the bacilli find their way into the circulation almost immediately, being rapidly deposited from the blood in the various organs.

4. The organs vary greatly in their power of holding up the bacilli, the liver being invaded to the greatest extent.

5. The number of bacilli in the organs rapidly decreases again after the first few minutes up to about two hours after inoculation.

6. From two to six hours there is a considerable increase, probably due to the body fluids having expended all their energy, thus permitting multiplication of the bacilli.

7. From six hours up to forty-eight hours there is again a decrease, rapid in the liver, but more gradual in the spleen. The second period of decrease is probably due to the action of the phagocytes, although recovery of the bactericidal action of the body fluids may also be a factor.

8. Neither the urine nor the bile appear to be channels of excretion under the conditions of the experiments.

9. The variations of temperature between the time of inoculation and that of bleeding afford no indication of what the results of the experiments are likely to be.

10. Experiments with rabbit serum "in vitro" give no clue as to what will happen "in vivo."

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ABSORPTION FROM THE PERITONEAL CAVITY.

PART III. — *The function of the diaphragm.*

B. H. BUXTON AND J. C. TORREY.

In the preceding papers we have shown how inert particles, like lampblack, or typhoid bacilli, reach the internal organs with extraordinary rapidity after inoculation into the peritoneal cavity. The experiments on which these conclusions have been based were given in detail, but no attempt was made to explain by what paths the particles or bacteria reach the organs. Coincidentally with these experiments we have been endeavoring to determine this point also, and the present communication deals with our investigations along this line.

Since bacteria (typhoid bacilli) can often reach the organs and become deposited in enormous numbers in the liver, spleen, and marrow within ten to fifteen minutes after inoculation, and particles of lampblack with equal rapidity, although apparently not to such a great extent, it is necessary to suppose an initial rapid rush to the interior organs. It can hardly be supposed that the initial rush is altogether due to the action of phagocytes which have already picked up the particles or bacilli and are carrying them off. We have indeed shown in Part I. that particles of lampblack in the earliest stages lie free in the veins and capillaries of the liver, and it is not until after two to four hours that they are found, for the most part, in the phagocytes. We find, also, strong evidence that the particles on their way to the organs are carried in a free condition, for they can be found free in immense numbers in the lymph trunks and nodes of the anterior mediastinum, whence they can readily pass into the thoracic ducts and the blood current. The source of the anterior mediastinal lymphatic trunks can be traced to the lymphatics of the diaphragm, so it appears highly probable that the diaphragm plays an important part in absorption of particles from the peritoneal cavity. Since most, if not all,

of the particles carried along the lymphatic trunks must pass through the lymph nodes connected with this system, it was thought that a study of the anterior mediastinal lymph nodes at various intervals after inoculation should prove of some interest.

The experiments have been conducted along two lines:

A. Examination of sections of the anterior mediastinal nodes.

B. Enumeration of the bacilli (typhoid) in the anterior mediastinal nodes by plating methods.

A. Sections of the anterior mediastinal lymph nodes.

If a guinea-pig is killed in five to fifteen minutes after injection of lampblack intraperitoneally, and the thorax opened, the anterior mediastinal nodes are found to be already quite black, and there is often a black line running along the anterior mediastinum on either side of the sternum in close proximity to the internal mammary vessels. The black line represents the anterior mediastinal lymphatic trunk. The most prominent and constant of these nodes are a pair, often three, in the first intercostal spaces. There are usually one or two other pairs lower down in the third or fourth intercostal spaces, but these are somewhat irregular both as to location and occurrence. These nodes will be designated as the "upper" and "lower" anterior mediastinals.

The sections on which this work is based were made by the paraffin method. They were cut for the most part six microns in thickness, and in many instances mounted serially. A variety of stains were used, such as hematoxylin and eosin, Gram's, and eosinate of methylene blue.

1. Sections of early stages.

a. Sections after injection of lampblack. — Although in fifteen minutes after injection of lampblack the nodes appear quite black, sections at this stage with a low magnification show that the particles lie in the peripheral afferent sinuses of the lymphatic channels, and the center of the node is only

slightly pigmented (Plate II., 1). In the lower nodes all of the afferent channels are filled with lampblack. Under a high power of the microscope the great majority of these particles are seen to be free, only here and there do we find a macrophage containing black granules (Plate II., 3). In the inner and efferent sinuses a considerable amount of free pigment may be found, but a much larger proportion of the particles are contained within cells than in the afferent plexuses. The cells are for the most part macrophages. A few microxycytes may be seen, but they have taken as yet no part in the phagocytosis. The upper nodes are not, like the lower, pigmented throughout their periphery, but only in the region where the afferent ducts enter the gland. As with the lower nodes, however, the greater part of the lampblack in the efferent sinuses of the upper pair has been taken up by macrophages, whereas at the periphery most of the particles are free. Although the microxycyte reaction is beginning in the lower nodes, these cells have not as yet invaded the upper pair.

An examination of the afferent lymphatic ducts of the lower nodes shows that at this early stage all of the lampblack particles have come from the diaphragm in a free state and as such enter the nodes. In passing through the gland, however, some of the particles are seized upon by wandering cells and detained, whereas the major part passes on into the efferent ducts entirely as free particles. A photograph of one of these efferent ducts filled with free particles is shown in Plate II., 5. The same is true of the upper nodes. The particles enter and leave the nodes in a free state, but in the sinuses are partly ingested by cells.

b. Sections after injection of chicken red cells. — Chicken red cells injected into the peritoneal cavity of a guinea-pig also reach the nodes in fifteen minutes, but in much smaller quantity than lampblack. The red nucleated cells are almost entirely free and stain well. Here and there macrophages have begun the attack and various stages of ingestion may be found. An early stage of ingestion may be found in Plate VII., 1, on the left.

After an interval of one hour the afferent channels of the lower nodes are gorged with chicken cells. Most of these are free, some are clumped together in groups of two or three, whereas others are contained within macrophages, where they may be found in various stages of destruction (Plate VII., 1). As is the case with lampblack, sections of the upper nodes do not contain so many chicken cells as the lower. In the afferent sinuses many of these cells are being taken up by macrophages. In the inner sinuses there are only a few chicken cells and these are for the most part free. There are quite a number of microxycytes to be seen and some of these seem to be attacking the foreign cells in a manner which has been described as occurring in the peritoneal fluid, *i.e.*, the microxycyte forms a center of attraction around which are grouped a number of chicken cells. Although the afferent sinuses are crowded with chicken cells they are rarely met with at this stage in the efferent sinuses. Plate VII., 1, shows the afferent sinuses of a node one hour after injection. Most of the chicken red cells are free, but some are contained within macrophages.

c. Sections after injection of bacteria. — Turning now to sections of mediastinal nodes after injection of an emulsion of typhoid bacilli, we find at the end of an hour a somewhat different condition of affairs than that seen after the injection of inert particles or foreign cells. Instead of the greater number of bacilli being free in the afferent sinuses of the node, nearly all have been taken up by macrophages. A few bacilli have also been conveyed by these wandering cells into the inner lymph channels. Throughout the node a very small minority of typhoid bacilli are lying in a free condition. Microxycytes are present in large numbers, as was the case with chicken cells, but they seem to take very little part in the phagocytosis.

It is rather surprising to find that such large bacilli as anthrax can be absorbed through the diaphragm of a guinea-pig and reach the anterior mediastinal nodes in fifteen minutes or less. Even chains of four or five bacilli in a free condition have found their way into the lymphatics of the

diaphragm. In the lower nodes these bacilli are present after fifteen minutes in much greater abundance than in the upper, but are confined in each case entirely to the afferent sinuses. Anthrax bacilli do not appear to be taken up by the phagocytes so readily as typhoid bacilli.

As would be expected staphylococci can be readily absorbed through the diaphragm. After one hour they are found in the afferent sinuses within the macrophages. A few cocci both free and in cells have also worked their way into the interior of the node. Microxycytes have been attracted in great numbers. In the afferent sinuses, where the cocci are present in abundance, all the available space is taken up by microxycytes. Although here and there a microxycyte may be found which has ingested several cocci, by far the greater number are clearly in the macrophages, possibly because these cells have been able to seize upon the cocci before the arrival of the microxycytes. It may be said, however, that the microxycytes which have wandered into the node show rather more marked phagocytic activities when the inoculation has been made with staphylococci than is the case with typhoid bacilli.

2. Sections of later stages.

a. Sections after injection of lampblack. — In Plate II., Fig. 2, is shown a photograph of a section of an upper mediastinal node under low magnification, twenty-four hours after injection of lampblack. It presents a very different appearance from that of the fifteen-minute stage. Much less pigment is found in the afferent sinuses, and what there is appears to be almost entirely contained within macrophages and microxycytes. The efferent sinuses on the other hand are now well filled with cells stuffed with lampblack.

As is shown by a high power of the microscope (Plate II., 4), the macrophages often contain so many particles that little or none of the protoplasm of the cell can be seen. The microxycytes have invaded the nodes in large numbers. Nearly every one has taken up a few particles, but none have gorged themselves after the manner of the

macrophages. In addition to the particles, some of the macrophages have also ingested microxycytes. A few free particles may still be found, but most of the lampblack is leaving the node within wandering cells. Although, as will be shown, foreign cells and some forms of bacteria disappear from the nodes within a short time, lampblack remains there very much longer. Even at the end of two months both the upper and lower nodes appear quite as black as at any previous stage.

b. Sections after injection of chicken cells. Later stages.— As regards the later stages with chicken cells, we find that at the end of eight hours almost every cell has been taken up by the macrophages. The efferent sinuses contain far more than the afferent. In the latter the macrophages have almost entirely digested the chicken cells. In many there is little left except the nuclei. The macrophages in the inner sinuses have stuffed themselves to a remarkable degree. They frequently contain from eight to fifteen of these cells, which are in all stages of destruction (Plate VII., 2). This digestion has progressed so far at the end of forty-eight hours that only occasionally a chicken cell may be found. Microxycytes are quite abundant, especially near the periphery of the node, but they take comparatively little part in the destruction of the foreign cells.

c. Sections after injection of bacteria. Later stages.— In four hours most of the typhoid bacilli have left the afferent sinuses and are found in abundance in macrophages within the inner and efferent lymph channels. Many of these macrophages which contain bacilli are vacuolated and stain poorly; a form of degeneration which is especially noticeable among those at the periphery of the node. At this stage, that is to say, in four hours after the injection of the typhoid bacilli, microxycytes are much more in evidence than after injection of inert particles. A few of these cells are already contained within macrophages, but those which are still free and presumably in an active condition seem to take little part in the phagocytosis. It is only here and there that a microxycyte containing typhoid bacilli can be seen.

Anthrax and staphylococci were found to have almost entirely disappeared from the nodes in four or five hours. Although apparently differing from typhoid in this respect, sufficient experimentation has not been carried on with these bacteria to permit us to lay much emphasis on this observation.

Colloidal platinum. — A few experiments were made with colloidal platinum prepared by Bredig's method of striking an arc between two platinum electrodes under pure water. The particles are ultra-microscopical, but on addition of an electrolyte increase in size and gradually precipitate out. One hour after injection of the colloidal solution very few particles could be found in the nodes, but this may be due to the majority of them being still of ultra-microscopic size. In four hours they are plainly visible and have been entirely taken up by macrophages. The position of the particles in the cells may be observed to much better advantage when this agent is used instead of lampblack, since it is semi-transparent, and the cells do not become so packed. Colloidal platinum evidently has little power of attracting microxycytes, for few of these wandering cells are found in the node.

B. Enumeration of bacilli by plating methods.

In addition to the section work, some experiments were made by plating out the anterior mediastinal lymph nodes after injection into rabbits of typhoid bacilli. The rabbits were the same as those used for the experiments already detailed in Part II., but in the earlier experiments the idea of testing the lymph nodes had not occurred to us, so that only from three to five results of each period can be recorded.

The lymph nodes were treated precisely as has been described for the spleen and other organs. The upper pair were picked out and rubbed through a fine wire gauze with fifteen cubic centimeters of salt solution. Since it is not easy to pick out the lower pair of nodes, the tissue where they lie was cut out and treated in the same way. Of the fluid one cubic centimeter was plated out and also fractions

of one cubic centimeter. From the colonies on the plates the total number of bacilli in the nodes could be roughly estimated. Since there is always a little uncertainty even with the upper pair, whether the nodes have been completely removed or not, the individual figures must not be taken too literally, but, regarded as a whole, there is no doubt that the lymph nodes may contain enormous quantites of bacilli, the upper pair always containing far more than the lower. It has been said that after injection of inert particles or bacteria into guinea-pigs sections appear to show that in the earliest stages the lower nodes are invaded to a greater extent than the upper. The accompanying tables under "at once" and "half hour" seem to contradict this statement, but it must be remembered that the upper nodes are always much larger than the lower; a disproportion, moreover, which is more marked in the rabbit than in the guinea-pig. It cannot be supposed that the small pieces of muscle and connective tissue attached to the nodes after cutting out can materially influence the results. Our sections show that lampblack, chicken cells, and bacteria are strictly confined to the lymph nodes and trunks, so that the possibility of large numbers of typhoid bacilli occurring in the tissues adjacent to the nodes may be excluded.

TABLE I.

Estimated number of bacilli occurring in the lymph nodes after intraperitoneal injection into a rabbit of half a culture of typhoid. Anterior mediastinal lymph nodes, upper pair.

Rabbit No.	IX.	VIII.	VII.	I.	II.	III.	IV.	V.	VI.
	At once.	30 min.	1 hour.	2 hours.	4 hours.	6 hours.	16 hours.	24 hours.	48 hours.
1...	25,000,000	6,000,000	.....	.....	.....	.....	1,500,000	.....	5,000,000
2...	.....	6,000,000	200,000	2,000,000	4,500,000	.....	.....	10,000,000	
3...	500,000	5,000,000	.....	.....	.....	.....	15,000,000		
4...	.....	.....	3,000,000	300,000	.....	1,200,000	2,000,000	3,000,000	60,000
5...	.....	1,500,000	60	.....	300,000	50,000	75,000	.....	350
6...	.....	600	12	.....	13	0	0	200,000	0
7...	120	.....	.....	6,000,000	25,000,000				

TABLE II.

Estimated number of bacilli occurring in the lymph nodes after intraperitoneal injection into a rabbit of half a culture of typhoid. Anterior mediastinal lymph nodes, lower pair.

Rabbit No.	IX.	VIII.	VII.	I.	II.	III.	IV.	V.	VI.
	At once.	30 min.	1 hour.	2 hours.	4 hours.	6 hours.	16 hours.	24 hours.	48 hours.
1	400,000	100,000	.....	.....	.....	.....	20,000	.....	10,000
2	.....	15,000	10	12,000	12,000	.....	.....	250,000	
3	25,000	25,000	.....	.....	.....	.....	10,000		
4	.....	.....	50,000	100	.....	1,000	5,000	120,000	600
5	.....	2,000	0	.....	150	3,500	100	.....	0
6	.....	0	0	.....	0	0	0	0	0
7	0	.....	.....	250,000	600,000				

Table I. gives the estimated number of bacilli in the upper pair of nodes at various intervals of time, and Table II. the corresponding numbers in the lower pair.

The rabbit numbers correspond with those of the previous tables given in Part II. For instance, the upper nodes of Rabbit No. 1 "at once" contain twenty-five million bacilli, and a reference to Rabbit No. 1 of Table IX. in Part II. will show the results which were obtained with the organs of this rabbit. The only exceptions are numbers 7 in two hours and four hours, which were not brought into the previous tables. It will be remembered that the tables in Part II. were arranged in such a way that the topmost row began with the highest figures, the figures gradually decreasing to the lowest row. On the whole, the same decrease can be noticed in these two tables, so it is evident that there is some relation between the numbers of bacilli found in the organs and those found in the lymph nodes. If the organs contain many bacilli the lymph nodes also contain many, and if there are few in the one case then there are few also in the other. To this general rule we find only two exceptions, both of which are rather marked, at one hour (II.) and at sixteen hours (I.). In these cases we may suspect that the lymph nodes have not been entirely cut out. In Table

II. variations are more in evidence, but with the lower nodes the uncertainty as to whether or not they have been actually included in the material removed is much greater than with the upper pair.

In the previous experiments on the number of bacilli occurring in the organs it was noticed that after a marked decrease in two hours there appeared to be an increase of the bacilli in the four and six hour periods, followed again by a decrease in sixteen hours. There is no evidence of the secondary rise in Table I., the numbers in the nodes after four and six hours being on the whole fairly comparable with those at the same level during the earlier periods.

