

ON THE RELATIONSHIP OF THE LEUCOCYTES AND CERTAIN ORGAN-EXTRACTS TO THE BACTERIO-LYTIC POWER OF THE BLOOD.¹

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SINCE Nuttall published his work on the bactericidal action of blood serum, a vast amount of research has been directed towards elucidating the intimate relations subsisting between the properties of the blood and the states of natural susceptibility or resistance to infective processes.

In recent years the subject has been expanded greatly by the labours of Metchnikoff, of Ehrlich, and of many others. For a long time an uncompromising opposition divided the workers in the field of immunity into two parties. Of late, however, a growing tendency towards a fusion of the rival schools of the humoralists and the supporters of the phagocyte theory, is manifesting itself. It is not surprising, indeed, that on many points, and these not the least important, a bewildering discordance of view still exists. One of the most interesting and vital of these issues relates to the rôle of the leucocytes as originators of bactericidal substances in the defensive mechanism of the animal organism.

At the suggestion of Dr. Allan Macfadyen I undertook an inquiry into this subject, especially as I was able to avail myself of certain new methods which will be described later, and which seemed to be peculiarly well fitted for an investigation concerned mainly with intracellular products. In view of the discrepancies of opinion already referred to I propose, before describing my own experiments, to give in broad outline a sketch of the principal results attained up to the present time.

I. ON THE RELATIONSHIP BETWEEN THE LEUCOCYTES AND THE ALEXIN OF SERUM.

Metchnikoff, in his recently published work on immunity, has set forth his views on this subject with admirable clearness. They may be summed up briefly thus:—He distinguishes between two forms of cytase—microcytase

¹ Thesis for M.D. of Aberdeen University.

and macrocytase. The former, derived from the polynuclear leucocytes, corresponds to the bacteriolytic complement, whilst the latter, a ferment peculiar to the lymphocytes, supplies the complement concerned in hæmolysis. The phenomenon of phagocytosis in naturally immune animals is explained by Metchnikoff, on the ground that in the leucocytes both cytase and immune body are present. Referring to the fact that the serum of the dog (an animal refractory to anthrax) is not bactericidal towards anthrax bacilli, he hazards the conjecture, that the reason may be found in the difficulty with which the cytases escape from the leucocytes, and also in the modifications they may undergo after being set free in the surrounding fluids. He suggests that in naturally resistant animals other soluble ferments may aid the cytases.¹

It is to be noted that all observers are not agreed as to the bactericidal properties of cellular effusions. It occurred to Metchnikoff that the divergent results might be due to the variety of leucocytes which happened to be in excess in the fluid. At his suggestion Gengou, using Buchner's method, tested, from this point of view, various exudates, some containing chiefly polynuclear, others mononuclear cells. He came to the conclusion that the former exhibited a bactericidal action, while the latter was quite devoid of any such action.

From a consideration of the phenomena observed in peritoneal effusions, called forth by bacterial injections, Beattie concludes that the evidence, on the whole, points to some alteration taking place in the micro-organism before it is ingested by the polymorphonuclear leucocytes, but not necessarily any actual degeneration. He thinks that the mononuclear cells play an important part in secreting a substance which produces changes in the blood serum, rendering it inimical to the life of the bacteria. Ainley Walker ranges himself on the side of the French school, and puts forward his opinion that complement is a leucocytic product only appearing in blood plasma or serum as a result of disintegration of leucocytes. On the other hand, Wright and Windsor take up the position "that it would seem difficult to assume that the bactericidal power of the serum is only a particular manifestation of a digestive power, or originally resident in the leucocytes, when we have realised that the serum exerts a digestive action on bacteria generally." They consider that in their researches the bactericidal effects are the result of definite chemical combinations, occurring between the bactericidal substance or substances in the blood and the affected bacteria.

