The Rôle *of the Phagocyte in Cerebro-spinal Meningitis.*

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[Plates 13 and 14.]

The more or less constant presence of the meningococcus in the spinal fluid of cases of cerebro-spinal fever, has led to many suggestions as to how this microbe gains admission to the spinal canal. It has been assumed that either there is a direct passage of the organism from the naso-pharynx to the cerebral meninges, or that transmission takes place through the blood or lymph channels.

The membranes which enclose the spinal fluid, however, present a serious obstacle to the passage of the microbe, and in fact it is doubtful if, in the living tissues, such a passage could take place.*

If passage by means of the blood stream or the lymph were easy, on the other hand, we might expect to find meningitis a frequent complication of streptococcal septicæmia—which is not the case.†

Though the spinal meninges are probably impervious to the passage of free meningococci, they certainly do not prevent the passage of leucocytes.

Normal healthy spinal fluid contains a few wandering cells, mostly of the lymphocyte variety. In cerebro-spinal fever the fluid is invariably crowded with large numbers of polymorph leucocytes, which then frequently contain many meningococci. In such cases we have often noticed that the microbes within the leucocytes have undergone little change, and show no obvious signs of degeneration or digestion. In staining reaction, moreover, they show no appreciable difference from those lying without the phagocytes.

* To obviate this difficulty it has been suggested that the meningococcus starts life as a filterable virus, and that in this form it successfully enters the canal. In none of our cases (with a Doulton filter) have we been able to obtain a filtrate that would give any growth when planted out on chocolate medium.

† No attempt has been made in the present paper to consider the question of the infection of the meninges by way of the lymph channels. If this is brought about by the flow of lymph from the nasal spaces through those surrounding the olfactory nerves to the subdural and subarachnoid spaces of the cranium, then we have to assume a peculiar susceptibility to infection on the part of the membranes surrounding these spaces, by the meningococcus, or otherwise we should find meningitis a frequent complication of the usual infections of the nose.

Is it possible that, under certain conditions, the meningococcus can remain alive within the leucocytes? If such should prove to be the case, then wandering leucocytes might convey living meningococci into the spinal canal and thus bring about infection.

More than a quarter of a century ago, Ruffer* drew attention to the fact that, in sections of the rabbit's tonsil, the leucocytes are frequently seen crowded with bacteria, which seem to have undergone little digestion. To all appearances they seem to resemble free germs.

Since then many similar observations have been made, notably by Metchnikoff,† and his pupils, Adami,‡ Nicholls,§ Ford,‖ and many others.

In 1895, Bordet¶ found that cholera spirilla, injected into the blood stream of cholera-immune animals, are taken up by the leucocytes before they are subjected to lysis by the circulating antibodies.

Metchnikoff,† Levaditi,** and Briscoe†† similarly have shown that red blood cells injected into previously immunised animals may be taken up by the leucocytes before they can be hæmolysed.

Rous and Jones‡‡ have shown that leucocytes can protect typhoid bacilli, after ingestion, from the toxic action of a N/150 KCN solution. This strength of KCN they showed was highly lethal to the unprotected germs.§§ They also proved that living leucocytes can shield these bacilli from the action of a strong homologous serum, as the serum is unable to exert any action on those germs within the leucocytes. They were able subsequently to recover living germs from these leucocytes.

McKee‖‖ has found that in ophthalmia neonatorum (gonococcal), the cells of the conjunctival epithelium can take up the gonococcus in large numbers, and that these cocci are not killed by the silver nitrate when the eye is subsequently washed out with this solution. Similar observations have been made on the urethral epithelium by other investigators. We have thus a clear explanation of the cause or source of recurrent infection, so frequent where this germ is concerned.

* Ruffer, 'Brit. Med. Journ.,' 1890.

† 'Metchnikoff, 'L'Immunité dans les Maladies Infectieuses,' Paris.

‡ Adami, Abbot, and Nicholson, 'Journ. Exp. Med.,' vol. 4 (1899).

§ Nicholls, 'Journ. Med. Resch.,' N.S., vol. 6 (1904).

‖ Ford, 'Journ. Hyg.,' vol. 1 (1901).

¶ Bordet, 'Ann. de l'Inst. Past.,' vol. 9 (1895).

** Levaditi, 'Ann. de l'Inst. Past.,' vol. 16 (1902).

†† Briscoe, 'Journ. Path. and Bact.,' vol. 12 (1908).

‡‡ Rous and Jones, 'Journ. Exp. Med.,' vol. 23 (1916).

§§ We did not find a N/150 KCN solution made up in Lock's solution toxic for the meningococcus after 3 hours' exposure to its action at 37° C.

‖‖ McKee, 'Ophthalmic Record,' Chicago, January, 1912.

It has been recently pointed out by several observers that it is doubtful if the polymorph leucocytes of the body are capable of successfully attacking and digesting bacteria of the acid-fast group. It has been shown by Tachernortusky* that extracts of these leucocytes, although containing the usual proteolytic enzymes, are remarkably deficient in lipase. They are probably unable for this reason to digest the waxy substance forming the characteristic feature in the organisation of this group of bacteria.

This is borne out by the work of Terry,* in Zinsser's laboratory, which shows that rat leprosy bacilli may be kept within the leucocytes for weeks without undergoing any apparent change or losing their acid-fast properties; whereas the same bacilli, as shown by Zinsser and Cary,† are rapidly digested when taken up by the spleen tissue cells grown in blood plasma.

In the light of the foregoing observations, it seemed to us that it should be possible to demonstrate experimentally the viability or non-viability of the meningococcus within the leucocytes in cerebro-spinal fever. The following experiments have been attempted with this object. The recovery of living cocci from the leucocytes of undoubted cases of cerebro-spinal fever ought conclusively to establish this point.

Our first object is to show that we are using a medium which is quite favourable to the growth of the meningococcus. Our experiments will give erroneous results, if, for example, a medium is used on which the organism will only grow when implanted in massive amount, for under such conditions we are not certain that the germ is dead, although it fails to grow. The tests should carry conviction on this point.

The medium used in our experiments is that described by one of us.‡ This is made from defibrinated bullock's blood and glucose, with trypsin agar as a base. To avoid the cumbrous title "blood-agar-glucose," it will be referred to briefly as "chocolate medium," from the similarity of its appearance to this substance.

To determine its power of growing the meningococcus, it was compared with a number of media. The results of this comparison will appear in detail elsewhere. Briefly, the conclusion is reached that if a 24-hour culture on chocolate medium is emulsified in distilled water and diluted down and planted out, then, presumably through a certain proportion of these germs being dead, it is found that a certain minimum number of organisms are necessary to the implantation for growth to take place. This minimum varies with the different media in accordance with their capacity for

* Quoted from Zinsser, 'Infection and Resistance,' p. 284 (1914).

† Zinsser and Cary, 'Journ. Amer. Med. Ass.' (1912).

‡ Crowe, 'Lancet,' November 21, 1915.

growing the meningococcus. It varies also with the character of the strain of meningococcus employed, as some grow very rapidly on artificial media, while others grow slowly.

The following Table gives roughly the number of germs of Strain "P," which, having been planted out in similar definite quantities on the following media, gave growth after incubation at 37° C., for 48 hours:—

Name of medium.	Growth positive.	Growth negative.
War Office legumen tryp-agar	50,000,000 germs	10,000,000 germs.
Buchanan's medium	—	100,000,000 ,,
Ascitic legumen agar	10,000,000 germs	1,000,000 ,,
Amino-acid agar (Cole's)	—	100,000,000 ,,
Amino-acid agar, mixed with fresh blood	10,000,000 germs	1,000,000 ,,
Blood agar	10,000,000 ,,	1,000,000 ,,
Blood smeared agar	10,000,000 ,,	1,000,000 ,,
Egg medium	—	100,000,000 ,,
Chocolate medium	1,000 ,,	—

In the above Table a thousand germs was the smallest number planted out. Chocolate medium will, however, readily grow the meningococcus in inoculations of 500 cocci. In implantations made from glucose broth cultures, this number is reduced still lower, as presumably, in this instance, all the cocci are alive. Experiment has shown that growth will then take place, on this medium, in implantations of as few as 20 or 30 organisms.