It is remarkable that the three later periods of sixteen, twenty-four, and forty-eight hours afford such high figures. These are the periods at which the spleen always, or very nearly always, shows a decided preponderance over the other organs in the numbers of bacilli it contains. It was observed in Part II. that this fact probably indicates a gradual shifting of the bacilli from the other organs toward the spleen, and this appears to hold good also for the mediastinal lymph nodes. But in this case the process is probably not so much a general shifting of the bacilli toward the lymph nodes as a holding up by the nodes of fresh supplies from the peritoneal cavity. After the initial rush in which the lymph nodes are overwhelmed, as it were, they probably settle down to their work, and later on more effectually prevent any further supply of bacilli from reaching the internal organs. In the lymph nodes, to judge of the appearance in sections, the macrophages take up and gradually digest the bacilli. This at any rate seems to be the probable explanation of the comparatively large numbers of bacilli occurring in the lymph nodes during the later stages, the macrophages probably remaining largely in the lymph nodes, where they digest the bacilli apparently more slowly than they digest chicken red cells, which in forty-eight hours have almost entirely disappeared.

The early infection of the anterior mediastinal nodes after intraperitoneal injection has not been recognized until lately.

Muscatello[1] in 1895 observed that in five to seven minutes after injection of carmine into the peritoneal cavity of dogs the mediastinal lymph nodes become colored. He expresses himself as surprised to find that this phenomenon has not been more frequently noticed, for he can find but one reference to it in the literature, that of Dunbar and Remy in 1882.

Durham[2] found that Indian ink reaches the anterior mediastinal nodes in about eight minutes after injection intraperitoneally in guinea-pigs, while cholera bacilli may be found in six minutes. The nodes are not connected with the omental lymphatics but with the diaphragmatic. Our experiments are quite in accord with the above and we may take it as fairly well established that there is a vigorous initial rush of particles or bacilli to the anterior mediastinal lymph trunks and nodes by way of the lymphatics of the diaphragm.

The next point for discussion is, "How do the particles pass from the peritoneal cavity into the lymphatics of the diaphragm?"

Von Reckinghausen in 1862 considered that he had demonstrated the presence of stomata in the diaphragm, and since then the existence of stomata in the diaphragm has been generally accepted and is taken for granted in the current text-books. According to this view it is easy to see how particles could pass through such openings into the lymphatics, since the peritoneum could be regarded simply as an enormous lymphatic channel in direct communication with the lymphatics of the diaphragm towards which peristalsis and the pumping action of respiration would direct the flow of lymph.

But recently the existence of the stomata has been strenuously denied. In the first place, Sabin[3] has very clearly demonstrated that the lymphatic channels and ducts form a system "sui generis," which has no connection ontogenetically with the connective tissue spaces or the large serous cavities. The lymphatics develop by a budding out from the endothelium of the subclavian veins and spread a network

of ducts all over the body by progressive budding out of blind sacs. Sabin regards the lymphatics as modified veins. One would not expect, therefore, the lymphatics to be in direct open communication with the peritoneal cavity, and Muscatello, as a result of his investigations, concludes that there is always a membrane covered on either side with lining cells between the lymphatics of the diaphragm and the peritoneal cavity. Nevertheless, he considers that the diaphragm is the only point of absorption from the cavity, and, since in the earlier stages he finds many of the particles free in the anterior mediastinal nodes, suggests that leucocytes after ingesting particles force their way through the membrane and are immediately followed by a rush of free particles which continues until the space closes up again, just as Thoma found a rush of red cells might follow the passage of a leucocyte through a capillary wall. In this way the smaller particles pass through in a free condition, the larger ones being taken up by the phagocytes.

MacCallum[4] agrees with Muscatello and, as a result of very careful and minute observations on the diaphragm, concludes that its lymphatics are not in direct communication with the peritoneal cavity, but that in places there is nothing between the two except the endothelium of the lymphatics on the one side, and the lining of the peritoneum on the other, with an exceedingly fine basement membrane between the two layers of cells. At such points the lymphatic channels are enlarged, forming lacunæ, and the free particles (carmine or Indian ink) can be forced through between the lining cells from the peritoneal cavity to the lacunæ of the lymphatics by the pumping action of the diaphragm. However, MacCallum accords the chief rôle to the phagocytes which take up particles in the peritoneum and wander through to the lacunæ. His drawings show that he considers the polynuclears (microxycytes) to be the chief agents in phagocytosis.

As to the presence or absence of a direct connection between the peritoneal cavity and the lymphatics of the diaphragm, we have not been able to decide from a histological standpoint. From its physical aspect, however, it seems

hardly possible that such relatively large objects as chicken cells or a chain of four or five anthrax bacilli could force their way through two layers of cells and a basement membrane and reach the anterior mediastinal nodes within fifteen minutes. There is no doubt that in this early stage, and in all probability in the later ones also, the chicken cells reach the lymphatic ducts in a free condition. It is not until they have reached the lymph nodes that their destruction by macrophages begins. The microxycytes are rarely able to ingest them and so their transportation by this means is out of the question. The diaphragm of a guinea-pig killed fifteen minutes after injection of lampblack shows, as has already been described, the lymphatic vessels marked out in black. Sections at this stage indicate that the polynuclears could have had little, if anything, to do with the transportation of the particles, for the lampblack in the lymphatics is entirely free, none is contained within cells, and it is not until the pigment reaches the anterior mediastinal nodes that any phagocytic action may be observed. The sections also show many particles clinging to the peritoneal side of the diaphragm and also apparently being forced through between the cells of the peritoneal endothelium. In the later stages, however, the wandering cells certainly play an important part in the transportation of lampblack to the lymphatics of the diaphragm. The lymph spaces are now crowded with microxycytes and macrophages filled with large black granules, more particularly the latter. Rarely, too, a wandering cell filled with particles may be seen in the act of crawling into the diaphragm between the endothelial cells of the peritoneal surface. Lampblack is also still passing through in a free condition.

Leaving unsettled the question of the presence or absence of "stomata," it may be said in conclusion that the structure of the peritoneal side of the diaphragm permits of a very rapid passage of inert particles, bacilli, and chicken blood corpuscles from the peritoneal cavity to the mediastinal lymphatic system; and that leucocytes have nothing to do with this "initial rush."

GENERAL CONCLUSIONS.

1. After intraperitoneal injection of suspensions there is an immediate rush of particles to the lymphatics of the diaphragm.

2. From the diaphragm the particles are carried along the anterior mediastinal lymphatic trunks, and through the lymph nodes, reaching the thoracic ducts and blood current almost immediately after injection.

3. Throughout their whole course from the peritoneal cavity to the organs the particles in the earliest stages are practically in a free condition, being taken up later by the phagocytes.

4. In the anterior mediastinal lymph nodes phagocytosis by the macrophages is the most prominent feature, the microxycytes taking comparatively little part. This applies, not only to inert particles and animal cells, but also to bacteria.

5. Whether the particles pass into the lymphatics of the diaphragm by stomata or gain entrance between the endothelial lining cells we have been unable to decide.

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ABSORPTION OF PARTICLES FROM THE PERITONEAL CAVITY.

PART IV. — *The function of the omentum.*

B. H. BUXTON AND J. C. TORREY.

We have fully discussed (Part III.) the part played by the diaphragm in absorption of particles from the peritoneal cavity, and have shown how the initial rapid rush of particles or bacteria in a free condition to the organs may take place by way of the lymphatics of the diaphragm. It now remains to consider whether there is any other channel by which the particles can leave the peritoneal cavity in a free state.

The omentum appears to furnish such an additional channel to some extent. Sections made from the omentum within an hour after injection of lampblack into the peritoneal cavity show that the afferent plexuses of the lymph nodes are filled with free particles. After two or three hours the particles have been taken up by the macrophages and lie principally in the sinuses of the node.

All this corresponds precisely with processes seen in the anterior mediastinal lymph nodes, so without going into further details we may take it as probable that the lymphatics of the omentum, as well as those of the diaphragm, are concerned in the initial rapid rush of particles to the organs, though probably to a less extent.

That there are any other parts of the peritoneal surfaces which participate in this initial rush seems doubtful. After injection of lampblack both ventral and dorsal abdominal surfaces of the peritoneum can be washed gently under the tap and every trace of pigment disappears from the surface. No black points can be observed, even with the aid of a pocket lens. Ricoux [1] has described minute lymphoid follicles on the abdominal surface of the peritoneum of guinea-pigs. The follicles consist entirely of macrophages which thrust out protoplasmic processes between the lining endothelial cells. The processes englobe particles (of carmine)

and then retract, drawing the carmine with them into the follicles, from which point the macrophages, containing the particles, wander into the lymph spaces and channels. In consequence of this process the abdominal wall, shortly after injection of carmine, appears covered with minute pink spots visible to the eye.

With lampblack we have been quite unable to confirm this observation of Ricoux, and even if he is correct in his statements, the process he describes is too slow to account for the rapid rush to the organs. We feel convinced that the abdominal parietes of the peritoneum are not concerned in it.

The same, so far as we have been able to observe, is the case with the mesentery. It can always be washed free of particles in early stages, although later the mesenteric nodes show infiltration of pigment. This infiltration is probably a secondary process, as suggested by Muscatello, the pigment arriving by way of the circulation; and also perhaps to some extent caused by phagocytes which have picked up particles in the peritoneal cavity, and worked their way into the mesenteric tissues.

Unlike the abdominal surfaces or the mesentery, the omentum becomes intensely blackened almost immediately after injection of lampblack, and the pigment cannot be washed off under the tap. The omentum, therefore, appears to play an important part aside from the rapid infiltration of its lymph nodes, already mentioned, since this infiltration alone would not account for its intensely blackened appearance. We find, indeed, that phagocytosis is exceedingly active on the surface of the omentum, and the phagocytes, after picking up particles, find their way into the omental tissues where their morphological characters become changed in a remarkable manner, as we shall see later. But the primary, immediate blackening of the omentum is mainly due to a deposit on its surface of fibrin in which the particles become entangled, and where they await the arrival of the phagocytes. In order to appreciate the details of the phagocytic activities of the omentum it will be advisable to

describe its normal histological appearance before attempting to deal with its functions under pathological conditions. Our remarks refer to rabbits and guinea-pigs.

Normal histology of the omentum. — If a piece of the omentum of a young rabbit is spread out over a microscopical slide and held up against the light,* it appears as a semi-transparent sheet dotted over with a number of opaque spots, the "tâches laiteuses" of Ranvier. Blood vessels course in various directions, some of which can be seen entering the milky spots, but others do not reach them. A milky spot is the precursor of a capillary network or tuft, and a large piece of the omentum spread out shows a tracery work of spots, marking out the courses of the future blood vessels (Plate III., 1). In a full grown rabbit the milky spots are all occupied by blood capillaries.

With the guinea-pig the milky spots are not apparent. The capillaries work their way out over the sheet singly and only form tufts exceptionally; the few tufts which may be seen not being preceded by a milky spot without vessels.

Let the omentum spread of the young rabbit be stained with eosinate of methylene blue, and examined under a low power of the microscope. The milky spots stand out as thick masses with the center often stained deeply in blue; some of the spots being already traversed by numerous capillaries. Plate III., 2, shows a milky spot without, and Plate III., 3, a milky spot with capillaries.

Over the surface of the omentum there are sometimes a number of violet dots, though these are not often present. They have no connection with the milky spots, and are more often present in the guinea-pig than in the rabbit.

The omentum can now be examined under a high power of the microscope (1/12 oil immersion lens).

1. The violet dots. — These are seen to be very large cells with a large round or ovoid nucleus, deeply stained in violet, the nucleus being surrounded by more or less scattered large violet granules. The cells appear to be very

* We shall call this an "omentum spread" for brevity.

unstable, and it is impossible to find any definite limit to the cytoplasm. Even the nucleus is generally broken up into granules.

These are the mast cells of Ehrlich. Westphal says that they do not occur in the rabbit, but we have certainly seen traces of them occasionally. In the guinea-pig they are often frequent, clinging mostly along the long axis of the smaller blood vessels. But they are most conspicuous on the mesentery of the guinea-pig, where they occur as large flat cells, apparently lying just below the surface epithelium (Plate VIII., 2).

2. The milky spots. — (*a*). Without capillaries: If one of the smaller milky spots is examined under a high power, it appears at first sight to be made up of a number of cells with branching processes of the nature of connective tissue cells. The nuclei are round to oval in shape, and from either end of a nucleus there is usually a relatively long process, the other branches being shorter and less conspicuous. There are also a few large round mononuclear cells apparently lying on the surface. Between the large round mononuclear cells and those with long processes, which may provisionally be called "elongated cells," may be seen a certain number of intermediate forms. The picture gives one the impression that the round cells are gradually working their way into the tissue, where they finally appear as the elongated cells. A few, both of the round and elongated cells, may contain small dark or reddish staining granules (Plate VIII., 3). In the center of the larger spots, in addition to the cells described above, there are masses of smaller lymphoid cells, stained deeply in blue. It is these cells which cause the center of the milky spot to appear deep blue under a low magnification.

b. With capillaries: In addition to the above elements, tortuous capillaries now traverse the milky spots in all directions. The lymphoid cells have increased greatly in number and appear to have changed somewhat in character. They now show a rim of deep blue basophile cytoplasm with a relatively pale-staining nucleus (Plate VIII., 1). The large

round mononuclears may still be seen in places, and the elongated cells to a great extent have arranged themselves so that they lie parallel with the axis of the capillaries (Plate IV., 3, may be referred to as an example of this arrangement). In this situation they appear with two long processes issuing from either end of the oval nucleus, the side branches being absent or inconspicuous. Occasionally, though very rarely, both large, round, and elongated cells may be seen to contain what is obviously the remains of an eosinophile cell or of a microxycyte. We may suspect, therefore, that in these large round cells we have the macrophages of Metchnikoff, and since we find remains of eosinophiles in the elongated cells also, the surmise already made that these are round cells which have worked their way into the tissue receives some support. It seems probable that the reddish granules previously described as occurring normally in the cells of a milky spot represent a later stage in the digestion of the eosinophiles, while the dark granules may be remains of nuclei or particles of extraneous matter picked up in the peritoneal cavity. We shall find these surmises fully confirmed when we come to deal with the omentum under pathological conditions, and a glance forward may be permitted here (Plate IX., 1), where is a complete history of the process of evolution when there is a large amount of pus in the peritoneal cavity.

3. The connective tissue meshwork. — Immediately around the blood vessels and milky spots the omentum forms a sheet of connective tissue covered on both faces by the endothelium of the peritoneum, but at a short distance on either side of the vessels or milky spots the continuous sheet becomes broken up into a meshwork, the trabeculæ of connective tissue being covered on all their faces by flat epithelial cells with vesicular, pale-staining nuclei. Here and there, also, are to be seen elongated cells with two, three or more branching processes like those of the milky spots, but as a rule more distinctly elongated. Occasionally a large round mononuclear cell or a polynuclear eosinophile cell may be seen on the surface. In the guinea-pig the meshwork

is much more open and the connective tissue of the trabeculæ much denser than in the rabbit.

As has already been remarked, there are no milky spots in the omentum of the guinea-pig, but in the neighborhood of the capillaries and in the meshwork the elongated cells can be found in large numbers. In the connective tissue of the meshwork they are very conspicuous, rarely presenting more than two or perhaps three very long, finely drawn out processes, in which reddish, eosinophile granules are much more in evidence than with the rabbit. We first observed these elongated cells in the guinea-pig and named them "trailers," a term which may be conveniently used to designate them.

4. The trailers (Plate IX., 2). — Although there is at first sight a very marked difference between such trailers as seen in the meshwork of the guinea-pig and the more branching cells of the milky spots of the rabbit, we think that the evidence is in favor of their being the same cells, simply varying in appearance on account of their environment. The tissue of the milky spots appears loose and somewhat of an areolar nature, so that the macrophages on entering it can put out processes in various directions. In the neighborhood of the capillaries or in the meshwork of the rabbit the connective tissue is denser, so we find the cells more distinctly elongated while the side processes are comparatively short and few in number. In the meshwork trabeculæ of the guinea-pig the tissue is much denser still, and here we find the typical trailer with its two, or at most three, very long, finely drawn out processes feeling their way along the narrow channels between the connective tissue bundles.

All these cells, wherever found, give evidence that they are, or have been, of a phagocytic nature; evidence which is more marked under pathological than under normal conditions, as we shall see later. The question whether or not these cells become fixed connective tissue cells will have to be postponed for discussion until we shall have become more familiar with the trailers under pathological conditions.

Ranvier appears to have been the first to describe the milky spots. He refers to them as being made up of connective tissue cells and lymphatic elements. With regard to the lymphatic elements Dominici[2] remarks that there are cells in the omentum of the rabbit comparable with lymphocytes. They predominate in the milky spots, and undergo various transformations. Some model themselves into endothelial, vasoformative or fat cells and incorporate themselves with such structures, while others preserve their rounded form, increase in size, and become macrophages. In his opinion the connective tissue cells can become mobile and change into cells identical with macrophages, and again the macrophages can become fixed connective tissue cells. Dominici's views are opposed to those of Retterer,[3] who maintains that the omentum develops as a double sheet of epithelium, and from the epithelial cells are derived the connective tissue cells and meshwork. The lymphoid cells of the milky spots also develop from the epithelium.