In a study of the serum complement in disease in man, Longcope observed a rise in complement associated with hyper-leucocytosis—a fact which harmonises with the conception of the polynuclear leucocytes as a source of alexin. In a similar research, with the same end in view, Guseff was unable to trace any relationship between the amount of alexin and the number and kind of leucocytes present in the blood. A recent contribution to the subject is furnished by Ascher, who, working in Pfeiffer's laboratory, could not demonstrate the presence of complement in the polynuclear leucocytes of animals immunised with cholera cultures. Lastly, Charlton Briscoe, in a research on the origin of the complement in the peritoneal cavity of guinea-pigs, conducted under the direction of Aschoff, obtained negative results. He was unable to determine any immediate relationship between the pseudo-eosinophile cells (microphages) and the presence of bactericidal complement. He believes that the complement is, like the immune body, constantly present in the fluid of

¹ The term "alexin" is used (as by most authors) either as referring in a general sense to the bactericidal substance of the leucocytes and of the blood, or in its more restricted meaning of complement. The terms "cytase" and "complement" are employed indifferently to signify the same thing, whilst the expressions "immune body," "fixative," "substance sensibilisatrice," "Zwischenkörper," and "amboceptor," are used more or less as interchangeable terms, the last two having a special relation to the intermediary body of a normal bactericidal serum.

the peritoneal cavity, and probably proceeds from the exudation or transudation of the blood serum.

II. ON THE NATURE OF THE ACTION OF NORMAL BACTERIOLYTIC SERA.

The dependence of bacteriolysis, in the case of an immune serum, on the interaction of two substances (complement and immune body) suggested a similar conception as to what occurs in normal bactericidal sera. This view is strengthened by the experiments of Ehrlich and Morgenroth, which show that normal hæmolytic sera exhibit a dual action analogous to that of an immune serum. As a matter of fact, Pfeiffer has found sensitising substances for *V. cholerae* in the serum of the normal goat. Bordet and Gengou investigated the relations of several normal sera to certain micro-organisms. They find, for instance, that normal horse serum has no "substance sensibilisatrice" for *B. pestis*. Again, Bordet inactivated horse serum by heat, and reactivated it for *V. cholerae* by means of guinea-pigs' alexin. Levaditi agrees with Pfeiffer and Moxter, that the bacteriolytic power of normal sera is due to a complement and to a normal "substance sensibilisatrice." The specific cases he studied were the peritoneal exudate and serum of the guinea-pig and those of the rat. He found that the polynuclear leucocytes were not responsible for the formation of this "Zwischenkörper," but he does not go further into the question of the site of its production. An interesting contribution to the study of natural immunity is to be found in the work of Malvoz. He examined the serum of the adult dog, an animal highly resistant to anthrax, and brings forward evidence to show that it contains in abundance specific *fixateurs* for anthrax bacilli, while that of the more susceptible young dog is destitute of such sensitising substances. He attaches much significance to these facts, and considers that they yield a satisfactory explanation of the difference in susceptibility in the two cases. Wechsberg has discovered that the inactivated serum of the normal rabbit contains amboceptors, which, with the co-operation of the alexin of the guinea-pig, have a bactericidal action on typhoid bacilli. Gruber believes that the killing of weakened races of bacteria by normal serum occurs by alexin alone, whilst for the destruction of very virulent races a specific "helping" substance, or "Zwischenkörper," is needed. Buchner stands alone in his idea, that the alexin of blood is a single body of the nature of a proteolytic enzyme. On the latter point, indeed, he is on common ground with Metchnikoff, who classes the cytases amongst the endo-enzymes, and thinks that they are allied to the trypsin group. It is not necessary to do more than refer to the much-debated problem of the unity or plurality of complements. Bordet, as the upholder of an alexin common both to bacteriolysins and to hæmolysins, and Ehrlich, in maintaining that a whole series of complements may exist in any one serum, have given proof at least of their remarkable ingenuity in experimentation. To complete this part of the subject it may suffice briefly to recall the position taken up by a group of bacteriologists, prominent amongst whom are Baumgarten, Walz, and Fischer. They look upon bacteriolysis in normal sera partly as an expression of osmotic changes, brought about by the transference of the micro-organisms into an unaccustomed medium, and partly as the result of insufficient nutritive materials in the medium.