We drew the conclusion from the above facts (1) that chocolate medium was well adapted to our requirements, and was suitable for testing the viability of the meningococcus, as far as this can be determined on artificial media; (2) that, given a reasonable number of germs to an implantation, failure to grow on our medium denoted the presence of almost a negligible number of cocci.

One of us has shown that nearly all strains of the meningococcus succumb rapidly in the presence of 0·85 per cent. NaCl solution, on exposure to its action for a short while.* All the strains of the meningococcus used in the following experiments were found to be highly susceptible to the toxic action of dilute NaCl. While a 0·85 per cent. NaCl solution is toxic, a 1·5 per cent. NaCl solution is more or less harmless. In the following experiments, taking fresh spinal fluid obtained from lumbar puncture of cerebro-spinal fever patients, and lightly centrifuging down the leucocytes, we have made use of this toxic action of a 0·85 per cent. NaCl solution to kill all the meningococci outside or attached to the leucocytes, while those enclosed within them are protected from its action.

* Shearer, "On the Toxic Action of Dilute Pure Sodium Chloride Solutions on the Meningococcus," 'Roy. Soc. Proc.,' B, vol. 89, p. 440 (1916).

Experiment 1.

Expt. 1.—Fresh "Sloan" spinal fluid, rich in meningococci, both within and without the leucocytes, was centrifuged lightly to bring down the leucocytes.

Treatment.	Growth, 24 hours.
(1) Leucocytic deposit was washed in 1 per cent. sterile glucose, centrifuged three times and planted out	Good.
(2) Leucocytic deposit was washed in 1 per cent. sterile glucose, centrifuged six times and planted out	Good.
(3) Upper portion of original centrifuged spinal fluid was centrifuged hard for 15 minutes and planted out	Good. Far the best.

In (1) fresh leucocytic deposit containing many meningococci within the leucocytes, was washed three times in 1 per cent. sterile glucose and centrifuged and planted out on a plate of chocolate medium. The glucose exerts no harmful action on the cocci, while it disorganises and probably kills the leucocytes through its hypertonic action. The washing has removed practically all the free cocci. At the end of 24 hours there was a good growth on the plate. This growth could hardly be accounted for by supposing it to be derived from the few free cocci that may have remained over from the washings.

To test this further, in (2) the deposit was washed six instead of three times and planted out in a similar fashion. The result was the same as in (1). Here the extra washing had no effect in lessening the amount of the growth, which was as great as in (1).

In (3) the upper part of the original centrifuged spinal fluid, containing few leucocytes but numbers of free cocci, was centrifuged hard for 15 minutes. On planting this out, as was to be expected, growth was immediate and greater than in (1) and (2).

The conclusion to be drawn from this experiment is open to question, as it cannot be said with any certainty that all the loose germs in (1) and (2) were removed by the repeated glucose washings.

Moreover, the glucose itself is a stimulant to the growth of the meningococcus. The following experiment brings out this point, but at the same time shows that this effect is not very great, and probably is not sufficient to disturb the result of our experiments.

A fairly thin emulsion of the meningococcus used in Experiment 1 (500 millions to the cubic centimetre) was made up in distilled water. Successive dilutions were made by mixing 5 c.mm. of this emulsion with 25 c.mm. of 1 per cent. sterile glucose broth, removing 5 c.mm. and mixing with a second 25 c.mm. of broth, and so on through eight dilutions. A sterile

camel's-hair brush was dipped in the highest dilution, and drawn across the surface of the plate. The remaining seven dilutions were treated in a similar manner. In the end there were eight parallel lines, taking up one half of the plate, each representing a dilution of the meningococcus in glucose. The other portion of the plate was treated in a similar manner with eight successive dilutions of the same quantity of emulsion, in distilled water. The plate was then incubated at 37° C. for 48 hours. A photograph of the plate is shown in fig. 3, after 24 hours' growth. The action of the glucose in accelerating growth will be seen from an examination of the successive lines on the one, as compared with the other half of the plate. A close comparison of the two top lines with the two bottom lines of growth brings out the action of the glucose. In this instance it is slight and inconsiderable. The action of the glucose, therefore, may be safely neglected as a source of grave error.

There is a further point to be considered with regard to this and all the following experiments. Are we certain that the leucocytes we are using in these experiments are alive and not dead?

We endeavoured to settle this point by two tests.

Firstly. By an examination of fresh leucocytic deposit of spinal fluid on the warm stage. This elucidated the fact that if the spinal fluid was freshly drawn by lumbar puncture from a cerebro-spinal fever case and was only cloudy and turbid in appearance, and not at all purulent, then practically all the leucocytes were alive, as they always showed vigorous amœboid movement when placed on the warm stage. If the fluid was purulent, then most of the leucocytes were dead.

Secondly. It has been shown by Rous and Jones* that a dilute solution of trypan blue in Ringer's solution readily stains the nuclei of dead leucocytes, while it will not touch those of the living cells. We have confirmed this point for human leucocytes found in the spinal fluid of cerebro-spinal fever cases. To this end, freshly drawn fluid containing leucocytes was taken which showed obvious amœboid movement on the warm stage. This was divided into two portions. The leucocytes of one portion were stained for a few minutes in dilute trypan blue in Ringer's solution, and then examined under the microscope; none of them took the stain. To the second portion a little alcohol was added to kill them, and they were then stained as before; all immediately took up the stain.

We then applied the trypan blue method of staining to a number of leucocytic deposits obtained from cerebro-spinal fever cases, such as those used in

* Rous and Jones, 'Journ. Exp. Med.,' vol. 23 (1916).

the following experiments. The trypan blue test bore out the results obtained with the warm stage. The leucocytes do not take up the stain as long as the spinal fluid is simply cloudy and turbid and no traces of pus present. This test, moreover, would seem to show that human leucocytes can remain alive for 2–3 days in the spinal fluid, when this is allowed to stand at room temperature under sterile conditions.

In the following experiments we made use of spinal fluid which we had every reason to believe, in view of the above tests, contained living leucocytes. In no instance did we use fluid showing the presence of pus cells. The results of the experiments themselves preclude, moreover, the possibility that the majority of the leucocytes were dead.

Experiment 2.

In this, as in the former experiment, leucocytic deposit of fresh "Sloan" spinal fluid, containing this time practically no free germs, but many within the leucocytes, was removed and washed. Part only was washed in 1 per cent. glucose. The remainder was washed in 0·85 per cent. NaCl. The washings in both instances were repeated 16 times, to make certain, as far as possible, that no free germs should remain.

Microscopic examination of the deposit after washing in glucose showed a slight disorganisation of the leucocytes, while those washed in saline showed no change. The normal saline, while not affecting the leucocytes, presumably exerted a toxic action on the free germs or those attached to the exterior surface of the leucocytes. Those within were protected from its action.

Expt. 2.—Fresh "Sloan" spinal fluid, containing no free cocci, but large numbers within the leucocytes, was centrifuged lightly to bring down leucocytes.

Treatment.	Growth, 24 hours.
(1) Leucocytic deposit of spinal fluid washed 16 times in 1 per cent. sterile glucose, and planted out	Good.
(2) Leucocytic deposit of spinal fluid washed 16 times in sterile 0·85 per cent. NaCl, and planted out	Good, but delayed: at end of 48 hours, same as (1).

In this experiment (1) we got a rapid and immediate growth covering nearly the whole of the plate at the end of 24 hours. In (2) the growth only attains to the amount of that of (1) at the end of 48 hours. In both (1) and (2) practically all free cocci have been eliminated by the repeated washings. Those remaining over or attached to the surface of the leucocytes in (2) have been killed or injured by the toxic effect of the saline. Those

attached to the surface of the leucocytes in (1) have been uninjured by the glucose. The immediate growth of (1), as compared with (2), is probably to be sought for in the fact that in (1) the glucose disorganises and destroys the leucocytes, so that the microbes they contain are more rapidly set free to grow, while in (2) the normal saline keeps the leucocytes longer intact, and their microbes are only set free to grow after a considerable interval.

Experiment 3.

This experiment was a repetition of 2, with spinal fluid from a case whose condition was critical when the lumbar puncture was made. The leucocytes were probably less favourable for the experiment than in the former instance. Practically the same result, however, was obtained. In figs. 1 and 2 are shown photographs of the growths of the glucose and saline deposits of the experiment respectively at the end of 24 hours. It will be seen that the glucose-washed deposit has grown much more than that in the normal saline. At the end of 48 hours the growth on the two plates was practically equal.