Jolly[4] considers the lymphoid cells as identical with the plasma cells of Unna. Opinions are divided, therefore, as to the origin of the lymphoid cells of the milky spots, and we may content ourselves with the fact that they exist in the forms already described. We shall not find them of any particular importance until we come to deal with immunized animals in a future series of papers.

When we first began examining the omentums of animals after infection of various substances, the observations were only intended as an adjunct to the work treated of in the three previous articles. It was only by degrees that we came to realize the full significance of the processes which take place on the omentum, and we had already worked out all that has been said by us on the subject of the trailers before investigating the literature on the subject, when we found that our observations had been anticipated.

We were under the impression that Ranvier's clasmatocytes were identical with Ehrlich's mast cells, and it was not until Ranvier's[5] articles came into our hands that we

discovered the clasmatocytes to be trailers under an older and more scientific name.

Ranvier at first in a series of articles in the Comptes rendues Academie des Sciences, 1890 and 1891, described the clasmatocytes as occurring in various situations in amphibia and mammals; in the latter being especially noticeable on the omentum of rats, rabbits, and guinea-pigs. He described them as being large mononuclear leucocytes which had wandered into the connective tissue and there, becoming fixed, secreted the granules which are such a marked feature in their cytoplasm. After a time the cytoplasm of the cells bursts, liberating the granules which are scattered free in the surrounding connective tissue — clasmatosis.

Later he injected carmine into guinea-pigs intraperitoneally, and after twenty-four hours found the carmine granules in the clasmatocytes. He suggests that the particles of carmine have been taken up by the large mononuclear cells which have then wandered into the connective tissue and become clasmatocytes. This is precisely our opinion, but Ranvier does not seem to have realized that the granules seen in the trailers of normal animals are probably granules derived from polynuclear cells and extraneous substances which have been ingested. We think that we have clear evidence that this is the case.

Renaut[6] in addition to the clasmatocytes on the omentum of the rabbit finds in the milky spots other somewhat similar cells containing minute acidophile granules. Such cells branch much more freely than the clasmatocytes and have a much greater resemblance to ordinary connective tissue cells. These cells he calls rhagiocrines (*ῥαγιον* — a small grape seed) and he considers them as a variety of connective tissue cell which secretes granules, whereas the ordinary connective tissue cells do not, their cytoplasm being homogeneous. The rhagiocrines are derived from the large round cells of the peritoneum. In a later communication[7] he describes the rhagiocrines as of a phagocytic nature, since twenty-four hours after injection of lycopodium powder the rhagiocrines are found to contain particles of the powder. Renaut's

rhagiocrines are obviously identical with the cells of the milky spots previously described by us (figured on Plate VIII., 3). We have already ventured our opinion that these cells cannot be differentiated from the "clasmatocytes" of Ranvier or our "trailers." Both are derived from macrophages which work their way into the tissues and there present somewhat different appearances according to the nature of the tissue.

Marchand[8] is of the opinion that the clasmatocytes are a variety of connective tissue cells which have no connection with macrophages, but possess the property of becoming phagocytic under inflammatory conditions. The clasmatocytes occur chiefly in the adventitia of the blood vessels.

The cells of the peritoneal fluid. — We have already touched on this subject in Part I., but we may summarize again, since these cells are of importance when studied in connection with the omentum. Kanthack and Hardy have established the presence of small and large lymphocytes in the peritoneal fluid, and eosinophile leucocytes. Amphophile polynuclears, or the microxycytes of Durham, are normally absent or few in number. The large lymphocytes (hyaline cells) are abundant. They possess a large vesicular nucleus and homogeneous, slightly basophile cytoplasm.

The large round mononuclear cells previously described as occurring on the surface of the omentum we take to be identical with Kanthack and Hardy's hyaline cells, and we have already indicated the important part they play as phagocytes. Eosinophile leucocytes are also abundant, and it is no doubt due to this fact that so many can be found on the surface of an omentum spread.

The omentum under pathological conditions. — The omentum of a guinea-pig, as already mentioned, is blackened almost immediately, even in five minutes, after injection of lampblack intraperitoneally, and remains pigmented for months. Three months is the limit of our observations. It is then still black. Shortly after injection the guinea-pig is killed and a piece of the omentum spread out on a slide. In many places the appearance presented by the photograph

(Plate IV., 1) of an unstained specimen immediately strikes the eye. The lampblack is not scattered diffusely over the surface but appears as a network of irregular black lines. The same may be observed in a stained specimen after injection of typhoid or other bacilli into a rabbit or guinea-pig.

On focussing down on these lines with a one-twelfth oil immersion lens the particles of lampblack or bacilli are found to be lying free in what appear to be narrow channels. The particles are not enclosed in phagocytes, and in the case of the bacilli they are all pointing along the long axis of the channel as if passing along in a current. The photograph (Plate V., 1) is one of typhoid bacilli thirty minutes after injection into a rabbit, and Plate V., 2, coli communis fifteen minutes after injection into a guinea-pig.

At first it was thought that these apparent channels must be the swollen lymphatics of the omentum into which the particles or bacteria had forced their way, much as they appear to do in the diaphragm, but this was soon found to be a mistaken idea. The strands of the network are simply fibrin which has been deposited on the surface of the omentum and has caught up the particles or bacilli in it. There were several reasons for suspecting that these apparent channels are not in reality lymphatics. In the first place they often seem to run a little way and then suddenly come to an end. Again, the strands run quite independently of the omental meshwork, nor could any nuclei be discovered in them. Their real nature was finally determined by means of Weigert's stain for fibrin, to which they unmistakably responded. The photograph (Plate IV., 2) was taken from the omentum of a rabbit two hours after injection of typhoid bacilli where the strands of fibrin were numerous, but contained few bacilli. The amount of fibrinous deposit varies very much. It may be extensive but often is quite inconspicuous.

We have dwelt somewhat at length on this subject since we have been unable to find in the literature any clear description of the appearance of the fibrin. Pierallini, who was

referred to in Part I., merely speaks of masses of fibrin, though Ranvier[9] in a short communication mentions that after injection of silver nitrate into guinea-pigs there is a deposit of fibrin like a spider's web on the surface of the omentum. Ranvier, 1896, also says that the lymphatics of the omentum follow the blood vessels but do not extend into the meshwork. Since abandoning the idea that the network described above represents the swollen lymphatics, we have been unable to find the lymphatic channels by which the omental nodes, found in sections to be quickly blackened, are supplied. However, apart from the question of the initial rush of particles to the organs, the omentum plays a very prominent part in the local phagocytic processes of the peritoneal cavity, and this phase of the subject will now be fully discussed.

To follow the argument it will be necessary to refer briefly to the processes in the fluid of the abdominal cavity. We have already described how with lampblack, after a period of leucopenia, the microphages (microxycytes) first come in, and later the macrophages. The same occurs also after injections of bacteria, but with them we have not found it so easy to follow the processes in detail.

I. THE OMENTUM IN EARLY STAGES AFTER INJECTION.

a. Chicken red cells. Early stages. — We may first examine the omentum spread of a guinea-pig which has been injected a few hours (4–8) previously with chicken red cells. We find on the surface and in the meshes an enormous number of large, round macrophages, singly or in masses, stuffed full of chicken cells and some containing also polynuclear leucocytes, more or less degenerated, many of the macrophages clinging to the strands of fibrin (Plate X., 1).

Not only are the macrophages a prominent feature, but a great many of the trailers may now contain relatively large, dark blue, almost black staining granules as well as the usual small, black and red granules which we have already described in the normal omentum. Some of the trailers may contain only dark blue granules. One cannot help

suspecting that the macrophages have collected over the surface of the omentum with the object of penetrating into the tissues, but are unable to do so until they have so far digested the chicken cells that they can carry the remains in with them, or in other words, until only the nucleus of the chicken cell is left.

Once the macrophages have entered the meshwork they trail along the narrow spaces between the connective tissue bundles. Occasionally, though very rarely, indeed, one may find a trailer containing the remains of one or two chicken red cells still retaining their cytoplasm (as figured in Plate X., 1, on left), but such an appearance is so very uncommon that one would hardly be justified in concluding that the cells are actually macrophages, which after englobing red cells have become trailers, any more than we could be certain about this with the trailers of the normal animal.

The colored drawing (Plate X., 1) is taken from an omentum spread five hours after injection of chicken cells, but trailers containing nuclei and more rarely one or two degenerated chicken cells may already be seen in one hour after injection. One hour, however, is the earliest period at which such trailers can be found. In fifteen and thirty minutes there is generally a considerable deposit of fibrin in which are entangled masses of chicken cells and hyaline cells of the peritoneal fluid, but the latter have only to a slight extent begun to englobe the chicken cells. After one hour, however, phagocytosis by the macrophages is very marked.

b. Lampblack. Early stages. — The appearances on the omentum are somewhat different from those with chicken cells. We find almost immediately after inoculation the usual deposit of fibrin in which are entangled lampblack particles and hyaline cells of the peritoneal fluid (macrophages), but the latter are already packed with black particles. Indeed, they are packed so full that they appear to be unable to enter the tissues, and it is not until six hours after injection that trailers begin to make their appearance. At this period, however, we find our surmises as to the origin

of the trailers from macrophages fully confirmed. Lampblack being indigestible undergoes no change in the cells, and there can be no question whatever that the trailers actually contain particles of the pigment which cannot possibly be mistaken for anything else. We find also a number of pigment-laden intermediate forms between the round macrophages and the elongated trailers. The macrophages appear to send out protoplasmic processes which insinuate themselves between the lining endothelial cells and penetrate into the connective tissue meshwork, dragging the particles with them (Plate X., 2).

We mentioned in Part I. that the macrophages appear to englobe chicken cells with much greater avidity than particles of lampblack. From our earlier observations on the peritoneal fluid we supposed this to be the case and have left the paragraph there as it was originally written several months ago. Our studies of the omentum, however, have gradually led us to consider that the greater avidity for chicken cells is only apparent. A macrophage which has englobed a dozen chicken cells presents a very remarkable appearance, whereas a similar number of small lampblack particles in a macrophage is not very conspicuous. Moreover, when free in the peritoneal fluid the macrophages are to some extent at a disadvantage, and small particles are probably more elusive than large ones. It is a well-established fact that ameboid cells can englobe particles much more readily if they are clinging to a surface over which they can crawl than when they float free in a fluid. The surface of the omentum, therefore, affords a better idea of the capabilities of the macrophages than the peritoneal fluid. Indeed, the macrophages entangled in the fibrin have a double advantage over those free in the fluid. Not only are they themselves supported, but they are in close proximity to particles which are fixed in the fibrin and not floating free.

This affords a clue as to why phagocytosis of lampblack takes place so much more rapidly than that of chicken cells in this situation. The particles of lampblack are so small

that the macrophages can pick them up quickly, one by one, until they are stuffed full in a very short time. To ingest a large chicken cell a great deal more work has to be performed, so it is distinctly longer before the macrophages show much evidence of their activity with such cells. Once the chicken cells have been taken up, however, the macrophages digest them very speedily, and it is not long before the load is lightened and the macrophages are able to penetrate into the tissues and from trailers, whereas the lampblack particles remaining unchanged delay the entrance. In fact, we are inclined to suppose that the primary overloaded hyaline cells do not enter the tissue at all, but that the trailers seen in six hours after inoculation of lampblack are derived from a fresh supply of macrophages which are not overloaded like the primary ones (an overloaded macrophage is represented in Plate V., 4).

II. THE OMENTUM IN LATER STAGES AFTER INJECTION.

a. Chicken cells. — In twenty-four hours the picture has changed very considerably. The large masses of macrophages stuffed with chicken cells are no longer a prominent feature. A great number of macrophages may still be seen, but the chicken cells contained in them have greatly degenerated, and little is left but the nuclei. On the other hand, trailers are very abundant, and many of them contain dark blue granules, no longer recognizable as nuclei of chicken cells, but probably representing their fragments. The majority of the round macrophages which are still left on the surface show signs of degeneration. They stain badly and are often riddled with vacuoles, the vacuoles probably representing the localities of the digested chicken cells. It appears probable that such cells will not enter the tissues, but have perished after performing their work of digestion.

In forty-eight hours the omentum has almost resumed its normal appearance, but few macrophages can be seen; some contain remains of chicken cells and are apparently dead, but many, which for the most part stain readily, show no traces of them, and are probably late comers for whom no

extra work of a pathological nature remains to be done. The chicken cells have all practically been disposed of. It is naturally understood that the rapidity of the process depends to a great extent upon the amount of the dose injected. If a large dose has been given, the destruction of the chicken cells occupies a longer period, but the final result is the same.

The remarkable similarity of the processes on the omentum with those in the anterior mediastinal lymph nodes, previously discussed, may be noted.

b. Lampblack. Later stages. — Since lampblack is indigestible, the later appearances are very different from those met with in the case of chicken cells. In twenty-four hours and forty-eight hours we still find the round macrophages loaded with pigment on the surface in large quantities, but there is distinctly an increase in the number of trailers containing black particles. Microxycytes are still numerous in twenty-four hours, and a large proportion contain pigment, but in forty-eight hours show a considerable decrease.

As has been remarked, the omentum remains black to the eye for a long time, and under the microscope, even one month after injection, we still find on the surface the masses of round macrophages packed with pigment, but they appear to have migrated largely to the neighborhood of the capillaries and small vessels. Or possibly newly-formed capillaries may have made their way to the macrophages and there ramified through the masses. But we are inclined to think the former view the more probable (Plate IV., 5).

At this stage — one month — however, the trailers have changed their characteristics to a great extent. Most of them still contain pigment, but the particles are for the most part now distinctly lying in vacuoles, and have undergone fragmentation, so that each vacuole contains a great number of exceedingly minute black granules.

Plate IX., 3, shows two trailers one month after injection of lampblack. The cells contain large vacuoles in which are minute fragmented particles. The photographs (Plate

IV., 3 and 4) were also taken from a preparation at this stage. No. 3 shows very well the manner in which the trailers tend to range themselves along the long axis of the capillaries and small vessels, and No. 4 represents a trailer with a vacuole in which the particles are partially fragmented.

In many cases vacuoles may be seen which are nearly or quite empty. This probably represents the "clasmatosis" of Ranvier — the bursting open of the cytoplasm and liberation of the granules — see page 62. It is interesting to note also that at this stage many very minute free granules may often be seen, although we do not feel certain that they are particles scattered in consequence of clasmatosis. It seems probable that the processes of fragmentation and clasmatosis here described represent an effort on the part of the organism to rid itself of the particles. Being now in such a minute state of division they would be more readily excreted by various channels than when in their original condition.

The polynuclear leucocytes. — It remains only to say a few words about the polynuclear leucocytes. In the earlier stages practically no microxycytes are to be seen, although there may be a considerable number of eosinophile cells on the surface of the omentum, many of them entangled in the fibrin. The eosinophiles do not appear to take any prominent part in the processes described, although rarely they may be seen to contain a few particles of lampblack. This is in accord with the opinions of most authors who seem to be generally agreed that eosinophiles are only feebly, if at all, phagocytic. In Plate X., 2, an eosinophile containing particles of lampblack is figured, but this is an exceptional case. Throughout the whole period covered by our observations a few eosinophiles may be seen in the omentum. They do not appear either to increase or to decrease in number and the part they play is, so far as can be judged, a purely passive one.

Microxycytes, however, arrive in vast numbers, beginning to appear in about one hour after inoculation, and increasing

in number up to eight hours after injection of chicken cells. In sixteen hours they are already less numerous, and in forty-eight hours are scarcely to be found. Probably they reach their maximum in about twelve hours, but we have not bridged the gap between eight and sixteen hours.

After injection of lampblack the microxycytes persist much longer than after chicken cells, being still very numerous in twenty-four hours, although in forty-eight hours there are comparatively few. We are inclined to suppose that the microxycyte reaction is as great in one case as in the other, but lampblack being indigestible, the macrophages are not able to turn their attention to the microxycytes and englobe them so early as in the case of chicken cells. During the first few hours microxycytes may be seen streaming out from the small vessels and capillaries in vast numbers, often followed by masses of red cells, so that there is a distinct though slight hemorrhage following the track of the vessels. The microxycytes do not ingest entire chicken cells, although occasionally one or two nuclei may be seen inside them. Nor have we observed on the omentum appearance mentioned in Part I. of a microxycyte forming a center around which is grouped a mass of chicken cells with partially eroded cytoplasm. But free chicken cells are rarely seen on the surface of the omentum after the first hour. By the time the microxycytes arrive all the chicken cells entangled in the fibrin have been englobed by the macrophages.