III. ON THE RELATIONS OF THE ALEXIN TO PLASMA AND TO SERUM.

The title of this section opens up a subject in the highest degree provocative of differences of opinion. Unfortunately the difficulties inherent in any

attempt to solve the problem by direct experiment are so great that the question seems destined to remain a source of controversy for years. Metchnikoff, influenced greatly by Gengou's paraffined tube experiments, clings tenaciously to the idea that the cytase cannot be demonstrated in blood plasma, and that in the cases where its presence has been detected in small amount, the reason is to be sought in its escape from the leucocytes by injury or after their death. Levaditi claims to have found evidence in favour of this view. Although denying emphatically that the cytases are secreted by the leucocytes, Metchnikoff admits that the fixatives in cases of acquired immunity may be secreted into the plasma by the phagocytes. In some cases, however, for example, where the blood serum has no preventive action, he believes they remain inside the cells. Buchner, Gruber, and Trommsdorf look upon the alexin as a secretion of the leucocytes. In a recent article, Petterssohn, in direct opposition to Gengou, states that the plasma of the circulating blood contains bactericidal substances. He believes, however, that the amount of alexin may be increased in certain cases by a separation from the leucocytes in shed blood. Ehrlich thinks that the complements are always free in the plasma. Finally, Sweet's work lends support to the view that a hæmolytic complement, which he has found in the serum of the rabbit, is present in the circulating plasma. The subject is admittedly a difficult one, and until fresh experiments are devised, whereby plasma may be prepared in a condition more nearly resembling that in the blood vessels, no statement can be positively made with regard to it.

IV. POSSIBLE SOURCES OF COMPLEMENT OTHER THAN THE LEUCOCYTES.

On this subject the amount of precise information available is very scanty. Landsteiner and Donath obtained an anti-complement by the injection of various animal cells. In experimental poisoning of rabbits by phosphorus, with consequent degeneration of the cells of the liver, Ehrlich and Morgenroth noted a disappearance of complement from the blood. They appear to regard the liver as a possible place of origin of complement. Ascher investigated the spleen, liver, and suprarenals in the fresh condition, for the presence of complement, but these were found to give no more than the corresponding blood or serum.

V. THE WRITER'S EXPERIMENTS.

General Account of Methods employed.

In the following experiments the method used for obtaining the freshly prepared contents of the leucocytes is one elaborated in this Institute by Rowland, and adopted in recent investigations on intracellular products by Hedin and Macfadyen, amongst others. The cells are triturated without admixture of sand or kieselguhr, at the temperature of liquid air, the low temperature rendering them so brittle that they are readily disintegrated in the ice formed from the moisture in the cellular mass. This cold-grinding process obviously presents a decided advantage over others, when it is remembered how easily the complement is affected by heat. Another practical point of value lies in the fact that, by its aid, the experiments may be commenced very shortly (two to three hours) after the exudate is obtained from the body of the animal. In Buchner's method, followed by Gengou and others, the cells are allowed to macerate in bouillon for twenty-four hours at 37° C. This procedure appears to be exposed to the risk of the destruction of the complement, or at the least of its conversion into complementoid. It may

be further pointed out that, as Conradi has shown, autolytic changes, leading to the formation of bactericidal substances, are liable to occur in cellular exudates kept for any length of time. As a consequence, the material used for the experiment may be quite different from that in the perfectly fresh condition.

In the work about to be detailed two main considerations were kept in view. First, the question as to whether the fresh intracellular products of polynuclear leucocytes contain bactericidal substances, as certain observers allege; and, second, failing any proof that such substances exist, whether the bacteriolytic agent in the serum owes its powers to substances originating in the leucocytes. In other words, is it possible that the white cells contain a complement, which, when added to a normal serum, inactivated by heat, is able to restore its bactericidal action? Having regard to the intricate problem of the unity or plurality of complements the questions at issue were, it was hoped, rendered capable of more accurate discrimination by limiting the experiments to those involving bacteriolysis; the complement was not, therefore, estimated by the aid of an inactivated hæmolytic immune serum. For determining the bacteriolytic effects sought for, the method of plating was resorted to, largely for the reason that the important work of Gengou, under Metchnikoff's guidance, might be controlled with greater exactitude, the same method having been adopted by this observer.

In all the experiments 0·25 c.c. to 0·5 c.c. of serum and cell-free exudate was used, and the disintegrated material available was divided equally amongst the various tubes containing it. A special precaution was taken, in the careful and repeated washing of the cells previous to grinding, in order that all traces of fluid exudate might as far as possible be removed; they were submitted to at least a fourfold centrifugalisation in normal salt solution. That this is a point of vital importance in all experiments of the kind may be appreciated from Ascher's results of careful as contrasted with inefficient washing of the leucocytes in his researches. When the cells were washed three times only, he found that a certain amount of bacteriolysis resulted. When the process was repeated four times, no evidence of granular degeneration was observed.