How far are we justified in drawing conclusions from Experiments 2 and 3? It is hard to believe that the abundant growths in both experiments with the glucose-washed deposit is due entirely to the accelerating action of the glucose on the few microbes remaining attached to the exterior of the leucocytes. That is, if we suppose all the microbes within the leucocytes to be dead. Taking into consideration all the facts of the case, we think there is a certain amount of evidence in favour of the view that the microbes were alive within the leucocytes.

Experiment 4.

Leucocytic deposit from the fresh spinal fluid (N. H.) was washed three times with sterile 0·85 per cent. saline and divided into two portions. One was kept intact, while the other was crushed with sterile glass powder. Samples of each were planted out, and the remainder were left to stand for three hours in a large bulk of normal saline. They were then thoroughly centrifuged and planted out.

Expt. 4.—Leucocytic deposit from spinal fluid (N. H.), containing many free and enclosed cocci, was washed three times in 0·85 per cent. NaCl, and divided into two portions. Portion (*a*) was kept intact, while portion (*b*) was crushed up with sterile glass powder. Samples of each were then planted out, and the remaining portions were then allowed to stand in sterile 0·85 per cent. NaCl for three hours. They were then centrifuged thoroughly and planted out.

Treatment.	Growth, 24 hours.
No saline—	
(*a*) Leucocytic deposit planted out immediately	Good.
(*b*) Leucocytic deposit crushed and planted immediately	Good; better than (*a*).
Saline for three hours—	
(*a*1) Leucocytic deposit planted out after three hours standing in 0·85 per cent. NaCl	Delayed and poor.
(*b*1) Leucocytic deposit crushed and planted out after three hours standing in 0·85 per cent. NaCl	One colony only.

At this point it seemed to us that it ought to be possible by making use of the opsonic technique to arrange an experiment which might provide a crucial test of our hypothesis.

The meningococcus, when first isolated from the spinal canal, is not susceptible of being taken up by the phagocytes in the presence of normal serum. There are said to be exceptions to this rule (Kolle and Wassermann), but so far in our work we have met none. During sub-culture the organism gradually loses the power of antagonising the action of the leucocytes, until after a month or six weeks of growth under laboratory conditions, a very considerable opsonic effect can be demonstrated. Immune serum on the other hand is a most powerful "opsoniser" of freshly isolated cocci. The contrast between an opsonic film prepared with normal serum and that prepared with immune is very striking.

Since it was our desire to obtain leucocytes filled with meningococci, clearly we were right to employ freshly isolated cultures, and sensitise the emulsion made from such a culture with the serum of the patient from whom the germ was isolated. As a control we desired to utilise leucocytes with very few germs inside them; the same emulsion sensitised by a non-immune normal serum gave us what we wished.

Remembering the poisonous action of the normal saline when applied to meningococci, we could readily free the leucocytes which we had charged or intentionally left uncharged from any loose germs which might not have been ingested, and in this way our results were not obscured by the growth of the organisms untouched by the phagocytes. We had to be careful, however, not to emulsify the culture used for the purpose of making the opsonic mixture in normal saline, since the toxic action of the saline would have come into play and seriously interfered with the result of the experiment. It would have been very difficult under these circumstances to appraise properly the leucocytic content, as many of the germs would have been killed by the saline beforehand.

Emulsion made up in 1·5 per cent. saline, however, completely avoids this difficulty, as this strength of saline has little or no deleterious action on the meningococcus and does not interfere with the leucocytes.

Having thus obtained leucocytes ready charged with germs, and also for control purposes other leucocytes almost free from germs, it only remained to destroy a certain proportion of the former by some means or other. To this end we again employed the method of grinding with sterile powdered glass. Finally, by submitting both crushed and uncrushed cells and also the control leucocytes to the action of normal saline, we were able to determine whether or no the organism within the intact leucocytes were still viable. Those which had been freed by the destruction of the leucocytes should have been killed by the unrestrained action of the normal saline.

For the sake of greater clarity, it is perhaps as well briefly to tabulate the conditions on which this crucial experiment depends.

They are as follows :—

1. An immune serum exerts a powerful action in stimulating the leucocytes to take up the germs. We can, therefore, fill leucocytes at will with meningococci.

2. Since freshly isolated meningococci are but slightly susceptible of being attacked by the phagocytes with normal serum, as a control, an opsonic mixture where normal serum is substituted for immune will provide us with leucocytes fairly free of meningococci.

3. We know from the foregoing experiments that normal saline will kill all meningococci except those ingested and protected by the leucocytes.

We can finally destroy by mechanical means some of the leucocytes containing meningococci, and resubmit both these and intact leucocytes to the action of normal saline. If our hypothesis that leucocytes protect meningococci from death is correct, then from the intact leucocytes growth will take place.

Provided also that the normal saline is poisonous to the germ, growth from the control leucocytes (treated with normal saline) will only be slight.

Experiment 5.

Opsonic mixtures { S = Coccus S + washed leucocytes and serum S (immune).
P = Coccus S + washed leucocytes and normal serum P.

Coccus S is a recently isolated coccus from patient S. suffering from cerebro-spinal fever. Serum of this patient and a normal man P. was drawn the day before the experiment. A 24-hour culture of coccus S was emulsified in 1·5 per cent. saline to prevent lysis, and the mixtures put up in equal parts of emulsion, serum and leucocytes, and incubated for

five minutes. A sample was spread on a film and the remainder of each specimen mixed with the saline and washed three times. A further sample was planted out 40 minutes after the mixtures were put up. After two hours half the centrifuged deposit of mixture S in saline was crushed with sterile glass powder. One and a half hours later, samples were again planted, and this procedure was repeated at intervals of 6 and 24 hours after the commencement of the experiment.

Microscopic appearance of the mixtures:—

(1) "S" mixture showed extreme agglutination, nearly all the leucocytes crowded with cocci.

(2) "P" mixture (control), no agglutination, only a few cocci in the near neighbourhood of the cells. It was doubtful if any microbes at all had been taken up by the leucocytes.

Experiment 5. (See figs. 6 and 7.)

Time.	S.*		P.*
I. 40 minutes in saline ...	Area planted, quite covered.		Very few colonies.
	Uncrushed.	Crushed (Cr.).	
II. 3 hours in saline	Area planted out quite covered	Area planted out quite covered	Very few colonies.
III. 6 hours in saline	Covered	No growth	Growth discrete.
IV. 24 hours in saline	Covered	No growth	Covered.

* S = Coccus S + washed leucocytes and serum S (immune).
* P = Coccus S + washed leucocytes and normal serum P.

In figs. 6 and 7 are shown photographs of the result of this experiment after 24 and 48 hours' growth, respectively.

Row I, P.—Very few colonies growing owing to the loose germs being washed away, whereas in S the leucocytes hold the organisms.

Row II.—The excellent growth in the crushed area (Cr.) shows that the manipulation with the glass powder did not destroy the vitality of the germs, although no leucocytes were left intact (confirmed microscopically).

Row III.—In column (Cr.) where crushed leucocytic deposit is planted, the free germs have succumbed to the action of the saline. There is no growth. On the left hand, however, where the leucocytes were intact, growth was maximal.

Row III and IV, P.—Growth steadily increasing. We must suppose that a few germs have been taken up by the leucocytes. These have probably

increased within their hosts; hence the progressive increase in the amount of growth. It is also possible that this growth is due to the blood being allowed to stand for such a length of time at incubator temperature. It was noticed that at the end of 24 hours' time it had undergone considerable hæmolysis. This would liberate an appreciable quantity of calcium salts which would in turn destroy the action of the saline. Thus a condition would be brought about which would be favourable to an excessive growth of the few germs that might have been taken up by the leucocytes. The accessory growth factor known to be present in the blood would also come into play, and, the inhibiting toxic action of the normal saline being abolished, rapid growth would take place.*

The main conclusion of our former experiments is therefore borne out again in Experiment 5. Taking Row III of this experiment, the large growth in the first area, where the leucocytes have remained whole, although exposed to the toxic action of the saline for six hours, as compared with the complete absence of any growth in the second area, where they have been crushed with glass powder and have come under the direct action of the saline, is very striking and is beyond dispute.

Experiment 6.

Emulsions of two strains were submitted to the action of the leucocytes. Strain W was an old laboratory culture, isolated from the spinal canal nine months previously. Strain S 1 had been isolated less than a fortnight.