The microxycytes, however, take up lampblack readily, and between four and eight hours after injection a large proportion of them contain particles. But they do not gorge themselves as do the macrophages. After taking up a few particles their activity seems to become exhausted and they then, so far as can be judged, remain passively inert, waiting to be taken up, particles and all, by the macrophages, so soon as the latter arrive in sufficient quantity. If there is any other physiological reason for their existence besides that of acting as assistants of the macrophages, it is not apparent from an examination of the omentum spreads.

CONCLUSIONS.

1. Almost immediately after injection of inert particles into the peritoneal cavity of a guinea-pig there is a deposit of fibrin formed on the surface of the omentum in which the particles and phagocytic cells of the cavity become entangled.

2. The phagocytic cells (macrophages) rapidly englobe the particles, becoming filled with small particles like lampblack within ten minutes, and with chicken red cells in about an hour.

3. If not overloaded the macrophages then enter the tissues and appear as long trailers or clasmatocytes.

4. If the particles are digestible, *e.g.*, chicken red cells, they are rapidly disposed of by the phagocytes.

5. If the particles are not digestible, *e.g.*, lampblack, they remain inside the macrophages and trailers for months. The particles in the trailers become fragmented, and after a time are discharged as minute fragments (clasmatosis of Ranvier).

6. The macrophages are the most active agents of phagocytosis on the omentum, the polynuclear leucocytes playing a minor part.

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ABSORPTION OF PARTICLES FROM THE PERITONEAL CAVITY.

PART V. — *The function of the omentum, continued.*

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For the experiments detailed in the previous section guinea-pigs were used, but in making parallel tests with typhoid bacilli, omentums of rabbits, the organs of which were studied in Part II., have been employed. The omentums of these rabbits, with the exception of a few of the earlier ones, were cut out and dropped into one per cent formalin, a spread being made about two hours later, after the plating out of the organs was finished. The spreads were dried and stained with eosinate of methylene blue. No special fixation is necessary, and the stained specimen can be mounted in Canada balsam.

The processes on the omentum of a rabbit which has been inoculated with bacilli are somewhat more complicated than with the guinea-pig, on account of the presence of the milky spots (Plate III., 1, 2, 3) in which Renaut's rhagiocrines, our modified trailers (depicted in Plate VIII., 3), are to be found in great abundance. These modified branching trailers often appear to become filled with bacilli almost immediately after injection of typhoid, and one of the objects of this study has been to attempt to determine if the original branching cells of the milky spots are actually of a phagocytic nature, or if those which are seen to contain bacilli after inoculation are newly formed branching cells derived from macrophages which have englobed bacilli and become quickly transformed. In addition to this there are many other interesting points which arise in the course of the study and these will also be discussed in detail.

The processes in the fibrinous deposit. Early stages. — Shortly after injection of typhoid bacilli there is the usual deposit of fibrin on the surface of the omentum, in which

may be entangled large masses of bacilli pointing chiefly along the long axis of the strands. This appearance has already been commented upon (page 64, Plate V., 1 and 2). If, according to the evidence on the plates, the bacilli appear to have been destroyed explosively (page 30), none can be seen entangled in the fibrin, and the deposit of fibrin itself is usually scanty. We shall return to this aspect of the phenomena later on, confining ourselves for the present to the first mentioned instances in which intact bacilli are entangled in the fibrin.

The bacilli at first lie free in the fibrinous deposit, but by degrees are taken up by the macrophages entangled alongside of them. This englobement is rarely complete in the earlier stages, the bacilli being far too numerous for the original macrophages to deal with. The macrophages already present, however, very quickly become filled with bacilli, and a single one may often contain fifty or more within half an hour after injection.

One of two things may now happen:

1. The bacilli may become pale staining or granular. Corresponds to few in the organs.

2. The bacilli may remain intact and increase in the fibrin. Corresponds to many in the organs.

The specimens naturally vary greatly, but in omentum spreads four to six hours after inoculation one or the other of these phenomena will be in the ascendant.

1. The bacilli become pale staining or granular. — In this case the bacilli will be found in the interior of the macrophages and perhaps also to some extent in microxycytes, which by this time will have arrived in considerable numbers. Probably no free bacilli will be found in the fibrin, and those in the cells appear either pale staining and somewhat swollen, or else as small, deep blue-staining granules, representing shrunken and distorted bacilli.

In the wash water and organs are few or no bacilli.

2. The bacilli remain intact and increase in the fibrin. — The main feature after four to six hours is the numerous, roughly spherical, packed masses of bacilli (Plate V., 3).

Some of the groups appear to be agglutination centers, in which the bacilli are now growing freely. Others appear to have started in the interior of macrophages, or occasionally in microxycytes; the phagocytes having probably taken up more bacilli than they could dispose of. Such cells often present a very remarkable appearance, being literally packed with vigorous bacilli, which stain perfectly and are evidently rapidly multiplying. When the phenomenon of packed masses of bacilli is predominant, there is usually relatively little microxycyte reaction.

In the wash water and organs bacilli are numerous.

It was noted in Part II. that rabbits killed at four and six hours after inoculation show much higher averages of living bacilli in the peritoneal cavity than those killed in one and two hours. The phenomena just detailed appear to support the correctness of the earlier observations. After inoculation there is probably an immediate reaction on the part of the animal, during which the greater part of the bacilli are destroyed, either explosively or during the first hour or two. In many cases, however, the reactive power becomes exhausted and those bacilli which remain alive are able to multiply again, not only when free in the peritoneal cavity, but also on the surface of the omentum, even though they have been taken up by the phagocytes.

The processes in the fibrinous deposit. Later stages. — Much the same condition as for four to six hours may hold good up to sixteen hours; the main feature may be granule formation or increase of bacilli in the fibrin. From what has been observed to occur it seems certain that those rabbits in which secondary multiplication of bacilli persists up to sixteen or twenty-four hours would succumb. In fact, on the omentum of one rabbit, which died twenty-three hours after inoculation (Part II., Table XI., 6), the phenomena of packed macrophages and dense masses of bacilli in the fibrin were present to an exaggerated degree. There was little microxycyte reaction.

The fibrin after sixteen and twenty-four hours in most cases is swollen, with indistinct outlines, and is surrounded by microxycytes which appear to be attacking it (Plate V., 5).

In forty-eight hours only traces of fibrin, packed with microxycytes, are left. If any bacilli can be seen at all they are pale or granular. It may be noted that rabbits killed forty-eight hours after inoculation are always on the highroad toward recovery. If inoculation is followed by death it always occurs within twenty-four hours.

Phagocytosis on the meshwork. — The foregoing descriptions refer to the processes as seen in the fibrinous deposit. Scattered over the meshwork will also be found a few macrophages, intermediate forms and trailers, which contain bacilli in all stages from intact to pale staining and granular. The condition of the bacilli contained in the phagocytes corresponds in each specimen with that in the phagocytes of the milky spots, in the account of which further description of the processes on the meshwork will be included.

Phagocytosis on the milky spots. Early stages. — The bacilli appear to be caught up and fixed over the milky spots in enormous numbers, and it is here that phagocytosis is most pronounced. In the macrophages and branching cells may be found bacilli, either intact or granular, the appearances in each particular instance corresponding very closely with those observed in connection with the fibrin. If granules predominate in the phagocytes of the fibrin they will predominate also in the cells of the milky spots, and the same applies if the bacilli remain intact.

We may now consider a typical case in which the rabbit was bled from the carotid immediately after inoculation (Table IX., 1, in Part II.) and the omentum removed as quickly as possible after the peritoneal cavity had been washed out. The rabbit was dead in ten minutes after inoculation and not over ten minutes more had elapsed before the omentum was taken out. The wash water contained many millions, and the organs were swarming with bacilli.

There is not much deposit of fibrin at such an early stage, but over the milky spots we find immense masses of intact bacilli, which stain well and give no evidence of granular degeneration. Closer examination shows that the bacilli are for the most part already enclosed in cells. Not only do the round macrophages on the surface of the milky spots contain enormous numbers, but a large proportion of the branching cells — the modified trailers — are also in the same condition. It is difficult to suppose that the macrophages have enclosed so many bacilli and transformed themselves to such a marked extent into branching cells in this short space of time. It appears more probable that the branching cells of the milky spots are themselves of a phagocytic nature. This supposition would lead us to infer that these cells are only partially fixed or anchored in the milky spots, from which position they send out processes, which, waving free in the peritoneal cavity, pick up bacilli floating in the fluid. There is no direct proof that this is actually the case, but it seems to be the best explanation of the phenomena observed.

It is true, however, that macrophages can transform themselves into trailers very quickly. This becomes evident on inspection of the meshwork, where even at this early stage transitional forms may be seen. The photograph (Plate VI., 3) shows early transitional forms, but many cells may be found in a much more advanced stage, and even a few typical trailers containing intact bacilli. Plate XI., 3, is a composite picture from the same specimen taken from three or four different fields of the microscope.

The transformation into trailers is effected much more rapidly when the macrophages contain bacilli than when they are loaded with unyielding masses of lampblack. It was observed in Part IV. that trailers containing lampblack do not appear in the meshwork earlier than six hours after inoculation into a guinea-pig.

The foregoing descriptions are typical of appearances on the omentum in any instance where large quantities of bacilli are found "at once" in the wash water and organs.

We may now consider instances in which the bacilli are

destroyed explosively, as recorded in Table IX., 5, 6, 7. No intact bacilli are to be found over the milky spots or elsewhere. Both macrophages and branching cells of the milky spots, however, may contain great numbers of ragged red and black granules with ill-defined margins and varying greatly in size. Such granules it is impossible to recognize as bacilli, but they undoubtedly represent remains of bacilli, since such a picture is not seen with the normal animal in the cells of which the granules are scanty and well defined. This rapid destruction may appear to have mainly taken place either extracellularly or intracellularly.

Extracts from notes of two cases tabulated in Part II. may be cited.

1. Table IX., 5. — No free bacilli or granules. Macrophages and cells of milky spots crowded with reddish indefinite granules (Plate XI., 2).

Wash water, 350,000. Organs, 0.

2. Table IX., 7. — Immense numbers of free bacilli on surface of milky spots and meshwork. The bacilli are shrunken and degenerated. Little evidence of phagocytosis (Plate XI., 1).

Wash water, 1,000. Organs, 0.

Unquestionably in the second instance the bacilli have been destroyed extracellularly, and it seems probable that this also occurred in the first case, the bacilli being then rapidly englobed and transformed into granules inside the cells.

Plate XI., 1. — Shows very well the way in which free bacilli are sometimes caught up in the meshwork lining the open spaces. The form of degeneration in this case is somewhat unusual, and probably represents a stage between the pale-staining, swollen bacilli, and the deep blue, small granules. The basophile substance seems to be massed in the center of each bacillus, leaving a pale-staining sheath.

Phagocytosis on the milky spots and meshwork. Later stages. — The bacilli caught up in the milky spots appear in later stages to undergo much the same fate as already

described for those in the fibrin. In four and six hours there may be a decided microxycyte reaction, and in such cases the phagocytic cells may contain pale-staining bacilli or granules. Sometimes, however, practically no bacilli or granules can be found at all. This condition probably represents an effective initial destruction of the bacilli. The pale-staining bacilli or granules observed in analogous cases, "at once" (*e.g.*, Table IX., 5, 6, 7), having been cleared off by this time.

The following are typical instances:

Four hours, Table II., 6. — Microxycytes plentiful. A few macrophages and trailers containing small granules. No intact bacilli.

Wash water, 4,000. Organs, 0.

Six hours, Table III., 6. — Microxycytes very numerous. No small granules or bacilli, either free or in phagocytes.

Wash water, 0. Organs, 0.

Generally, however, at four and six hours the microxycyte reaction is not yet very pronounced, and in such cases, although most of the phagocytic cells contain pale-staining bacilli or granules, there are sure to be some macrophages which are becoming packed with growing bacilli, and also probably a few masses of agglutination centers. It is not often that the animal is able entirely to prevent secondary multiplication. The secondary multiplication, however, does not often last long, for, in the rabbits killed sixteen hours after inoculation, it was evident in only one case out of five. In four cases there was a good microxycyte reaction, but in the fifth little reaction, while centers of multiplication were numerous and far advanced. The temperature, moreover, had fallen from 102° to 99°, and this rabbit (Table IV., 1) would undoubtedly have died in a few hours.*

Of six omentums examined twenty-four hours after inoculation, not one showed any centers of multiplication. A few pale-staining bacilli and granules might be found in some specimens, but in all of them there were swarms of

* The temperatures of our normal rabbits varied from 100° to 103°. If, after inoculation, there was a fall below 100° which persisted, the rabbit died.

microxycytes. All of these rabbits would certainly have lived. It has already been mentioned that in one rabbit which died in twenty-four hours centers of multiplication were numerous. After forty-eight hours bacilli or granules can rarely be found at all, and there is generally a considerable decrease in the number of microxycytes. The rabbit has overcome the infection and has practically recovered. Nevertheless, the surface of the omentum usually shows marked changes which may be considered under the next heading.

The endothelial and connective tissue cells of the omentum. — The endothelial lining cells normally possess large, pale-staining, vesicular nuclei. During the first hours succeeding inoculation the cells undergo little or no change, but in sixteen and twenty-four hours the nuclei are usually much swollen and often project into the meshes (Plate VI., 4). Durham considers that the swollen endothelial cells peel off from the surface and become phagocytic — equivalent, therefore, to macrophages. We have never seen any evidence that this is the case, and are strongly of the opinion that the endothelial cells of the peritoneum have no phagocytic attributes whatsoever. Ricoux* mentions that he has never found grains of carmine in the interior of endothelial cells of the peritoneum.

In forty-eight hours after injection of chicken red cells into a guinea-pig the omentum, as already remarked (page 68), has almost resumed its normal appearance, and the same may be said after the injection of lampblack, although with the latter macrophages and trailers containing pigment are abundant.

Typhoid bacilli, however, in forty-eight hours after injection into a rabbit show evidence of having caused much more serious inflammatory processes on the omentum. Microxycytes and macrophages have decreased in number, but the omentum is generally much thickened, the meshwork being entirely obscured, or very nearly so. The connective tissue

* Thèse de Paris, 1898, p. 31.

appears edematous, and perhaps increased in amount, and is traversed in all directions by newly formed capillaries. The endothelial nuclei are very indistinct, and may not be distinguishable at all. In the connective tissue are very large numbers of branching, apparently connective tissue formative cells, far more numerous than in the normal animal.

Ranvier observed similar phenomena as occurring after injections of silver nitrate and considered that the endothelial cells transform themselves into connective tissue cells. We have never found any evidence that this is the case, and, knowing that the macrophages containing granules of any kind can enter the tissues and become trailers, it is rather natural to infer that macrophages without granules may enter also and become branching formative cells.

Relations of the macrophages, trailers, and connective tissue cells. — We do not feel at all certain that the macrophages, once they have entered the tissue and become trailers, can be sharply differentiated from ordinary connective tissue cells. If the trailers continue their ameboid movements after having entered the tissue and gradually carry off particles to the spleen or lymph nodes, where they resume their existence as macrophages, they must do so very slowly, since in one or two months after injection of lampblack the trailers show evidence of having existed as such for a considerable time. The fragmentation of the particles and the clasmatosis, commented upon on page 69, point to this conclusion.

It may be, then, that the very numerous branching cells found forty-eight hours after injection represent formative cells derived from macrophages which have not picked up anything in the shape of granules from the peritoneal fluid, or have already disposed of such granules.

To summarize this point: we have found no evidence that endothelial cells and macrophages are interchangeable, but it is by no means impossible that macrophages, trailers, and connective tissue cells are all one and the same cell under different conditions of environment, acting as scavengers or formative cells, according to the needs of the organism.

A few observations bearing on this subject may be quoted from the literature.

Dominici (Arch. de Med. Exp., Vol. 14, 1902, p. 20) concluded from studies of the omentum under mild inflammatory conditions that macrophages and connective tissue cells are identical. He agrees, however, with Ranvier that the endothelial lining cells of the omentum can transform themselves into connective tissue cells.

Maximow (Ziegler's Beiträge, Vol. 35, 1904, p. 93) provoked inflammation of connective tissue in rabbits and white rats, and decided that the chief part of regeneration is due to proliferation of preëxisting fibroblasts, but the possibility of mononuclear wandering cells also transforming themselves into fibroblasts cannot be excluded.

Borrel (Annales Pasteur, Vol. 7, 1893, p. 593) induced tuberculosis of the lung by means of intravenous injection and, from a study of the lesions at various intervals, arrived at the conclusion that after primary invasion by polynuclears the tubercles are formed by macrophages, which, on their first arrival, pick up the polynuclear cells, and after digesting these, transform themselves into the spindle-shaped epithelioid cells.