Finally, it may be mentioned that microscopic examination of the ground débris of the cells showed that none had been left intact. Deeply stained, irregularly shaped bodies were suggestive of fragments of nuclei.

The first four experiments were of an orientating character, and I do not feel disposed to attach much significance to the results obtained. For this reason the details need not be given.

EXPERIMENT V.—A large rabbit, which had received an intrapleural injection of 10 c.c. plant-casein emulsion eighteen hours previously, was bled to death. The pleural cavities contained a copious turbid exudate. This was pipetted into normal salt solution, with a trace of sodium oxalate, and was

centrifugalised. As a result of an accident, part of the leucocytic deposit was lost; consequently the ultimate yield was small. It was disintegrated at liquid air temperature, and then mixed with an equal bulk of broth. The exudate stained by carbol-thionine showed chiefly polynuclear cells. A young broth culture of *B. typhosus* was used in this and the subsequent experiments. It is to be noted that in this experiment the centrifugalisation of the cells in salt solution was admittedly imperfect.

TABLE I.

	A.	B.	C.	D.
1. Disintegrated leucocytes + broth + <i>B. typhosus</i>	1440	0	0	0
2. Supernatant cell-free fluid of exudate	2700	92	0	0
3. Supernatant cell-free fluid of exudate (heated 55°-60° C. half an hour)	1520	1110	∞	∞
4. 1+3	2680	56	37	10
5. Serum	960	0	0	0
6. Serum (heated 55°-60° C. half an hour)	2720	abt. 3000	∞	∞
7. 1+6	1600	0	0	0

A. = Plates made immediately after addition of typhoid organisms, in order to serve as a control.

B. = Plates made three hours after.

C. = Plates made six hours after.

D. = Plates made twenty-one hours after.

The interest of this experiment is to be found in the apparent bactericidal action manifested by the contents of the polynuclear leucocytes. As no special pains were taken to remove traces of the fluid portion of the exudate, the result entirely loses its value, although it emphasises the importance of the care requisite for this step of the experiment. In the similar experiments, carried out by Gengou, only two washings of the cells were considered necessary. It would seem in view of this result and of Ascher's, to which reference has been made, that revision of the technique in this respect is called for, in order to place his conclusions on a reliable basis. It is clear, on this account also, that the reactivation of the exudate and serum (4 and 7) cannot be held to be established. The bactericidal action of the cell-free exudate and serum is in accordance with the results of subsequent experiments.

EXPERIMENT VI.—A large, full-grown rabbit was injected with 6 c.c. of an alkaline emulsion of plant-casein in each pleural cavity. Eighteen hours after the animal was bled to death. Over 20 c.c. of turbid exudate were removed from each cavity and mixed with physiological salt solution. 5 c.c. were pipetted into a small centrifugal tube without the addition of salt solution. All the material was spun. The cellular deposit was thoroughly mixed with normal salt solution, by means of stirring with a sterile glass rod. In this way the cells were washed with fresh salt solution four times. After the cellular mass had been freed from excess of moisture, it was ground at liquid air temperature for two hours. For the same length of time a portion of the serum was kept at the same temperature. Films of the fresh exudate, stained by carbol thionine and by Jenner's stain, showed 80 per cent. of

polynuclear leucocytes. Reaction of ground leucocytes found to be distinctly alkaline to litmus paper.

TABLE II.

	Immediately after.	2 Hours after.	18 Hours after.
1. Ground leucocytes + broth + <i>B. typhosus</i> .	3500	4000	∞
2. Cell-free exudate heated	2470	∞	∞
3. 1 + 2	3400	3350	∞
4. Serum	2500	0	0
5. Serum heated	1230	1200	∞
6. 1 + 5	3710	3500	224
7. Liquid air serum	3140	0	0
8. Liquid air serum heated	5470	4000	about 10,000

This experiment points conclusively to an absence of bactericidal substances in the polynuclear cells. No reactivation of the inactivated exudate has occurred, and although the comparatively small number of colonies in the third plating seems to indicate a partial restoration of the bacteriolytic power of the serum, the experiments which follow may be anticipated so far as to remark that the result has only on one other occasion been obtained. The fact that the serum kept for the same length of time as the leucocytes, at the low temperature, has undergone no diminution in its bactericidal power forms a useful control, and meets a possible objection, that the alexin in the white cells is destroyed at the low temperature. It is evident that the serum alexin is not affected by it in the slightest degree. This corresponds with the experience of Courmount, Chanaz, and Doyen, who found that the agglutinating properties of a serum were unaltered after exposure to -180° C. for twenty minutes.