Opsonic mixtures:

A.—Coccus W + leucocytes + normal serum.
B.— „ S1 + „ + „ „
C.— „ W + „ + immune serum.
D.— „ S1 + „ + „ „

Microscopic appearance of the mixtures after 15 minutes' incubation :—

"A."—Showed no agglutination, but the leucocytes contained many cocci, many shades of cocci, and the staining of nearly all of them within the cells was poor, suggesting partial digestion.

"B."—Showed no agglutination, and very little ingestion of the germs. Organisms stained well.

* It has long been remarked that all blood media grow the meningococcus with great readiness; similar results can be obtained with watery and alcoholic extracts of blood. One of our number (C. S.) hopes to show in a forthcoming paper that in nasal secretion such a body is also present, which is undoubtedly of the nature of an accessory food body in all its properties. It is possible that this body present in blood is similar to that found in the nasal secretion, the nasal secretion, in short, obtaining it from the blood.

"C."—Showed an intense degree of ingestion, and nearly all the free organisms aggregated into clumps.

"D."—Showed extreme agglutination and some degree of ingestion, but not nearly so much as in "C," a rather curious result, probably due to rapid agglutination preventing the full play of the leucocytes.

A portion of each mixture washed in 1·5 per cent. saline, and freed from leucocytes by the centrifuge, was planted out immediately, and in every instance growth took place. The remaining portions of the mixtures were shaken up with a large bulk of normal saline and incubated at 37° for two hours, then lightly centrifuged. The upper portion was afterwards drawn off and recentrifuged hard for a quarter of an hour, whilst the deposit was planted out. Finally, the deposit of the recentrifuged upper portion, which would contain free germs, was also planted out. The result is shown in the following Table.

Experiment 6.

Mixture.	Planted immediately to show viability of culture.	Planted after 2 hours in normal saline.	
		Leucocytic deposit.	Free germ deposit.
"A"	Good growth	No growth	No growth.
"B"	Good growth	One or two colonies	No growth.
"C"	Good growth	Good growth	Good growth.
"D"	Good growth	Good growth	Good growth.

At first sight this result was incomprehensible, since it appeared that the immune serum of a patient (mixtures "C" and "D") had no bacteriological effect on his own germ or on a laboratory culture. Yet both were apparently killed by the serum of a normal man. But consideration of the foregoing experiments shows that in point of fact the result is quite consistent. The explanation would appear to be as follows:—

Mixture "A."—The old laboratory culture was taken up and killed by the leucocytes whilst free organisms succumbed to the normal saline.

Mixture "B."—This gave an identical result with Experiment 5 (*q.v.*). All loose germs were killed by the normal saline, whilst from the leucocytic deposit one or two colonies arose from odd germs, which had been ingested and protected from the action of the saline by the leucocytes.

Mixture "C."—Here the dominating factor in the situation seems to be the agglutination, which, with an undiluted immune serum, may take place in less than a minute. Under the influence of this serum, aggregations of germs are taken up by the leucocytes, and, owing to the crowding of the

cells, fail to get digested. At the end of 15 minutes' incubation, the aggregations are very large, and the germs cannot be acted on by the normal saline. Living germs can, therefore, be demonstrated both within and without the leucocytes after two hours' incubation in normal saline.

In regard to mixture "D," the same explanation would apply.

Finally we would like to draw attention to the fact that in some cases of cerebro-spinal fever, the leucocytes of freshly drawn spinal fluid frequently show the meningococci growing out from them in large numbers. The germs can be seen filling the interior of the cells and actually bursting them open in places and growing forth in dense masses.

In one case under our care, "Hayes," this condition was very obvious. In fig. 8 is shown a microphotograph of some of the freshly drawn leucocytes of this case. In the centre of the figure is a large polymorph cell which has been burst open on one side by a dense mass of meningococci. A close examination of the fluid showed that almost every second cell was in a similar condition. Staining with trypan blue showed at the same time that relatively few of these cells were dead. It is clear that in instances like these the meningococci are plainly alive within the leucocytes.

Having now shown that the meningococcus can be alive within the leucocytes, the suggestion that the disease appears as the result of accidental carriage of the organism into the spinal canal by emigrating leucocytes assumes some credibility. Should this suggestion prove true, then in it we have an explanation of the fact that the disease is so seldom transmitted direct, but usually through the intervention of a "carrier." Ingestion of the germs by the phagocytes would have to be an essential factor in the propagation of the disease. As we have seen, the meningococcus when freshly isolated, is insusceptible of being ingested by the phagocytes in the presence of normal serum.

It is also true of the meningococcus when isolated from the naso-pharynx of a patient suffering from the disease in the early stage. In a recent case, where the organism was present in the naso-pharynx on the third day of the disease, a very thick emulsion of the germ was incubated with normal serum and washed leucocytes for a quarter of an hour at 37° C., and no trace of ingestion of the germ by the phagocytes could be observed.

At various times a considerable number of strains have been examined in this connection, and although they gave rather variable results in certain instances, on the whole we found that the further removed an organism is from the case in which it caused the disease, either in point of time or passage from throat to throat, the more susceptible does it become to

ingestion on the part of the phagocytes. At the same time it also becomes weaker, and may succumb to the lethal action of the serum or of the serum and the leucocytes combined. Thus the examination of the leucocytes when tested with an enfeebled throat strain shows them to be gorged with "shades" and poorly stained cocci. The substance of the leucocytes is vacuolated, suggesting the complete digestion of some of the germs taken up. This appearance is never seen in recently isolated spinal strains even when ingestion of the germs by the phagocytes is obtained with an immune serum.

If it were possible to trace a case of disease to a certain "carrier," eliminating any other possible source of infection, we ought to be able to demonstrate phagocytosis of the organism when isolated from the "carrier," although no ingestion on the part of the phagocytes would be observed in the same germ when isolated from the patient.

We were fortunate in coming across such a case, where the course of infection seemed to be beyond doubt. A patient developed cerebro-spinal fever after he had been in hospital a few days. None of his hospital contacts were "carriers" of the meningococcus. On investigating the camp from which he came we found one "carrier" with whom he had been in close contact in the same hut. In the presence of normal serum the germ from this man's throat was readily attacked by the phagocytes to a considerable degree. No ingestion on the part of the phagocytes could be demonstrated, however, after the passage of the germ through the patient. These strains, both spinal and nasal, we presume were the same, as they both behaved identically when tested with Gordon's monovalent serum Type 2. In this instance, then, it is hard to avoid believing that the man was infected from this "carrier," and it is interesting in the light of our experiments to note that the germ at the time of infection in this case was rapidly attacked by the phagocytes in the presence of normal serum.

Summary.

As the result of the foregoing experiments we think we have obtained good evidence for thinking that under certain conditions the meningococcus can be taken up by the leucocytes but not killed by them. In the case of freshly isolated strains we have seen that the leucocytes will not take them up at first. With old laboratory cultures, on the other hand, ingestion on the part of the phagocytes takes place with great rapidity. In a short time the germs are killed and completely digested by the leucocytes.

This happens also with the majority of the nasal strains we have examined from chronic "carriers" although they show great individual differences.

In the intermediate stage between the fresh spinal condition and the naso-pharyngeal state, it can be shown experimentally that they are taken up, but not killed, by the leucocytes. They can be recovered from them after a period of 24 or 48, or even 60 hours, and grown on artificial media. If we can believe they behave similarly within the body, then we can understand how they might be carried into the spinal canal and there set up infection.

It might also explain why direct infection (apart from the "carrier"), seldom, if ever, takes place in cerebro-spinal fever; that is from patient to patient, attendant, or physician, etc., the phagocytes refusing to take up the germs in their virulent condition. In the "carrier," on the other hand, in the majority of instances, the germs have lost their virulence so completely that they are taken up and immediately killed and digested.

In conclusion: if the method of infection is by leucocytic conveyance, then the reason why direct infection is so uncommon is clear. The virulent organism is unsusceptible of being attacked by the phagocytes. The longer the germs grow in the "carrier" throat, the more easily will they be ingested until a time is reached, when, on ingestion, they are also destroyed. Somewhere between these two extremes, infection may produce the disease. The organism is sufficiently weakened to give in to the leucocytic attack, but not to lose its life in the battle. Should infection occur at this point, the leucocytes will pick them up from the mucous membrane of the naso-pharynx, and in the course of their wanderings will sometimes carry them into the spinal canal. There the liberated organisms will set up the disease, at the same time re-acquiring the power of resisting the attacks of the leucocytes in the presence of normal serum.