The part played by the microxycytes. — We have remarked in Part IV. that the microxycytes do not englobe chicken cells, but will take up a little lampblack, indigo or other small particles, and then appear to remain passive until they deliver themselves up, particles and all, to be englobed by the macrophages. This process is not so readily followed with typhoid bacilli. Englobement of bacilli or granules by microxycytes is not always to be seen on the surface of the omentum, but from what has already been said, it has become clear that if microxycytes do not arrive in great numbers within four to six hours after injection, the macrophages which have already taken up living bacilli seem incapable of digesting them effectively, and centers of secondary multiplication become more or less manifest. It appears from a close study of large numbers of omentum spreads that the

microxycytes themselves are of minor importance as phagocytes in the disposal of typhoid bacilli, but in some way or other assist the macrophages in their work of destroying living bacilli, possibly by virtue of some secretion.

This property of the microxycytes may not apply so forcibly in cases where the granules are formed very quickly after inoculation, although even in such specimens a marked influx of microxycytes is always to be seen.

Whenever microxycytes, after four to six hours, have arrived in great force, the granules always greatly predominate over intact bacilli. Sometimes intact bacilli cannot be found at all, and even the granules are comparatively scanty. In such cases englobement of the microxycytes by the macrophages will be found to have commenced; a feature which becomes much more conspicuous in sixteen, twenty-four, and forty-eight hours, when all stages from the round macrophages to the typical trailer, containing one to four or five microxycytes, may often be traceable within a very few fields of the microscope. Plate IX., 1, showing transitional forms, was taken from a specimen sixteen hours after inoculation.

A frequently noticeable feature in spreads made in sixteen to twenty-four hours is an attack upon the fibrin strands by the microxycytes. Plate V, 5, shows this very clearly, the fibrin appearing swollen with irregular outlines. Clinging to it are swarms of microxycytes, whose business appears to be solely to destroy the fibrin, since the bacilli and granules had already entirely disappeared in this particular specimen.

The granules. — That typhoid bacilli after injection into the peritoneal cavity do not so readily become granular, as is the case with cholera bacilli, is generally recognized, and it is not necessary to refer to the numerous observations which have been reported. Nevertheless, as we have already learned, on the omentum a change of this nature may often be observed. The bacilli at first preserve their natural form, but take the blue stain slightly, and appear pale and often somewhat swollen. They then seem to shrink into small, deeply-staining

granules, which later lose their basic properties and are found as somewhat indefinite reddish masses, chiefly inside the trailers and cells of the milky spots. The transformations occur generally within the phagocytes, but degeneration of the bacilli can sometimes be seen to have occurred extracellularly. This is often evident in the earliest stages after inoculation when there has been explosive destruction, and has already been commented upon.

In addition to the small granules which are obviously degeneration forms of the bacilli, since all intermediate forms can be traced, other very large, almost black granules may occasionally be found in enormous numbers contained within macrophages or trailers (Plate VI., 1, 2). It was at first supposed that these granules represent a peculiar form of degeneration of typhoid bacilli, but this was a mistake, since they have also been observed in one instance on the omentum of a rabbit which had received no injection and was intended as a control. The liver of this rabbit presented an extreme case of coccidiosis, and in all of the four specimens where such granules have been a prominent feature, there has been some lesion of the liver observed. We are of the opinion that the large black granules indicate some previous pathological lesions, débris from which has found its way into the peritoneal cavity.

Leucopenia. — The question of the leucopenia occurring in the peritoneal fluid shortly after injections was touched upon in Part I., and may be considered here in relation to the appearances presented by the omentum.

Opinions are divided as to whether leucopenia is due to actual destruction of cells (Metchnikoff) or to a clumping of the cells and their deposition on the omentum or other peritoneal surfaces (Durham). We have never during our studies of the omentum met with any evidence that the hyaline cells or the eosinophiles — that is to say, the principal cells of the normal peritoneal fluid — degenerate or break up. On the contrary, immediately after injections, we find both kinds of cells entangled in enormous numbers in the

fibrin, and they can also be observed scattered freely over the meshwork, and over the milky spots.

All these cells, wherever found, appear perfectly normal, and the hyaline cells (macrophages) are obviously in an exceedingly active condition. The cells appear to be simply deposited on the omentum; whether they are swept to it mechanically, or exercise some selective power of their own, it is impossible to say.

Before concluding, a few more words may be said about the milky spots, since it has become evident in the course of the discussion that they play an important part in the disposal of typhoid bacilli.

1. The milky spots and inert particles. — One experiment was made with lampblack, the rabbit being killed one hour after injection. The same features predominated as after injection of bacilli. Under a low magnification the milky spots appear dotted with black (Plate VI., 5), and examination under a high power shows that the pigment is partly contained within cells, while some lies free on the surface. The round macrophages are packed with black granules, and many of the branching cells also contain considerable quantities of pigment.

Comparatively little free pigment can be found over the meshwork, and it appears certain that a milky spot, apart from the phagocytic nature of its cells, is able to fix free particles or bacilli on its surface.

2. The nature of the milky spots. — In its earliest stages a milky spot appears to be formed by macrophages which enter the tissue and become partially fixed as phagocytic branching cells. Small lymphoid cells then appear and group themselves in the center of the spot, followed by the capillaries, which work their way through the meshwork to each milky spot as it increases in size. In short, the fully formed milky spots possess histologically all the elements of a lymph follicular structure, and our studies of the milky spots under pathological conditions have made it evident that they also fulfil the functions of lymph follicular tissue.

It may be mentioned that in the course of these studies we have examined the omentums of about ninety rabbits and seventy-five guinea-pigs.

CONCLUSIONS.

After intraperitoneal injection of typhoid bacilli into a rabbit:

1. The bacilli become fixed in immense numbers on the surface of the omentum.

2. Some may lie free in the fibrinous deposit or over the surface of the milky spots.

3. Some may be contained in macrophages.

4. The bacilli may be rapidly destroyed either extracellularly or intracellularly, or may partly remain intact for some time.

5. If they are rapidly destroyed there is a good microxycyte reaction in four to six hours.

6. If in four to six hours the microxycytes do not arrive in great numbers, secondary centers of multiplication become evident.

7. The secondary centers of multiplication are no longer present in sixteen or twenty-four hours, if the rabbit appears to be recovering.

8. If the rabbit is dying or dies between sixteen and twenty-four hours secondary centers of multiplication are marked features.

9. As with inert particles, the macrophages take the chief part in phagocytosis. Nevertheless, the macrophages cannot effectively dispose of the bacilli unless there is a considerable microxycyte reaction.

10. The stages of degeneration of the bacilli appear to be:—

    1. Pale staining and swollen.
    2. Aggregation of chromatin in center, leaving pale-staining sheath.

3. Small basophile granules.
4. Indefinite eosinophile granules.

1, 2, 3 have been observed to occur extracellularly.

1, 3, 4 have been observed to occur intracellularly.

(We are greatly indebted to Dr. Martha Tracy for the photographs and colored drawings which accompany this series of articles.)

The Journal of Medical Research, Vol. XV., No. 1, July, 1906.

PLATE I.

FIG. 1. Lampblack particles in microxycytes. Two hours after injection. x 1,500.

FIG. 2. Macrophages containing microxycytes. Twenty-four hours after injection of lampblack. x 1,000.

FIG. 3. Macrophages containing lampblack particles. Some remains of microxycytes are barely visible. Twenty-four hours after injection. x 1,000.

FIG. 4. Chicken red cells surrounding a microxycyte. The cytoplasm of the chicken cells adjacent to the microxycyte appears eroded. The microscope shows that the central cell around which the chicken cells are grouped is a microxycyte, although it is not clear in the photograph. Four hours after injection. x 1,200.

FIG. 5. Macrophage englobing a number of chicken cells. Twenty-four hours after injection. x 1,200.

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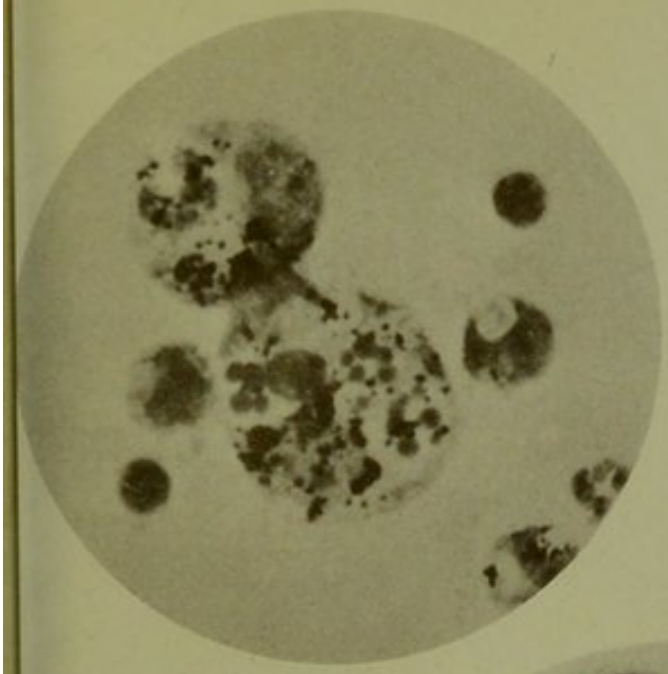
Fig. 5. Macrophage englobing a number of chicken cells. Twenty-four hours after injection. x 1,200.



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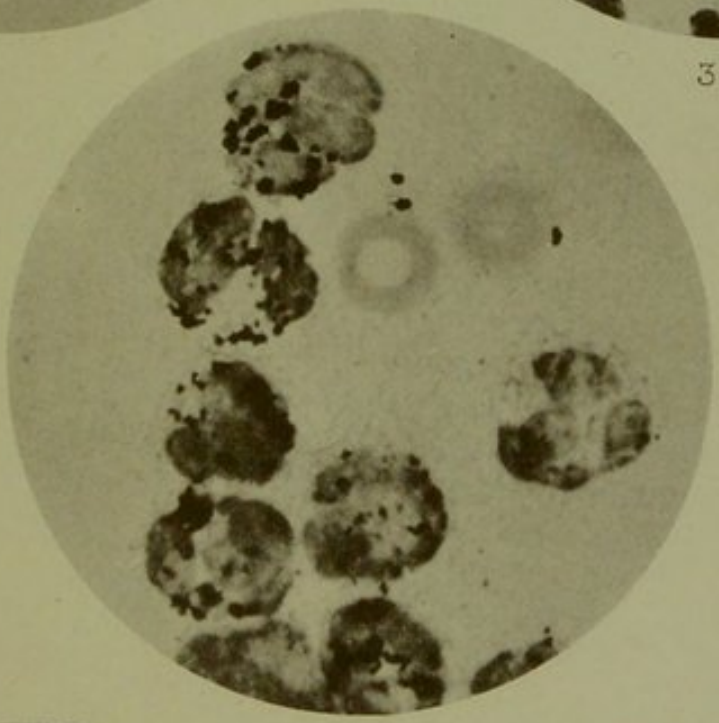

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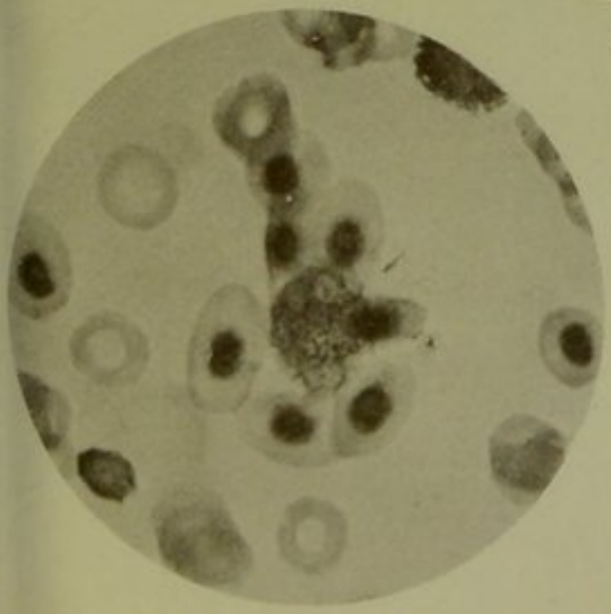

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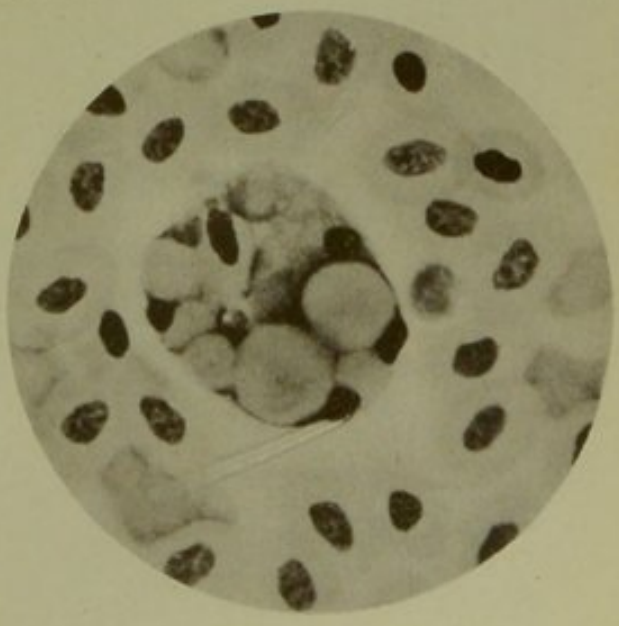

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PLATE II.

(Sections of anterior mediastinal lymph nodes after injection of lamp-black into guinea-pigs.)

FIG. 1. *Fifteen minutes after injection.* x 25. The pigment is massed chiefly in the afferent lymphatic plexuses. There is very little in the interior of the node, except in some of the larger sinuses.

FIG. 2. *Twenty-four hours after injection.* x 25. The pigment lies chiefly in the interior sinuses.

FIG. 3. *Fifteen minutes after injection.* x 800. From the afferent lymphatic plexus. The pigment lies free in the channels.

FIG. 4. *Twenty-four hours after injection.* x 800. From an interior sinus. The pigment is chiefly contained *in* macrophages.

FIG. 5. *Fifteen minutes after injection.* x 800. Efferent duct of a lymph node filled with free particles.

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Fig. 5. Fifteen minutes after injection. x 800. Efferent duct of a lymph node filled with free particles.

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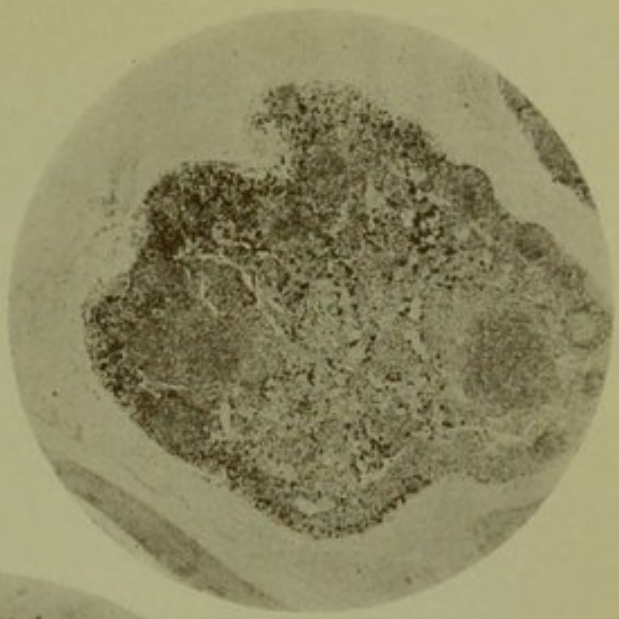

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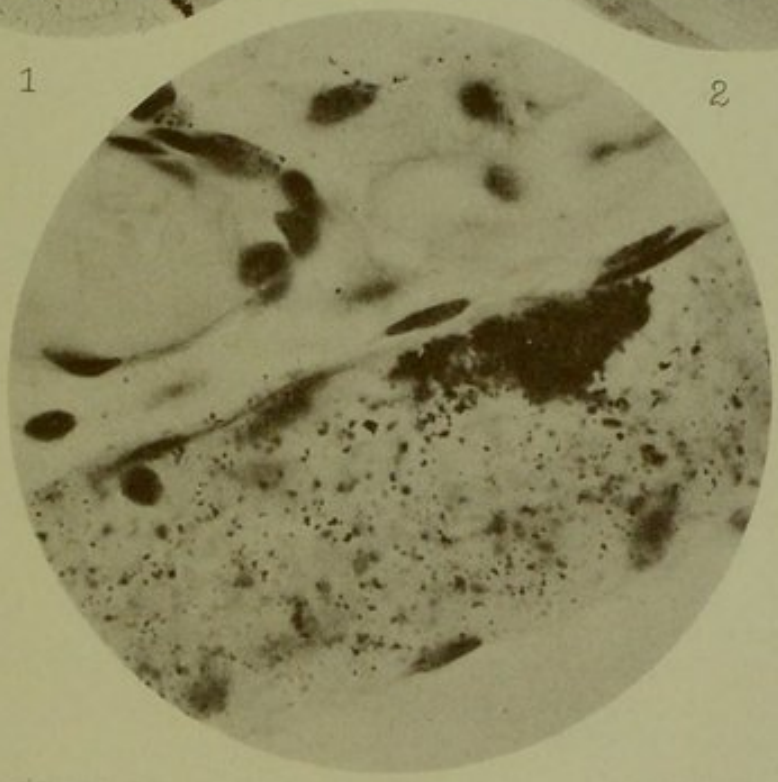