EXPERIMENT VII.—A rabbit, which had been injected with $6\frac{1}{2}$ c.c. casein in each pleural cavity, was bled to death. About 10 c.c. of fluid on each side. Part of exudate unmixed with salt solution spun. Cells in remainder spun down and washed in normal saline three times. The exudate was blood-stained, so that red cells formed part of the deposit. Excess of fluid removed from cells, which were then disintegrated at liquid air temperature for half an hour. For the same length of time, part of the supernatant serum of the exudate and of the blood was kept in liquid air. Reaction of ground substance distinctly alkaline. Film of exudate, stained by Jenner's stain, shows 95 per cent. polynuclear leucocytes.

TABLE III.

	Immediately after.	2 Hours after.	18 Hours after.
1. Ground leucocytes + broth + <i>B. typhosus</i> .	704	272	0
2. Cell-free exudate	1248	0	0
3. Do. frozen	864	9	58
4. 2 inactivated	860	704	∞
5. 1 + 4	1248	640	∞
6. Blood serum	640	3	0
7. 1 + inactivated serum	768	512	16
8. Frozen serum	992	0	0
9. Do. heated	480	832	190

The value of the results obtained from the leucocytic juice in this experiment is discounted to an equal extent with that in Experiment V., and the same criticism is applicable. For the same reason the reactivation experiments may be put out of court. The results given by the frozen exudate and serum are in agreement with those in the previous experiment.

EXPERIMENT VIII.—A rabbit, injected as before with casein eighteen hours previously, bled to death. 8 c.c. exudate in each pleural cavity, with considerable admixture of blood. Cells spun and thoroughly washed. Deposit of white and red cells, ground at liquid air temperature for seventy minutes. Serum of rabbit, immunised with *B. typhosus*, obtained for this experiment. 95 per cent. polynuclear cells in film, stained by Jenner's stain.

TABLE IV.

	Immediately after.	2 Hours after.	18 Hours after.
1. Ground cells + broth + <i>B. typhosus</i> . .	1904	2400	∞
2. Supernatant serum exudate	2016	1824	22
3. Do. inactivated	2832	2800	∞
4. 1+3	3296	2048	∞
5. Normal rabbit serum	2032	4	0
6. Do. inactivated	2640	1616	∞
7. 1+6	3040	1456	∞
8. Immune serum	1920	...	0
9. Do. inactivated	2720	1120	∞
10. 1+9	9600	∞

Here, again, no bactericidal action is manifest in the case of the polynuclear leucocytes. The reactivation experiments have also proved negative. The endeavour to render active a heated immune serum has not succeeded; this coincides with Ascher's similar experiments with cholera immune serum.

EXPERIMENT IX.—Procedure as before. 15 c.c. exudate in each pleural cavity. Deposit, after spinning, consisted of equal parts of white and red cells. Ground at liquid air temperature for one hour. White cells of exudate contained 95 per cent. polynuclears.

TABLE V.

	Immediately after.	3½ Hours after.	6½ Hours after.
1. Ground cells + broth + <i>B. typhosus</i> . .	1856	4800	∞
2. Supernatant serum exudate	2464	7	0
3. Do. inactivated	1520	1024	∞
4. 1+3	992	2320	∞
5. Normal serum	528	0	0
6. Do. inactivated	624	1632	248
7. 1+6	576	1072	1680
8. Immune serum, kept two days on ice, frozen at -180° C. for seventy minutes	1824	0	0
9. Same immune serum inactivated . .	1584	∞	2048
10. 1+9	2176	∞	1680

The results accord with those in the last experiment.

EXPERIMENT X.—Details of procedure as in previous experiments. Animal killed by chloroform. 15 to 20 c.c. exudate obtained from each pleural cavity. Deposit consisted equally of leucocytes and red corpuscles. It was ground at -180° C. for one hour. The proportion of polynuclears was 95 per cent.