Appendix.

The important part played by virulent non-ingestible strains of the meningococcus, as compared with virulent indigestible ones, in the light of the foregoing experiments, renders necessary some consideration of Rosenow's* remarkable results with virulent and non-virulent strains of the pneumococcus.

Rosenow has brought forward certain experiments to show: first, that a non-virulent pneumococcus strain does not absorb opsonin from a normal serum, and that it is always non-ingestible; secondly, if well washed it will become indigestible. He considers these properties to be brought about through the possession on the part of the virulent cocci, of a specific

* Rosenow, 'Journ. Inf. Dis.,' vol. 4 (1907).

substance which he calls "virulin"—presumably this attaches itself to the microbes as a covering, as it is removed by washing; thirdly, a non-virulent strain of the pneumococcus may be rendered virulent by incubating it a certain time in a saline extract of a virulent one. When thus treated it was no longer indigestible, and would kill a guinea-pig, which he proved it would not do previous to this treatment.

His experiments, however, do not carry conviction. The loss of opsonic effect which he describes might be explained by the fact that the extract in which the avirulent pneumococci had lain would itself neutralise the opsonin, the death of the guinea-pig being the result of the injection, along with the non-virulent cocci, of some of the virulent toxins in which they had been placed. He explicitly states that the washing of these cocci was "rapid." It does not seem to be necessary to postulate a specific "virulin" to explain the result.

The importance of this point has led us to undertake some experiments on the same lines with the meningococcus, since this organism resembles the pneumococcus, in that a presumably virulent strain is not susceptible of being taken up by the leucocytes.

We can confirm Rosenow's finding up to a certain point, that a non-virulent indigestible meningococcus strain, when grown in glucose-serum broth to which a certain quantity of a killed freshly isolated non-indigestible meningococcus culture extract had been added, is no longer taken up by the leucocytes. We prefer to ascribe our finding, however, to a neutralisation of the opsonic properties of the serum, by the fragments and debris of the killed extract. A certain amount of this debris had been taken up by the leucocytes. Moreover, if Rosenow's contention is correct, that virulence depends on a specific "virulin" and that this "virulin" is taken up by the non-virulent pneumococci, so that they are now transformed into proper strains, that are not taken up by the leucocytes; then if this quality is in any way similar to that found under natural conditions, it should be retained by these cocci on subculture. This, however, did not hold in the case of our meningococci. The ingestible condition was immediately lost on the first subculture. It is clear that the treatment they underwent did not in any way invest them with a virulence similar to that of the freshly isolated meningococcus, which invariably retains its ingestible condition through a number of subcultures.

FIG. 1.

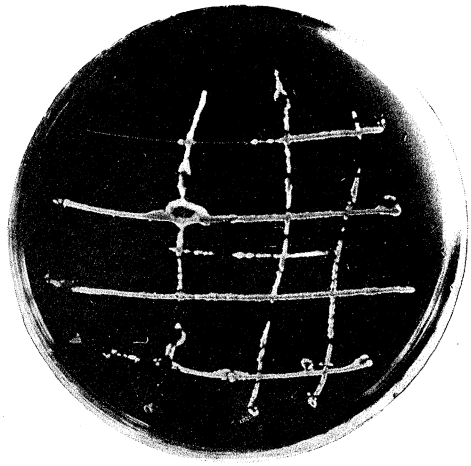

FIG. 2.

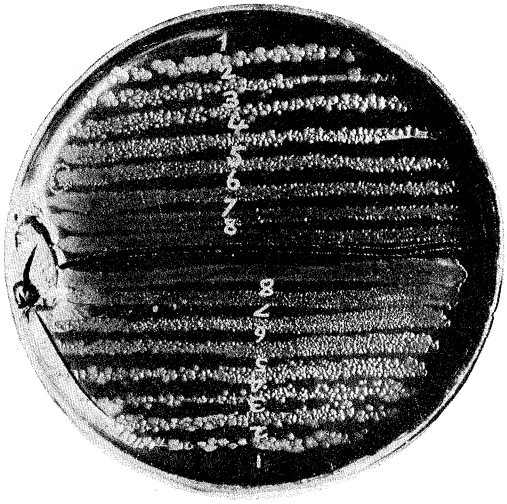

FIG. 3.

FIG. 4.

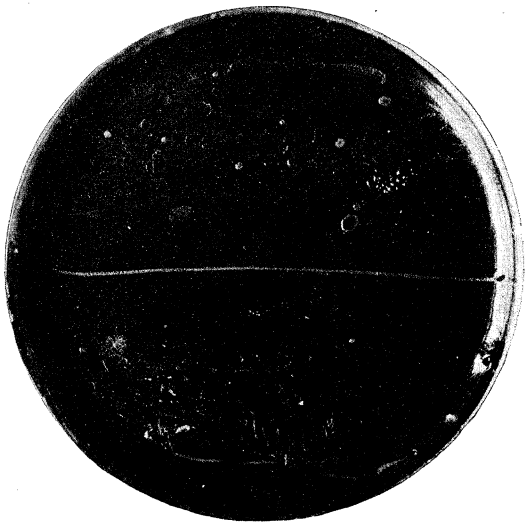

FIG. 5.

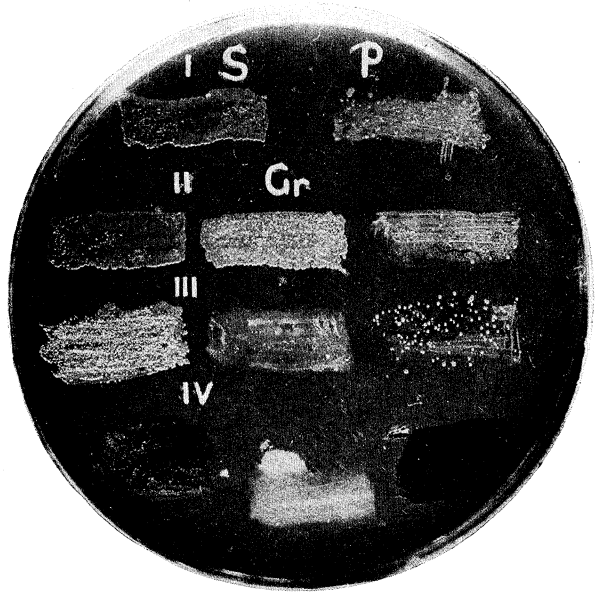

FIG. 6.

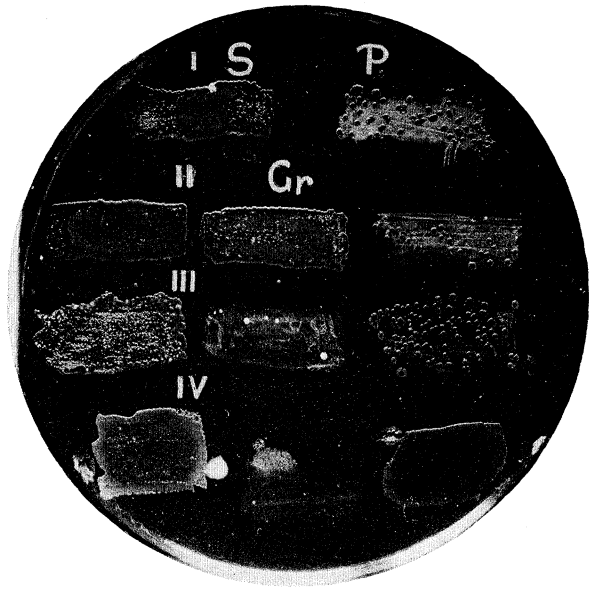

FIG. 7.

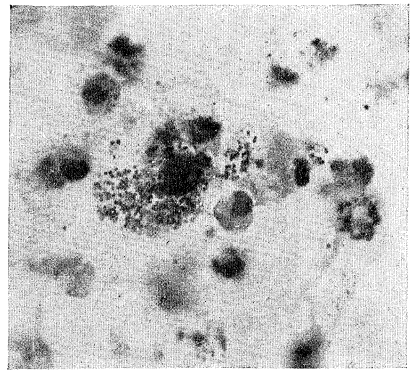

FIG. 8.