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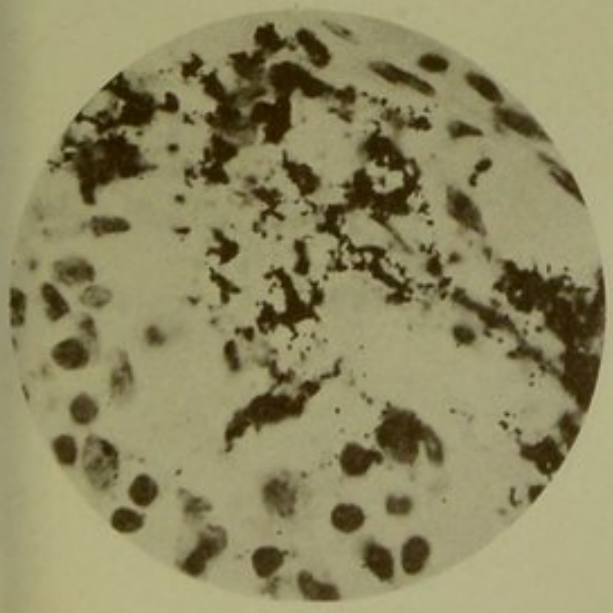

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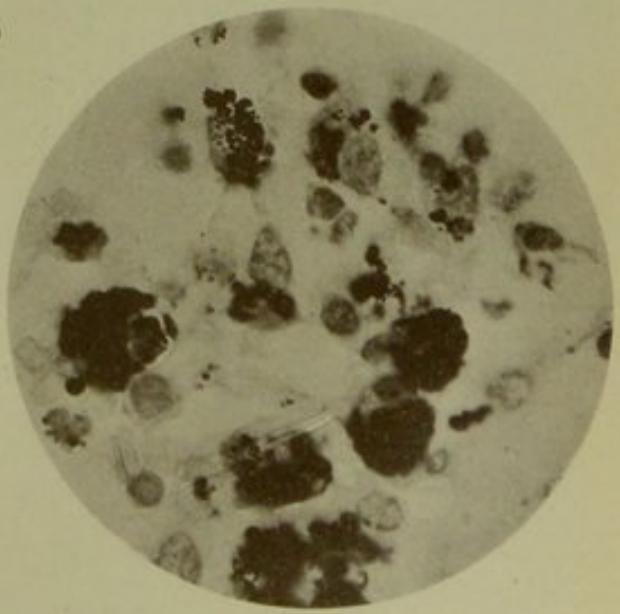

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PLATE III.

(The normal omentum of a young rabbit.)

FIG. 1. A large omentum spread. × 18. A tracery work of milky spots indicates the course of the future blood vessels. The capillaries are working their way from the main vessels towards the recently formed milky spots.

FIG. 2. A milky spot without capillaries. × 25. The deeper shade of the center is due to the presence of small lymphoid cells.

FIG. 3. A milky spot with capillary network. × 25. The artery and vein enter the milky spot together and from the opposite side capillaries are leaving the network to work their way towards the next milky spot.

Plate III.

(*The normal omentum of a young rabbit.*)

Fig. 1. A large omentum spread. x 1½. A tracery work of milky spots indicates the course of the future blood vessels. The capillaries are working their way from the main vessels towards the recently formed milky spots.

Fig. 2. A milky spot without capillaries. x 25. The deeper shade of the center is due to the presence of small lymphoid cells.

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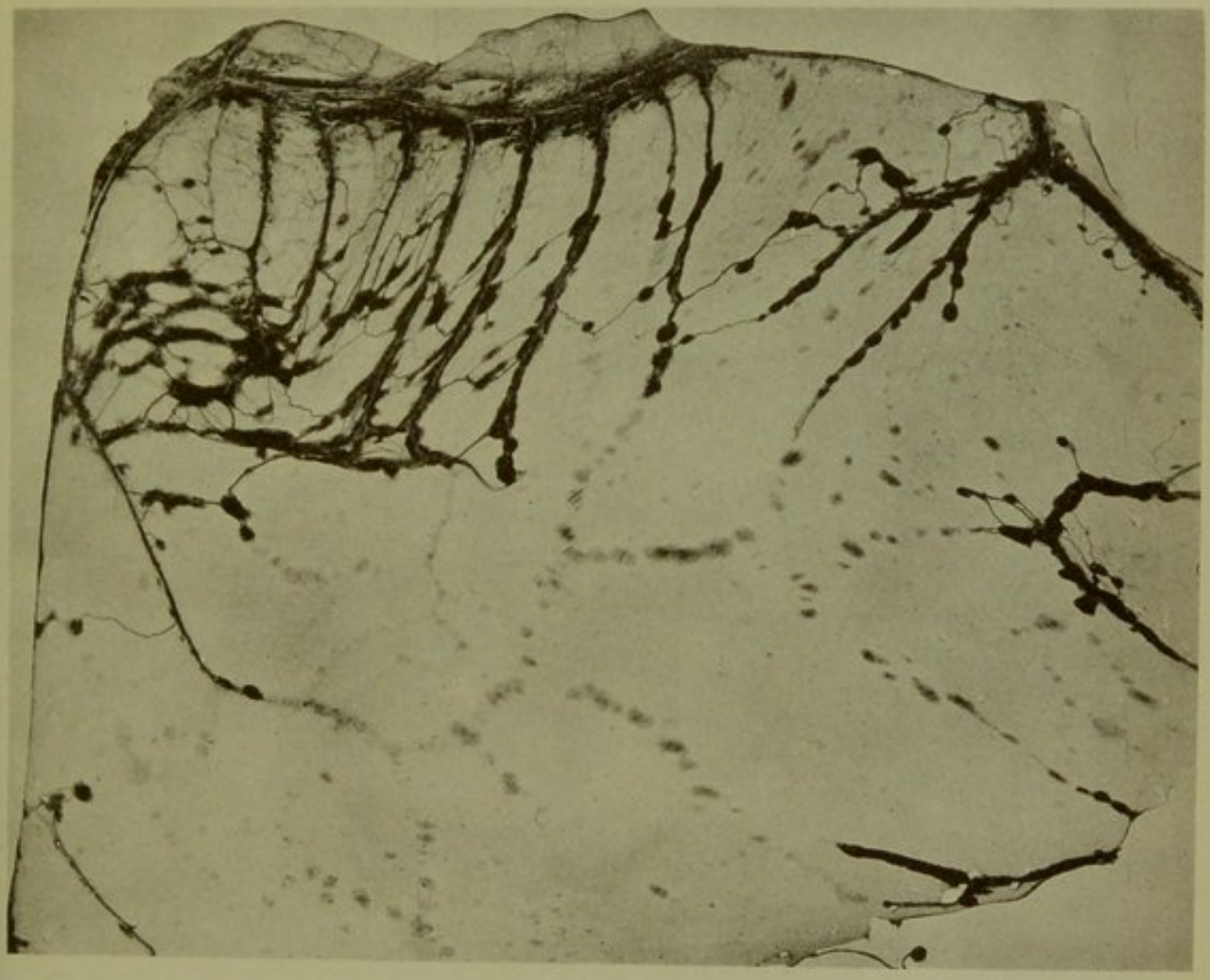

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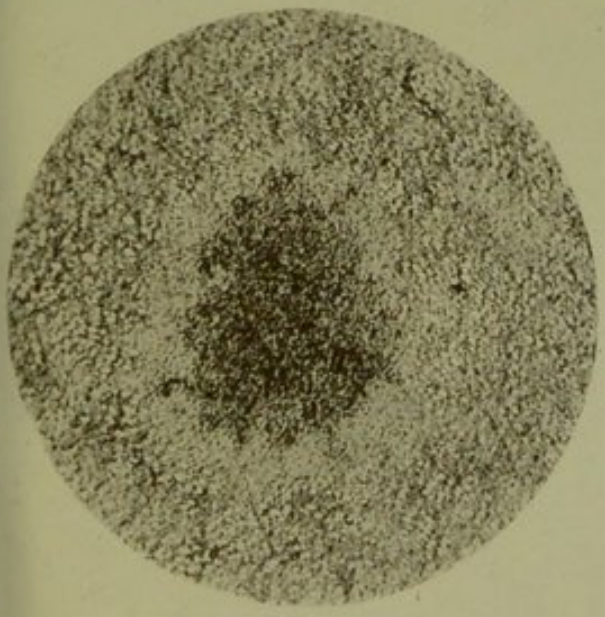

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PLATE IV.

(The omentum under pathological conditions.)

FIG. 1. Lampblack in fibrin network. × 25. Thirty minutes after injection into a guinea-pig. Unstained specimen.

FIG. 2. Fibrinous deposit. Weigert's stain. × 25. Two hours after injection of typhoid bacilli into a rabbit.

FIG. 3. Trailer lying along capillary. × 900. One month after injection of lampblack. The particles have become fragmented and are lying in a vacuole, the outline of which is not distinct. The two long processes of the trailer show clearly.

FIG. 4. Trailer in meshwork. × 900. One month after injection of lampblack. The particles are clearly contained in a vacuole, and are partially fragmented. The nucleus of the trailer is more deeply stained than those of the endothelial cells lying around it.

FIG. 5. Lampblack massed around capillaries. × 50. One month after injection. The particles are all contained within macrophages.

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Fig. 5. Lampblack massed around capillaries. x 50. One month after injection. The particles are all contained within macrophages.

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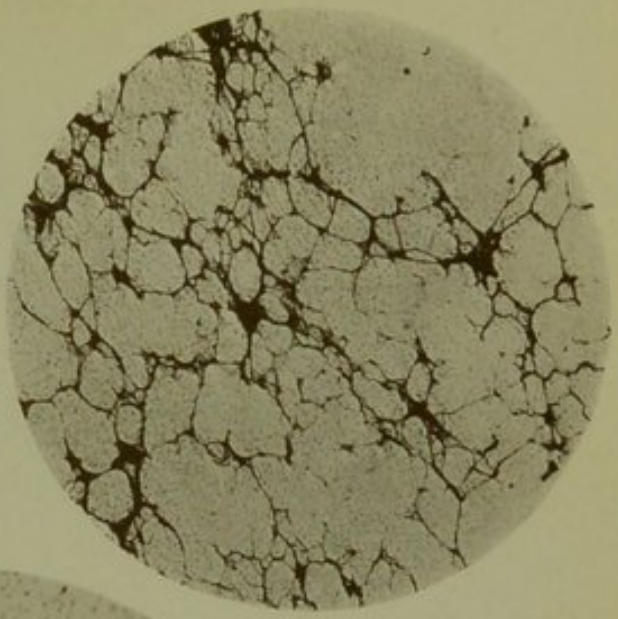

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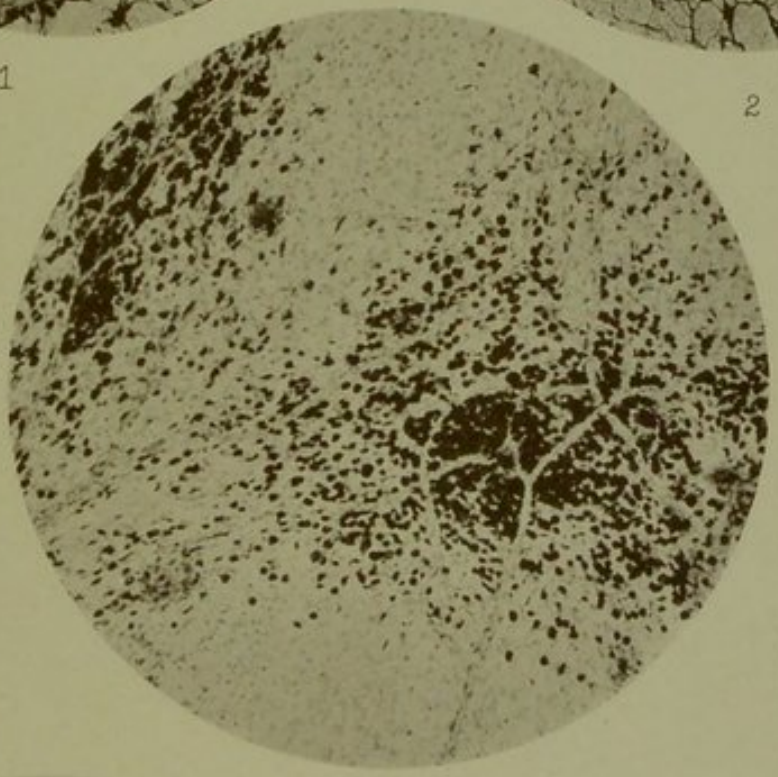

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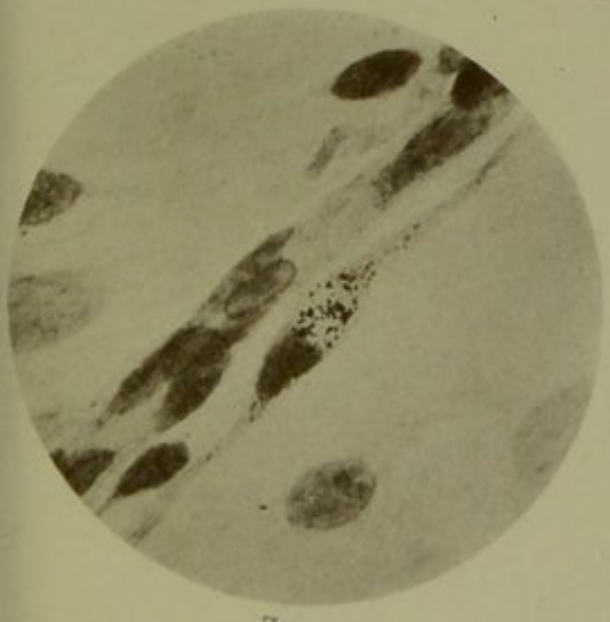

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PLATE V.

(The omentum under pathological conditions.)

FIG. 1. Typhoid bacilli in fibrin. × 1,000. Thirty minutes after injection into a rabbit. The bacilli lie free on the fibrin, pointing along the long axis of the strand.

FIG. 2. Bacillus coli communis in fibrin. × 1,000. Thirty minutes after injection into a guinea-pig.

FIG. 3. Typhoid bacilli in fibrin. × 1,000. Six hours after injection into a rabbit. Centers of secondary multiplication. Some centers are forming in the interior of macrophages, and some are starting from agglutination clumps, but this difference cannot be made out in the photograph.

FIG. 4. Indigo in phagocytes. × 1,000. Twenty-four hours after injection into a guinea-pig. Below is a macrophage packed with pigment, and above a microxycyte also containing pigment. The trailer between these two cells contains less pigment than the macrophage. Probably the latter is packed so full that it will be unable to enter the tissues and transform itself into a trailer.

FIG. 5. Fibrin attacked by microxycytes. × 50. Twenty-four hours after injection of typhoid bacilli into a rabbit. Both bacilli and granules have entirely disappeared.