TABLE VI.

	Immediately after.	5 Hours after.	24 Hours after.
1. Ground substance + 0.8 per cent. NaCl solution + <i>B. typhosus</i>	145	2720	∞
2. Ground substance + broth + <i>B. typhosus</i>	1152	∞	∞
3. Ground substance + broth + <i>B. typhosus</i>	608	∞	∞
4. Supernatant serum exudate	928	224	∞
5. Do. inactivated	512	∞	∞
6. 1+5	1072	∞	∞
7. Normal serum	960	0	0
8. Do. inactivated	928	1440	1248
9. 1+8	134	2304	∞

Nothing calls for special remark in this experiment except that the cell-free exudate has exhibited no bactericidal action. The tube containing it was found to enclose a soft fibrinous clot. It appears very probable that, as Petterssohn has stated, the alexin is readily absorbed by the fibrin. In this way the bacteriolytic effect of a serum or exudate in contact with a clot may be diminished or may entirely disappear.

EXPERIMENT XI.—Rabbit, injected eighteen hours previously with 7 c.c. casein in each pleural cavity, killed by chloroform. In left pleural cavity, 8 c.c. fluid; in right cavity, very little exudate. In both, masses of clot with entangled leucocytes. Cell-free exudate obtained by centrifugalising. Clot broken up in sterile salt solution, and cells thoroughly freed from plasma. Deposit contained moderate proportion of red cells. Cells disintegrated at liquid air temperature for half an hour. Leucocytes consisted of 90 to 95 per cent. polynuclear cells. Part of leucocytic extract used at once for experiment; the rest kept for twenty-four hours at 37° C. before use.

TABLE VII.

	Immediately after.	3½ Hours after.	18 Hours after.
1. Leucocytic extract + <i>B. typhosus</i>	592	1040	∞
2. Do. + <i>B. coli</i>	544	2240	∞
3. Do. + <i>B. enteritidis</i> (Gaertner)	1926	∞	∞
4. Exudate + <i>B. typhosus</i>	1008	512	17
5. Do. inactivated	4160	∞	∞
6. 1+5	934	1376	∞
7. Serum	528	0	0
8. Do. inactivated	368	288	∞
9. 1+8	336	672	5
10. Leucocytic extract, kept twenty-four hours at 37° C.	256	1536	∞
11. Leucocytic extract, kept twenty-four hours at 37° C., inactivated	704	864	0
12. 10 + inactivated exudate	1136	∞	∞
13. 10 + inactivated serum	544	∞	∞

It was a natural conjecture that the negative results recorded in the previous experiments might conceivably be due to the fact that the fresh leucocytic contents included a complement which, however, was in a condition of an inactive precursor. In the résumé of the views held with regard to the nature of the alexin it has been shown that the majority of observers are agreed in regarding it as an enzyme. If this is so, and especially if Metchnikoff be correct in his belief that it is an endo-enzyme, *i.e.* that it fulfils its functions within the cell, then a pro-enzyme might possibly be present in leucocytes obtained fresh from the animal.

Accordingly, I carried out an experiment in which dilute acetic acid was added to fresh leucocytic juice, with the view of effecting a conversion of the latter into its active modification. As, however, typhoid bacilli are exceedingly sensitive to acetic acid, the method was abandoned in favour of allowing the material to remain at incubator temperature for twenty-four hours. It will be seen that this, as well as the fresh material, was not able to restore the activity of the heated exudate and serum except in the case of Series 9. It is interesting to note that this experiment is comparable to those made by Gengou, in that the material was not used till the lapse of twenty-four hours, and yet the result is totally different. In explanation of this difference, I need only refer to the remarks appended to the foregoing experiments. The bactericidal action of the heated twenty-four hours' leucocytic extract seems anomalous. It will be seen that the polynuclear leucocytes contain no bactericidal substance for *B. coli*, a result which does not accord with Gengou's experience.

EXPERIMENT XII.—Procedure similar to that in last experiment. Cells which included an equal number of red corpuscles, ground at -180° C. for half an hour. Leucocytes mainly polynuclear.

TABLE VIII.