DESCRIPTION OF PLATES.

Experiment 3.

FIG. 1.—Photograph of planted out leucocytic deposit (1) of spinal fluid washed four times in 1 per cent. sterile glucose, after 24 hours' incubation at 37° C.

FIG. 2.—Photograph of planted out leucocytic deposit (2) of spinal fluid washed 16 times in 0·85 per cent. NaCl, after 24 hours' incubation, at 37° C.

FIG. 3.—Photograph of plate after 24 hours' incubation, to show the action of glucose on the growth of the meningococcus. Upper half of the plate shows eight dilutions of the germ in 1 per cent. glucose, while the lower half shows the same number of dilutions in distilled water.

The top and bottom line show the highest dilutions in each case.

Experiment 4.

FIG. 4.—Photograph of plate with leucocytic deposit of washed spinal fluid planted out immediately. In upper portion of plate the deposit has been planted out untouched. In the lower, the deposit has been crushed with sterile glass powder. Growth about the same on both portions of the plate, possibly slightly greater on the crushed area.

FIG. 5.—Photograph of leucocytic deposits similar to the last, but in this case they have been treated with 0·85 per cent. NaCl for three hours before being put on the plate. The upper portion of the plate represents the uncrushed while the lower shows the crushed deposit. Four or five colonies are showing after 24 hours' incubation in the upper half, while only one has developed in the lower half of the plate.

Experiment 5.

FIG. 6.—Photograph of plate used in Experiment 5, showing growth obtained at the end of 24 hours' incubation at 37° C.

FIG. 7.—Photograph of the same plate at 48 hours' incubation.

FIG. 8.—Microphotograph × 1000 showing in centre a large polymorph cell from freshly drawn spinal fluid being burst open by growing meningococci.

FIG. 1.

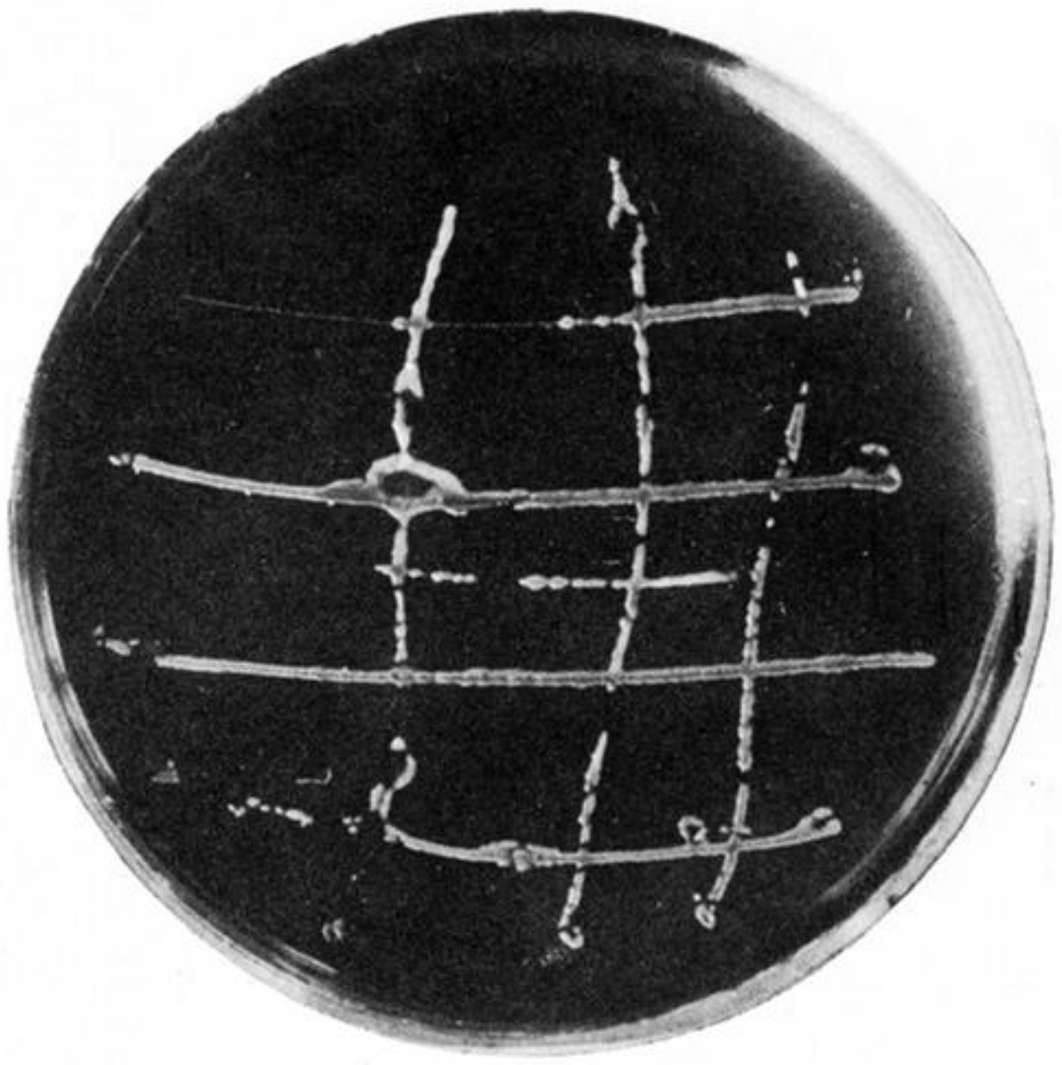

FIG. 2.

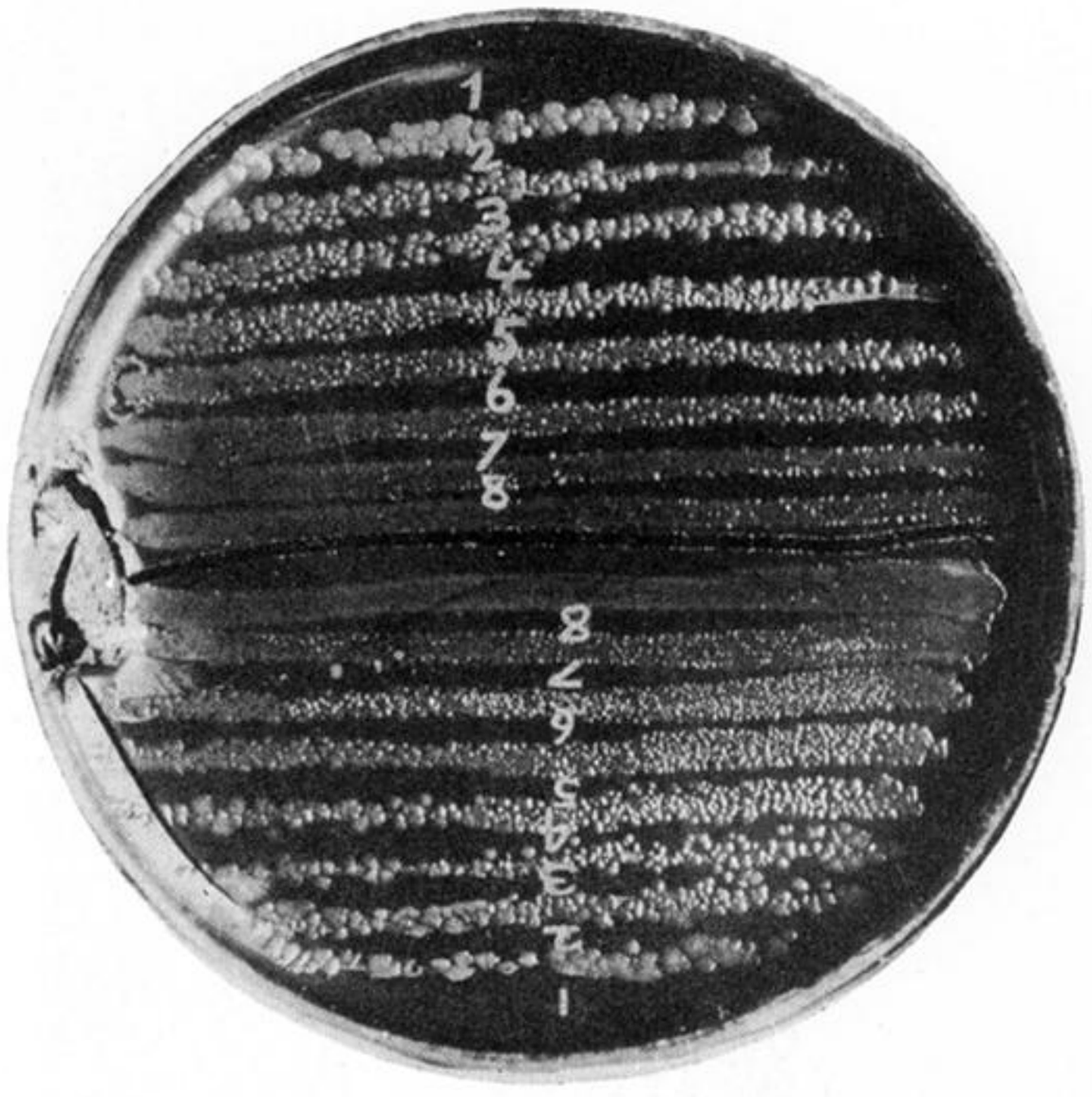

FIG. 3.