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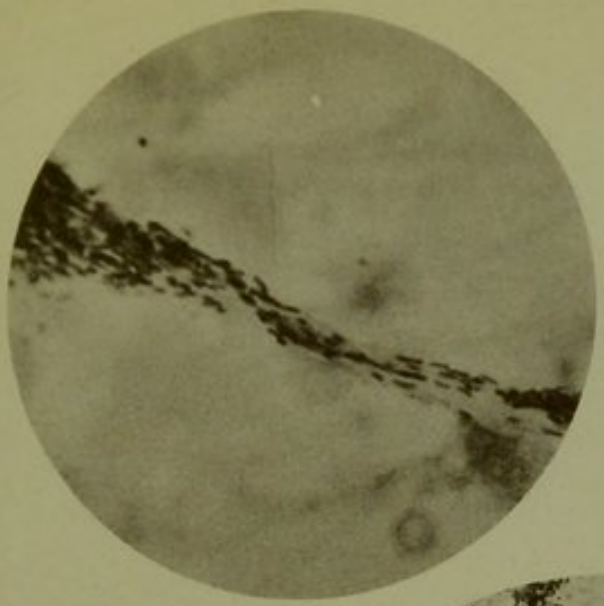

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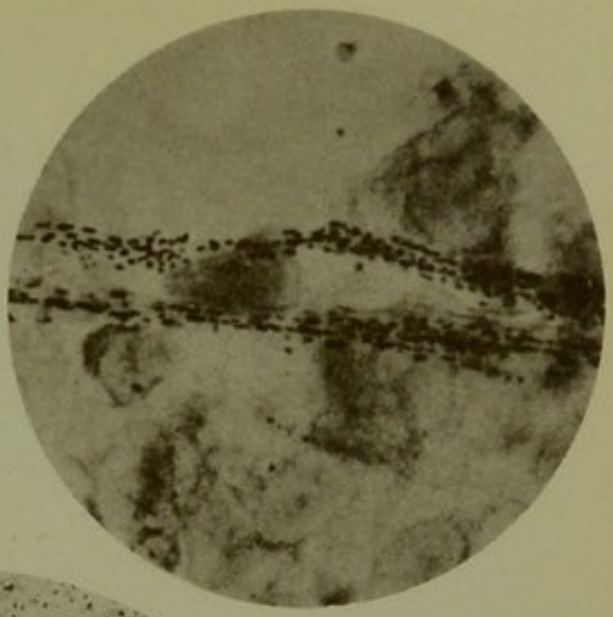

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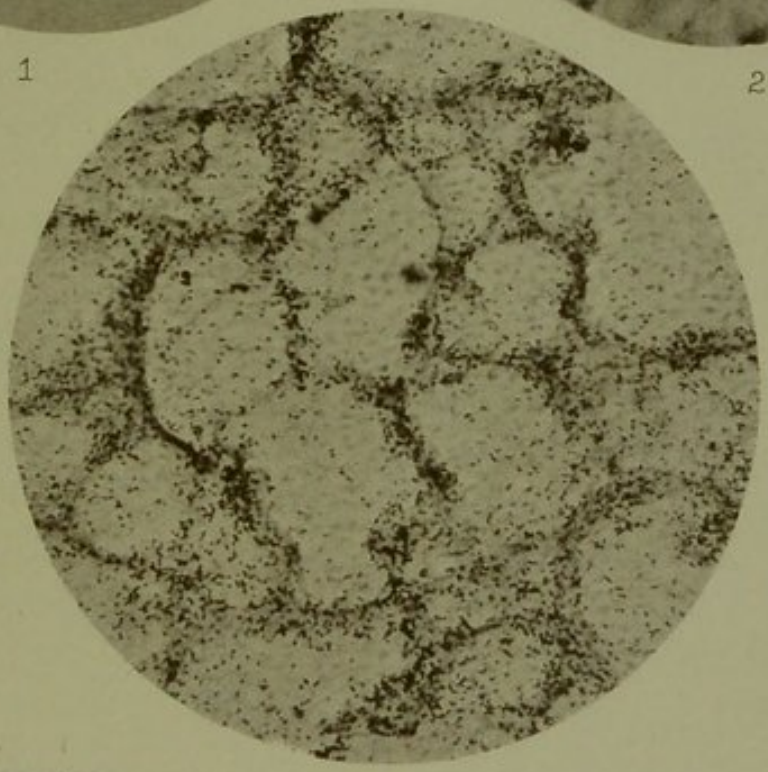

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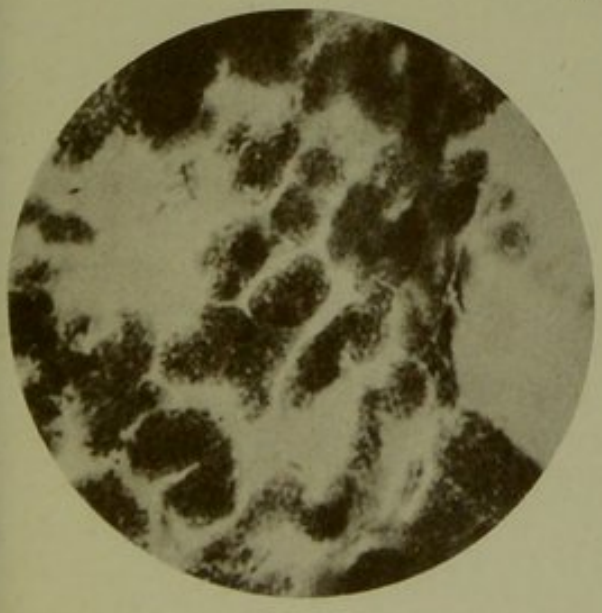

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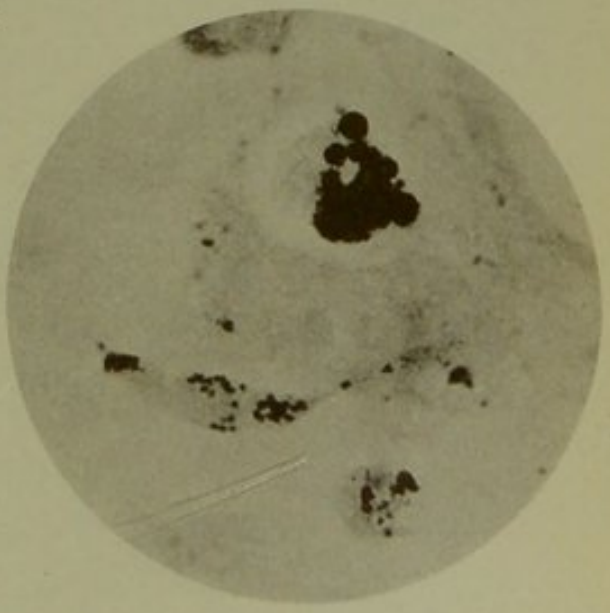

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PLATE VI.

(The omentum of the rabbit under pathological conditions.)

FIG. 1. Large granules in cells of milky spots. x 800. Twenty-four hours after injection of typhoid bacilli. No bacilli or small granules can be seen. The large granules probably represent débris from some previous pathological condition and are not degeneration forms of typhoid bacilli.

FIG. 2. Large granules in trailer. x 800. From the same specimen as Fig. 1. Two branching connective tissue cells without granules can also be seen.

FIG. 3. Typhoid bacilli in macrophages. x 800. From the meshwork of the omentum almost immediately after injection (see Part II., Table IX., 1.). One round macrophage is seen and three early intermediate forms, all containing numerous bacilli. Plate XI., 3, is composite drawing from the same specimen.

FIG. 4. Swollen endothelium. x 800. Sixteen hours after injection of typhoid bacilli. The nuclei are swollen and project into the lumen of the meshes.

FIG. 5. Lamphlack over a small milky spot. x 50. One hour after injection into a rabbit. The pigment is massed over the milky spot; very little can be seen on the meshwork.

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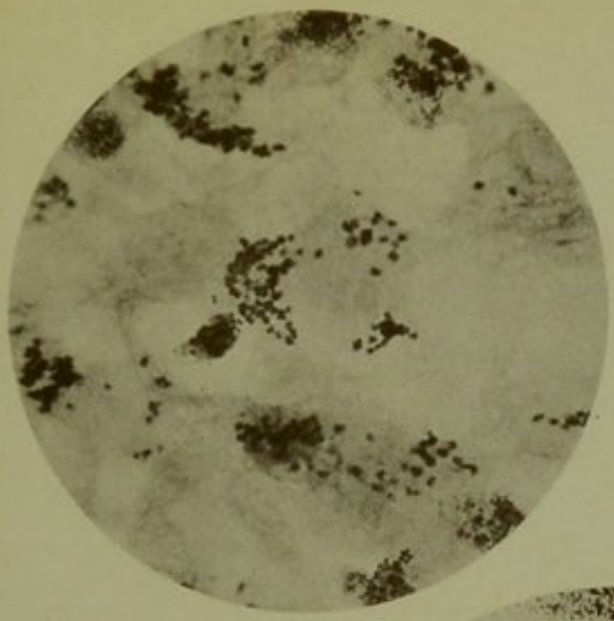

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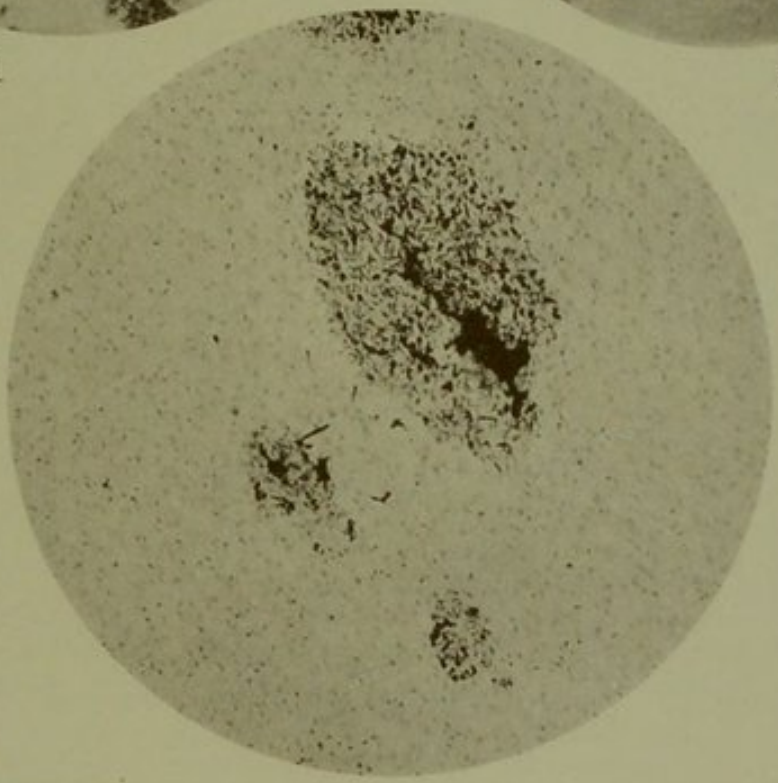

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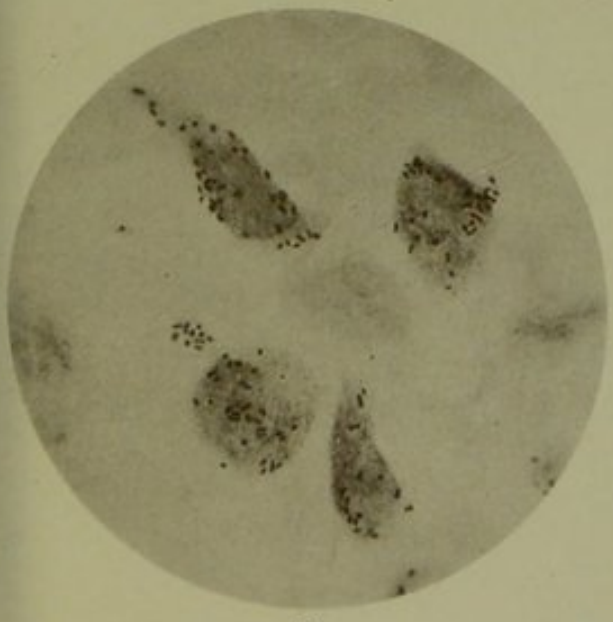

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PLATE VII.

(Sections of anterior mediastinal lymph nodes after injection of chicken red cells into guinea-pigs. Drawn with camera lucida. About 1,200 diameters.)

FIG. 1. One hour after injection. Afferent sinuses with free chicken red cells. Two macrophages are shown: one on the left just beginning to engulobe red cells, and another already stuffed full.

FIG. 2. Eight hours after injection. Chicken red cells engulobed by macrophages lying in the interior sinuses. None in the follicular structures. A few free red cells may still be found.

Plate VII.

(*Sections of anterior mediastinal lymph nodes after injection of chicken red cells into guinea-pigs. Drawn with camera lucida. About 1,400 diameters.*)

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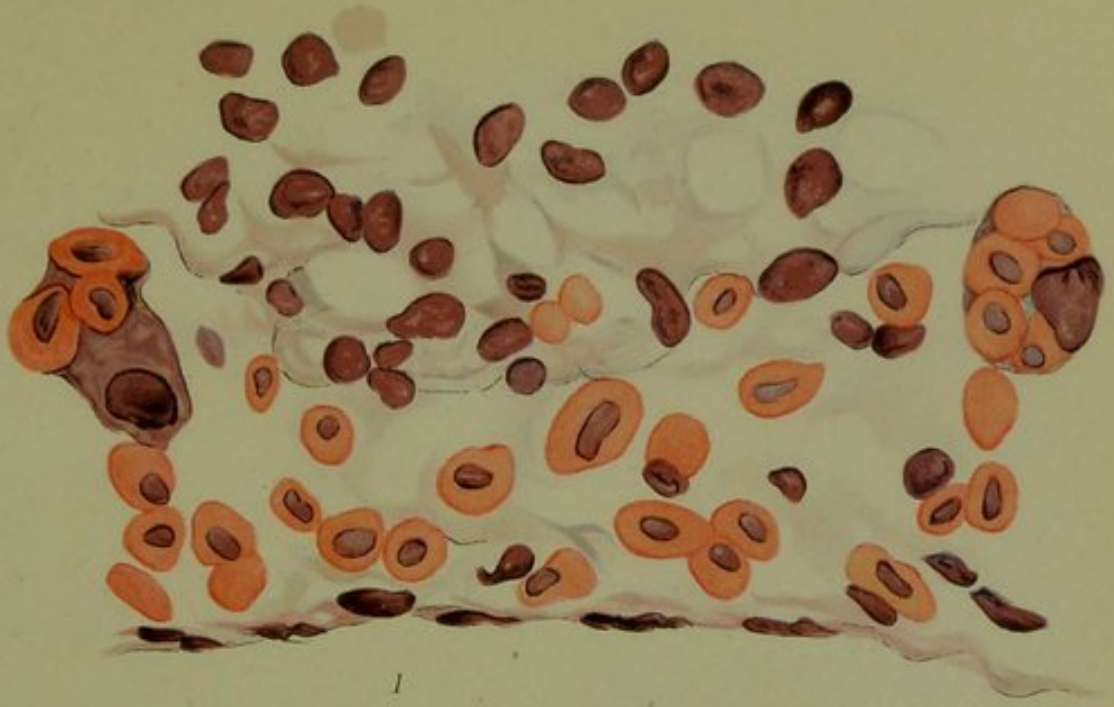

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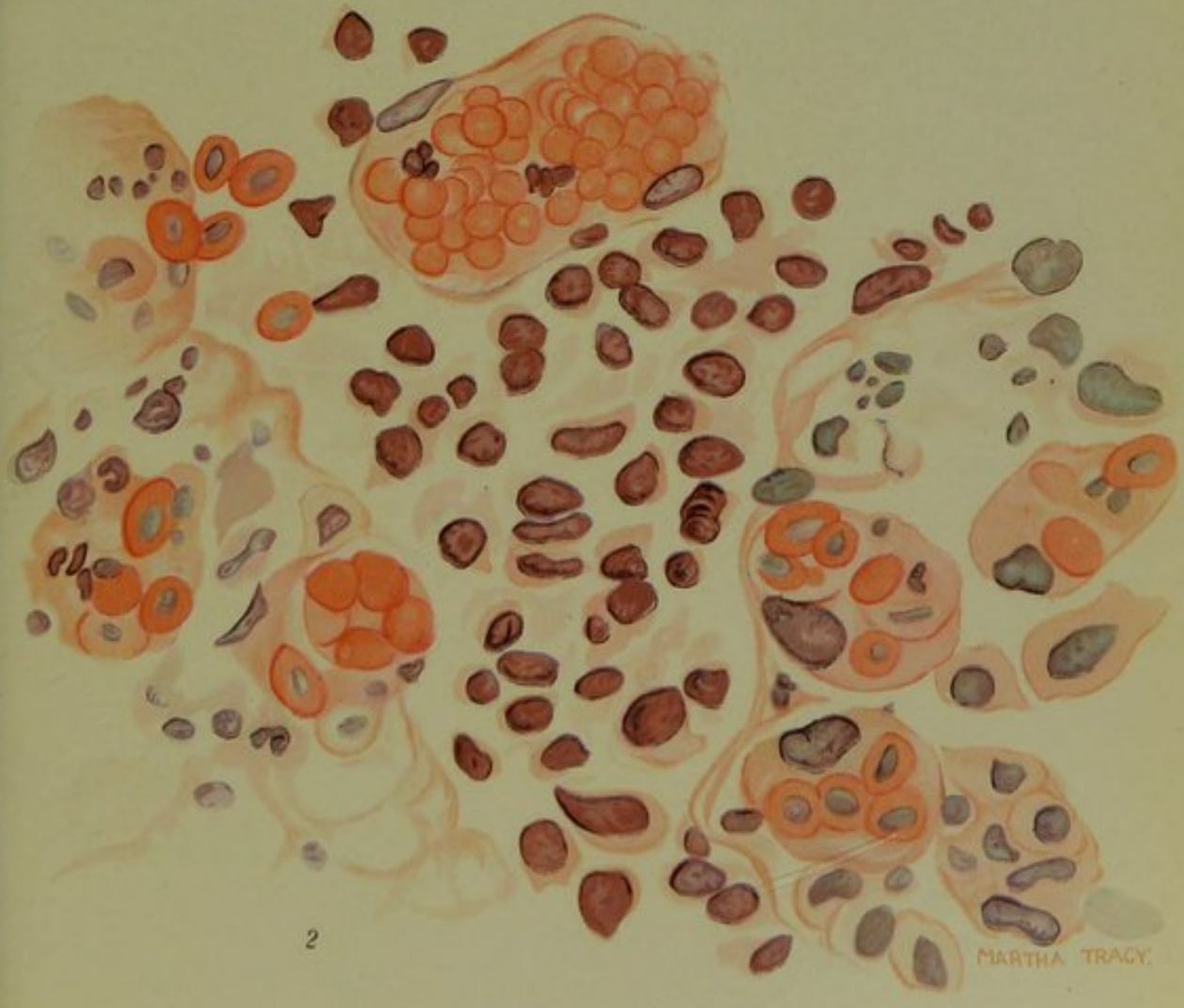

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PLATE VIII.