FIG. 4.

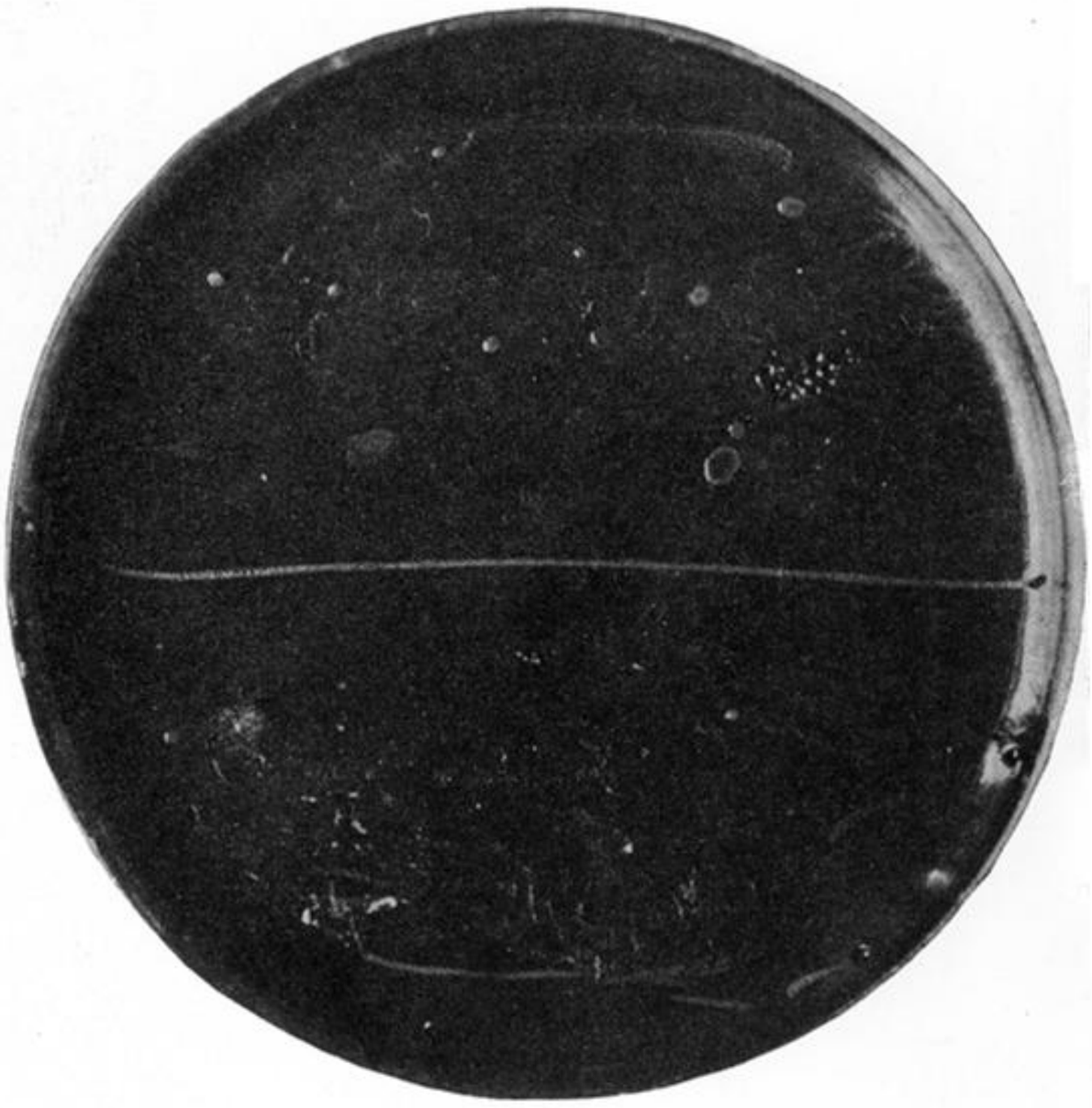

FIG. 5.

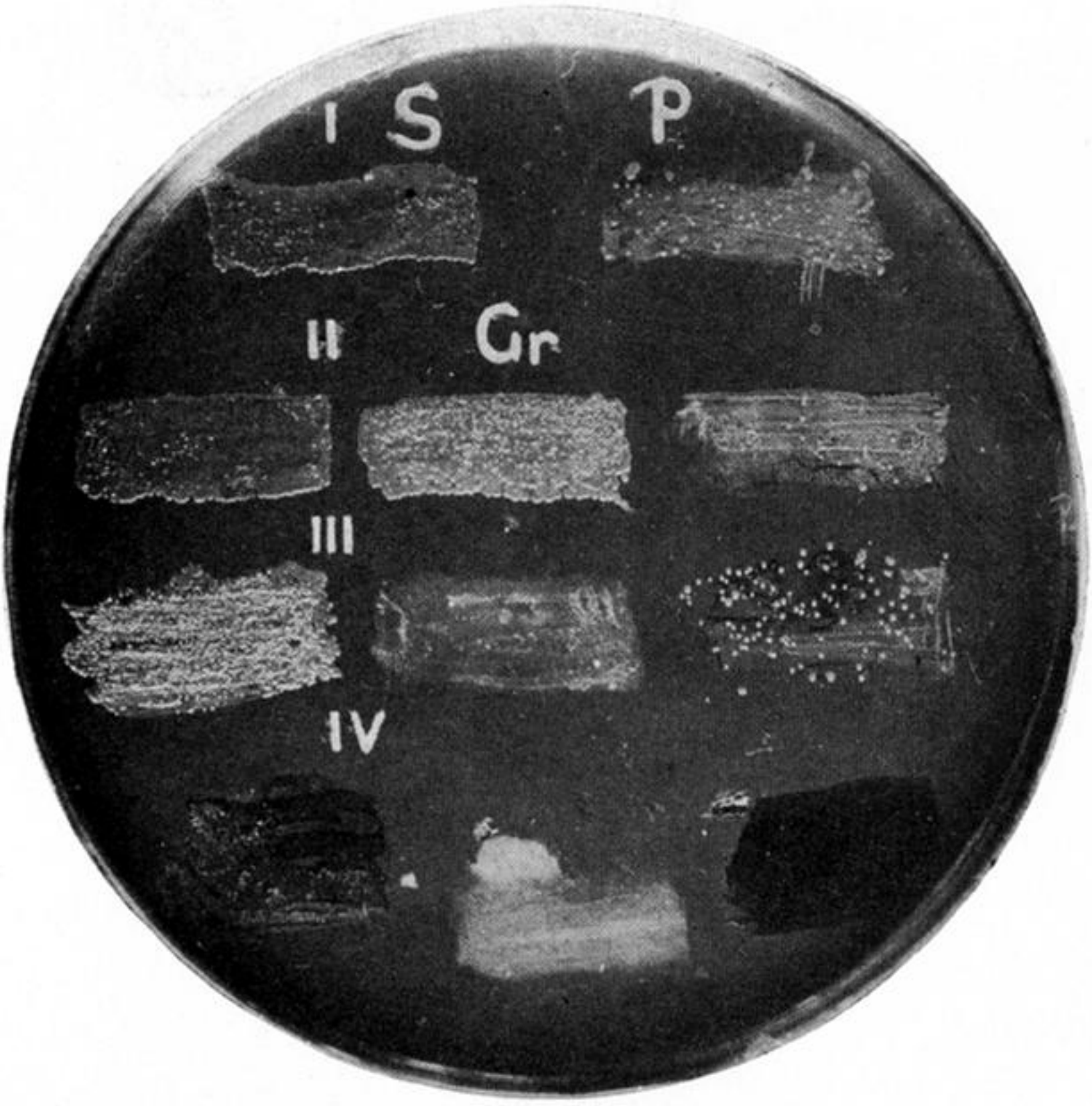

FIG. 6.

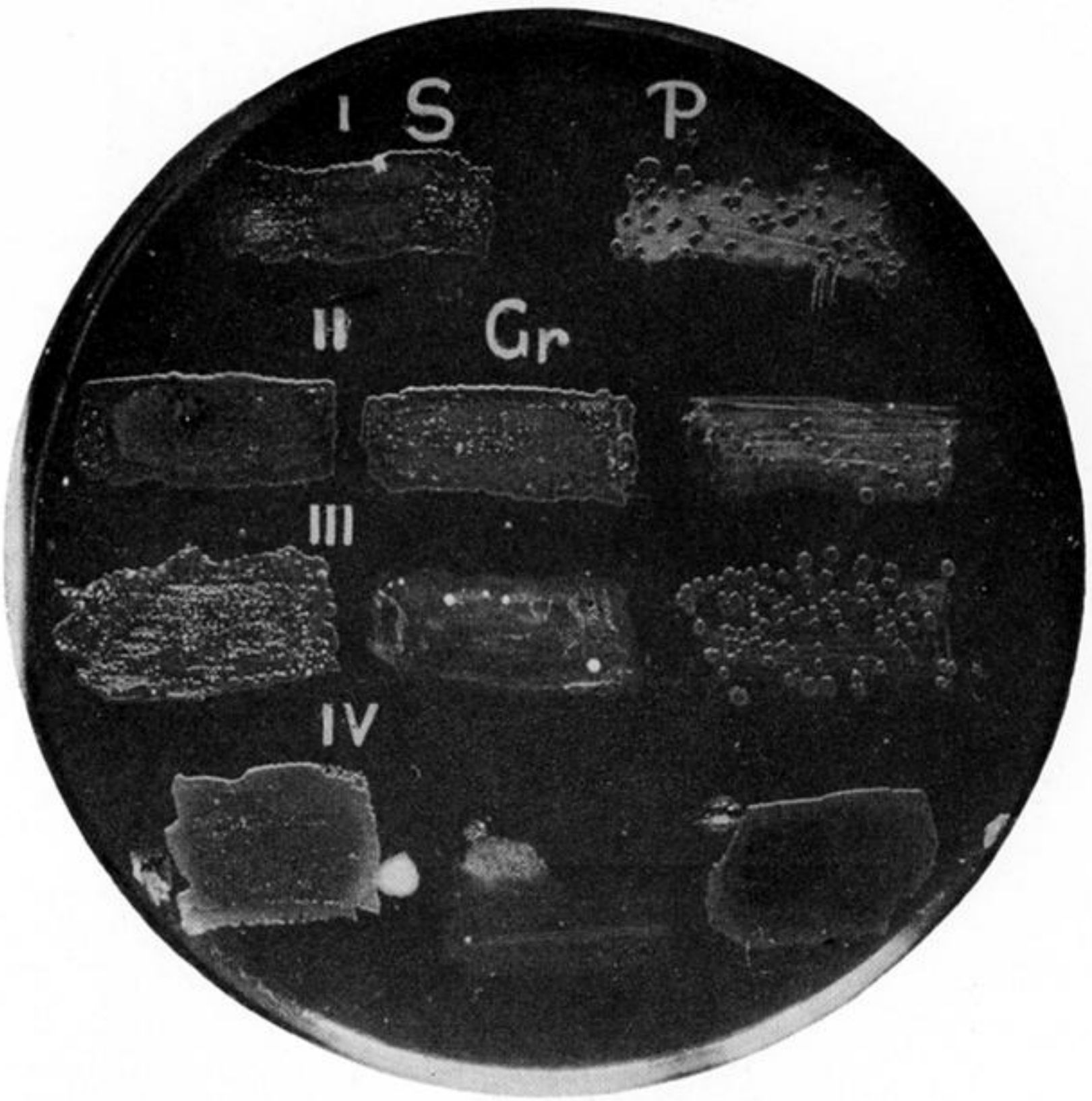

FIG. 7.

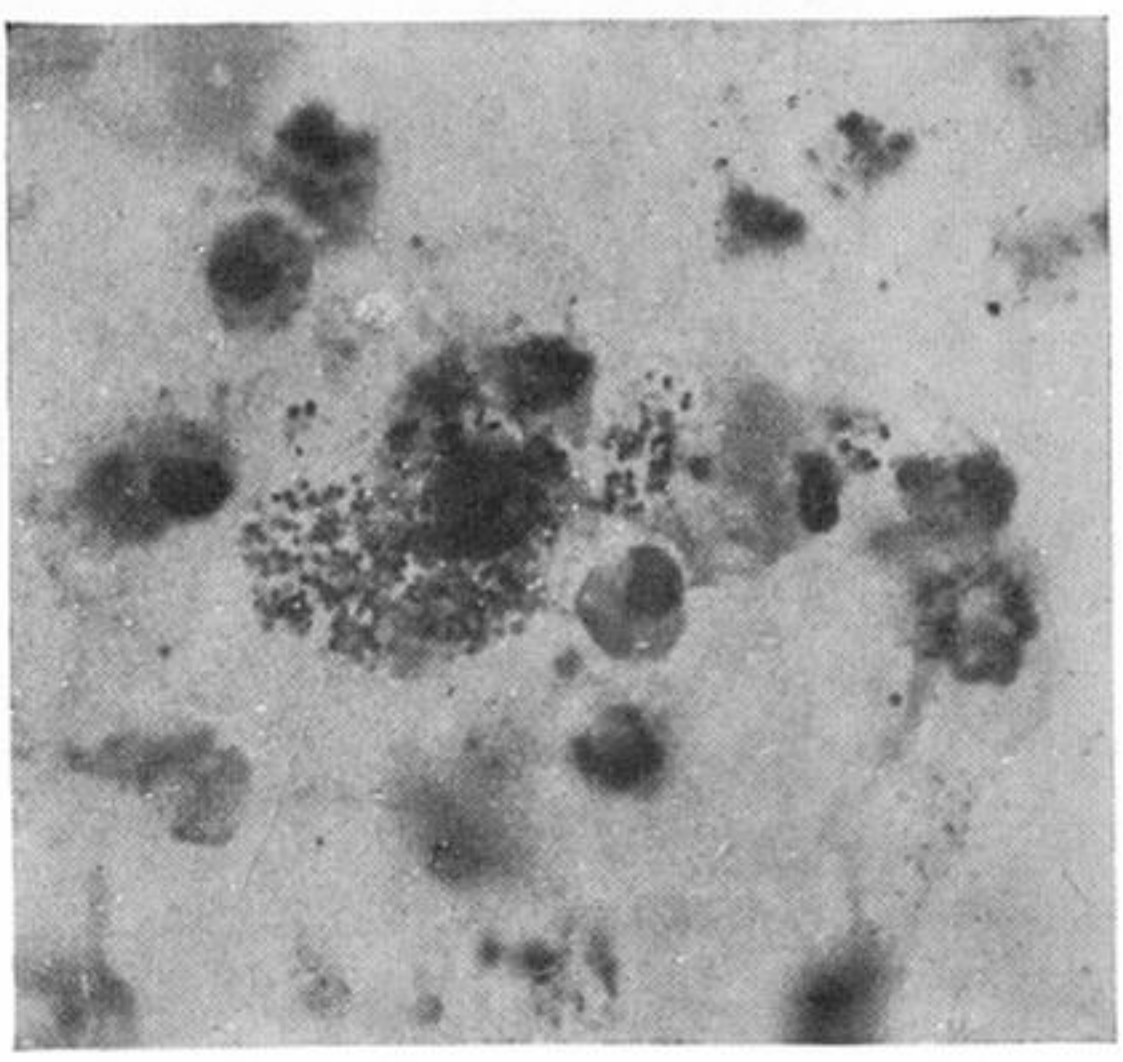

FIG. 8.