(Normal structures. Drawn with camera lucida.)

FIG. 1. From the normal omentum of a rabbit. x 1,000. Basophile lymphoid cells lying around capillaries

FIG. 2. From the normal omentum of a guinea-pig. A mast cell. x 1,000.

FIG. 3. From a normal milky spot of a rabbit. x 1,000. Macrophages and branching phagocytic cells. One macrophage contains the remains of a microzyte. A few small red or blue granules can be found in the branching cells.

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Absorption.

PLATE IX.

(Drawn with camera lucida. About 1,500 diameters.)

FIG. 1. Sixteen hours after injection of typhoid bacilli into a rabbit. On the omentum, in almost every field of the microscope, phagocytes containing microzycytes could be found. The drawing shows one round macrophage, one typical trailer and two intermediate forms, each containing remains of microzycytes. The cells also contain a few dark granules, representing degenerated bacilli.

FIG. 2. Normal omentum of rabbit showing typical trailer containing small granules, probably derived from an oxyphile cell or extraneous particles.

FIG. 3. From the omentum of a guinea-pig one month after injection of lampblack.

Trailers with vacuoles filled with fragmented particles. In the cell figured on the right the fragmentation is only partial.

Compare with Plate IV., Figs. 3 and 4.

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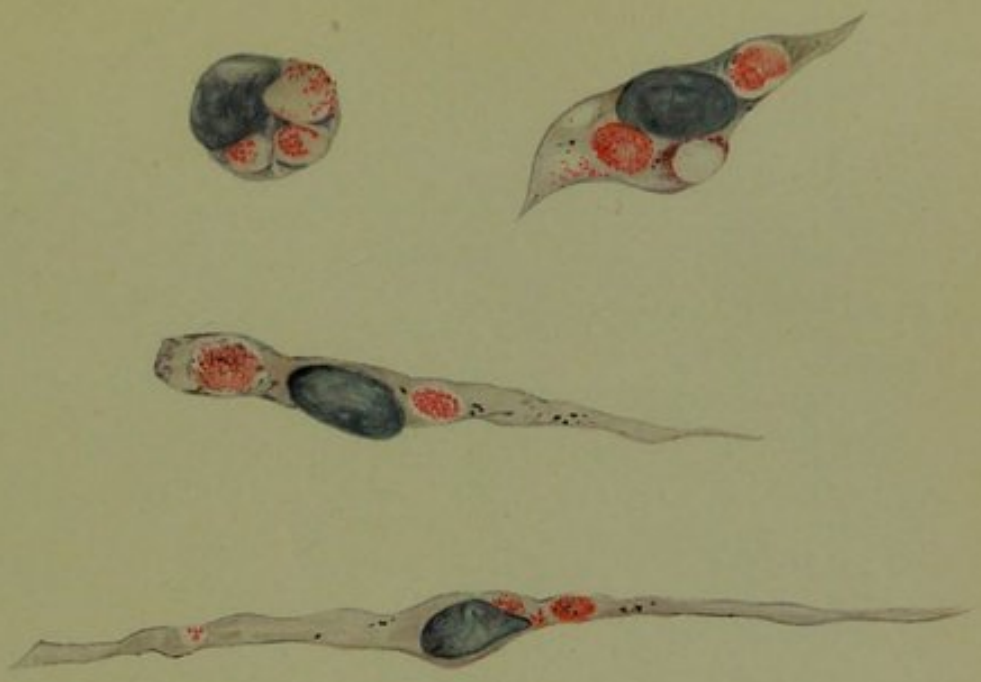

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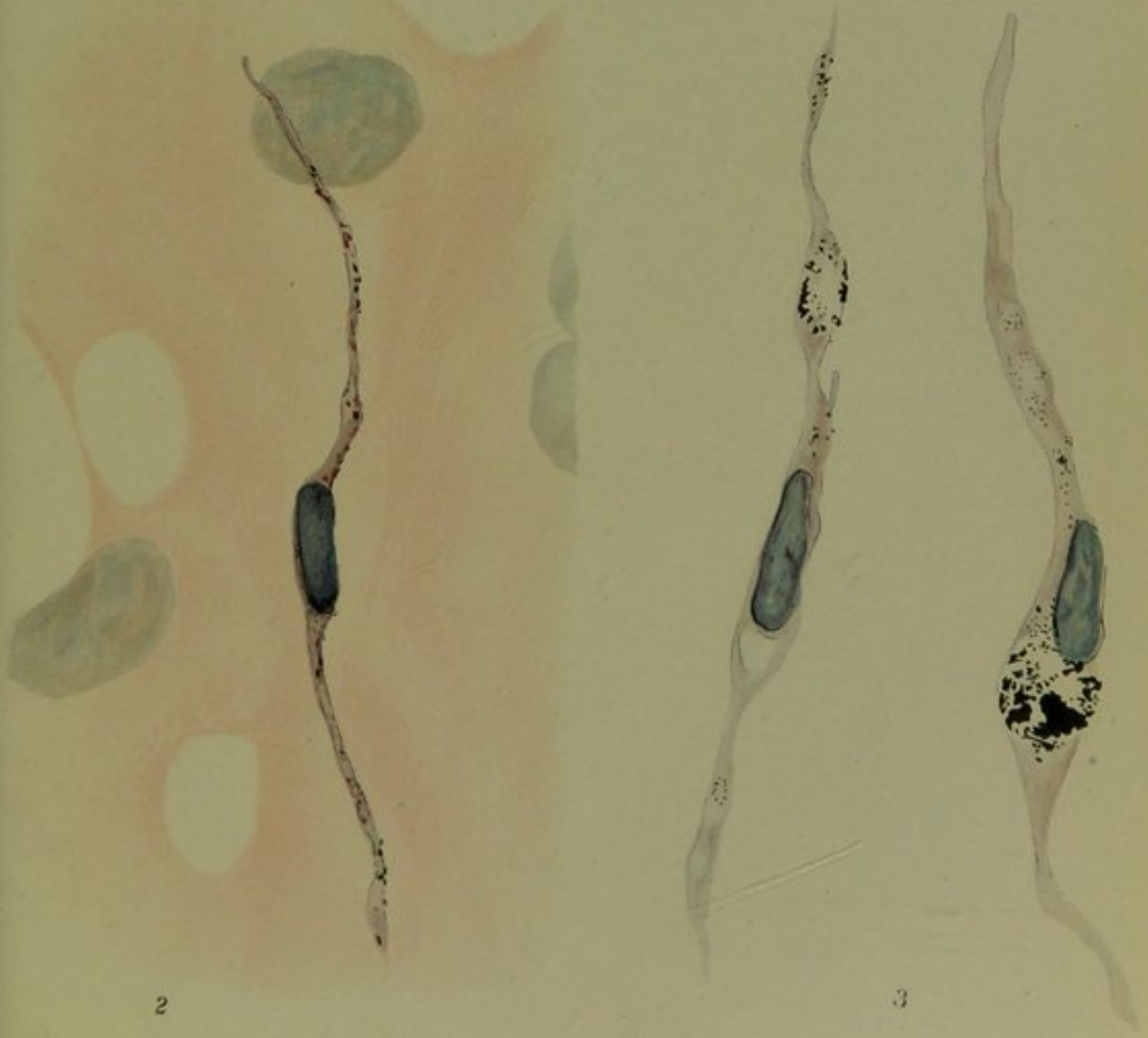

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PLATE X.

(Phagocytosis on the omentum. Drawn with camera lucida. About 1,400 diameters.)

FIG. 1. Five hours after injection of chicken red cells into a guinea-pig. The cells have been englobed by macrophages. On the right is a strand of fibrin, crossing the meshwork, with macrophages clinging to it. In the meshes is figured a large, free macrophage containing chicken cells and remains of microxycytes. Another macrophage is clinging to the wall of the mesh.

In the trabeculæ are two trailers: one containing nuclei of chicken cells, and the other, on the left, with remains of two chicken cells, the nuclei of which are not visible.

FIG. 2. Twenty-four hours after injection of lampblack into a guinea-pig. In the meshwork are figured four phagocytes, showing transitional forms from a round macrophage to an elongated trailer. The phagocytes contain particles of lampblack and remains of microxycytes. Two free microxycytes and one eosinophile cell, all containing a little pigment, are also figured.

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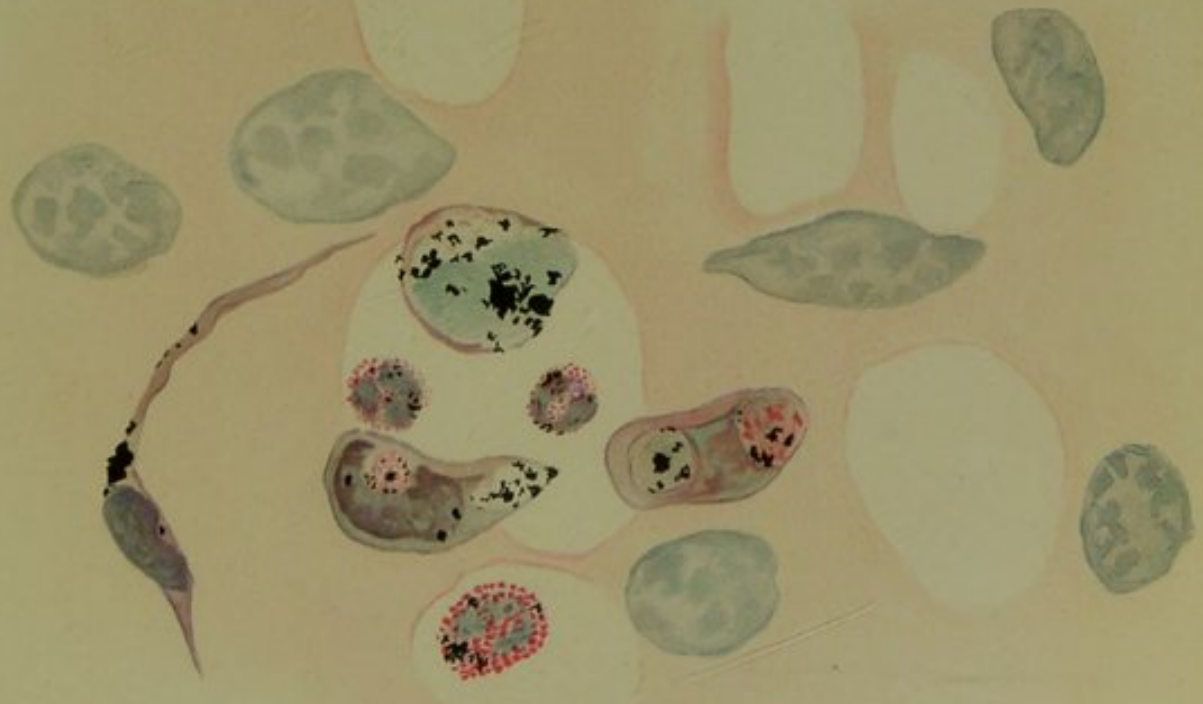

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PLATE XI.

(The omentum immediately after injection of typhoid bacilli into a rabbit. Three types of result may occur. Drawn with camera lucida. About 1,400 diameters.)

FIG. 1. Explosive extracellular destruction of bacilli.

The bacilli lie free in the meshwork. They have undergone a peculiar form of degeneration, the chromatin being massed in the center of each bacillus, leaving a faintly staining sheath.

See Part II., Table IX., 7. Wass, 1,000. Organs, o.

FIG. 2. Explosive intracellular destruction of bacilli.

From the edge of a milky spot the branching cells are filled with indefinite reddish and blue granules representing the remains of bacilli. No free bacilli or granules.

See Part II., Table IX , 5. Wass, 350,000. Organs, o.

FIG. 3. No explosive destruction of bacilli. Composite drawing. Meshwork showing a macrophage, intermediate forms, and a trailer, all containing intact bacilli.

See Part II., Table IX., 1. Wass, many millions. Organs, full of bacilli. Compare with Plate VI., 3, from same specimen.

Note. — In each figure a few pale endothelial nuclei may be seen.

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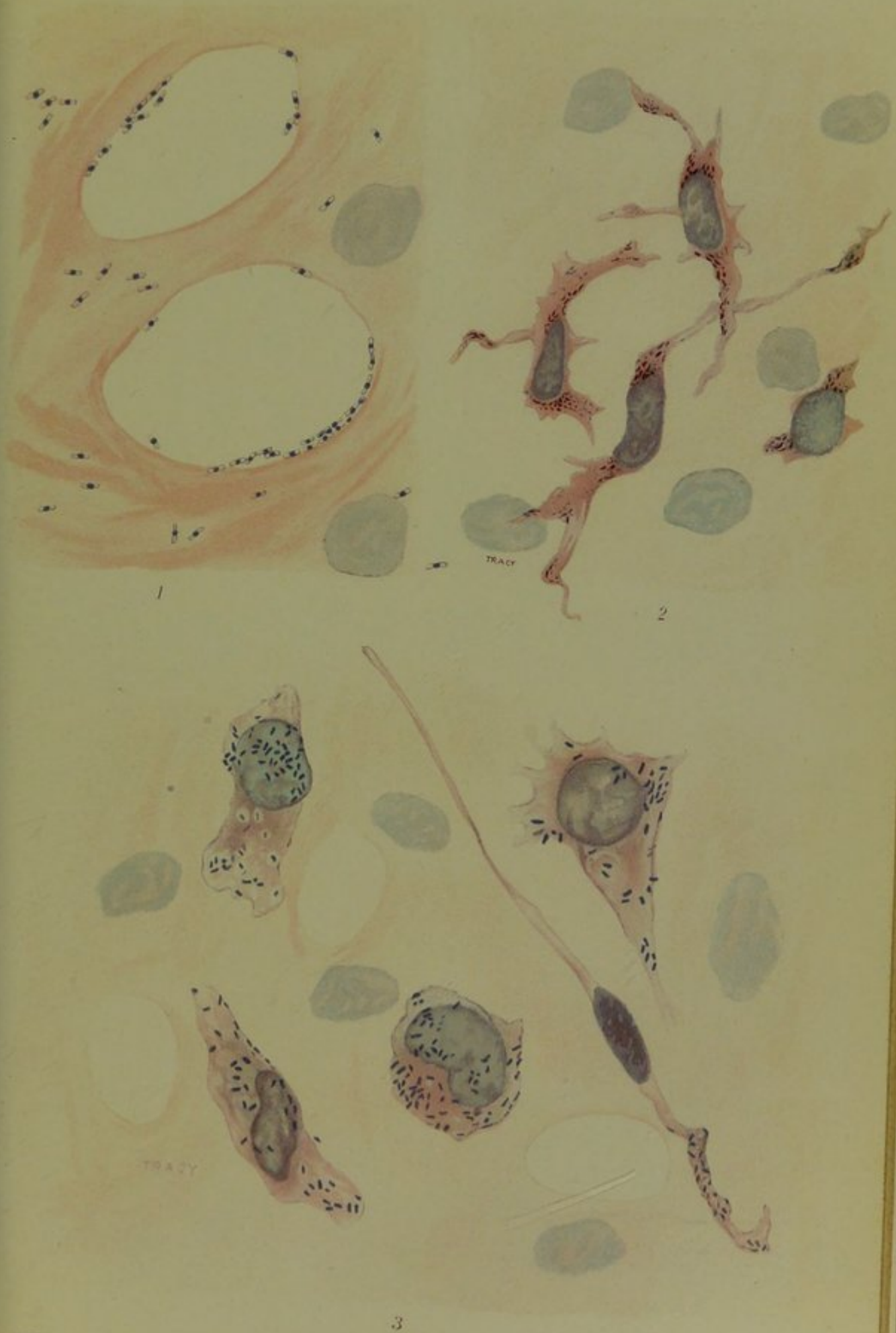

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