	Immediately after.	4 Hours after.	7 Hours after.	18 Hours after.
1. Serum + <i>B. typhosus</i>	424	0	0	...
2. Do. + <i>B. enteritidis</i> (Gaertner)	2720	∞	∞	...
3. Cell-free exudate + <i>B. typhosus</i>	1024	∞	∞	...
4. Do. + <i>B. enteritidis</i> (Gaertner)	2160	∞	∞	...
5. Fresh leucocytes + <i>B. typhosus</i>	864	∞	∞	∞
6. Leucocytes, kept thirty-six hours, at 37° C. + <i>B. typhosus</i>	912	∞	∞	∞
7. Leucocytes, kept thirty-six hours, at 37° C. + <i>B. enteritidis</i> (Gaertner)	4480	∞	∞	∞
8. Leucocytes, kept thirty-six hours, at 37° C. + inactivated serum + <i>B. typhosus</i>	560	∞	∞	∞
9. Leucocytes, kept thirty-six hours, heated + <i>B. typhosus</i>	860	∞	∞	∞

The main results confirm those of Experiment XI. It will be observed that *B. enteritidis* (Gaertner) is much more resistant to rabbit serum than *B. typhosus*.

EXPERIMENT XIII.—A large rabbit received, in each pleural cavity forty-eight hours previously, 10 c.c. of washed guinea-pig's erythrocytes, suspended in physiological NaCl solution, containing a trace of sodium oxalate. Another rabbit, eighteen hours previously, injected as before with 6 c.c. casein. Both rabbits killed by chloroform. The latter yielded about 30 c.c. exudate, containing comparatively few red corpuscles. Stained by carbol-thionine, it showed 90 per cent. of polynuclear cells. In the thoracic cavity of the former there was a small amount of blood-stained fluid. The pleuræ were covered with a thick reddish fibrinous material which, when examined, was found to consist almost exclusively of mononucleated cells, some with the remains of red corpuscles in their interior. Both exudates were spun, and the deposits washed and mixed.

The mixture was then disintegrated at liquid air temperature for forty minutes.

TABLE IX.

	Immediately after.	4 Hours after.	18 Hours after.
1. Ground leucocytes + <i>B. typhosus</i> . . .	384	1328	∞
2. Do. inactivated . . .	496	∞	∞
3. Normal serum (polynuclear) . . .	864	0	0
4. Do. inactivated . . .	2112	∞	∞
5. 1 + 4 . . .	1328	768	2496
6. Normal serum (mononuclear) . . .	1360	0	0
7. Do. inactivated . . .	2560	∞	1408
8. 1 + 7 . . .	1664	752	∞

The object of this experiment was to determine whether a mixture of the intracellular products of polynuclear and mononuclear leucocytes had a bacteriolytic action. It seemed not unlikely that the alexin, if an enzyme, required the co-operation of another body before it was able to produce its effect. A similar mode of action has received prominence of late from the study of the influence of entero-kinase on the activity of pancreatic juice. From this point of view, it seemed a reasonable supposition that such an auxiliary body might be furnished by the mononuclear cells. Levaditi has found that no amboceptors exist in the polynuclear leucocytes of the normal guinea-pig, but he makes no suggestion that they originate from the mononuclear leucocytes. Metchnikoff evidently holds the macrophages responsible for the formation of immune body in the case of artificial cytotoxines. It remained to be seen whether they supply an amboceptor in cases of natural immunity. So far, however, as this experiment goes, it would seem to negative such a possibility.

TABLE X.

	Immediately after.	3 Hours after.	18 Hours after.
1. Ground cells + <i>B. typhosus</i> . . .	512	3580	∞
2. Serum . . .	960	0	0
3. Do. inactivated . . .	544	0	10
4. 1 + 3 . . .	464	0	∞

EXPERIMENT XIV.—Rabbit injected intrapleurally with casein forty-eight hours previously. Exudate, chiefly in left cavity, collected, spun, and cells freed from plasma. A considerable admixture of red corpuscles. Deposit ground at -180° C. for half an hour. Film, stained by Jenner's stain, shows 50 per cent. mononuclear leucocytes, some containing two or three polynuclear cells in their interior.

This result confirms that of the last experiment.

EXPERIMENT XV.—Rabbit treated as before; killed nearly forty-eight hours after; moderate exudate, consisting largely of mononuclear cells—50 per cent. Disintegrated at -180° C. for half an hour.

TABLE XI.

	Immediately after.	4 Hours after.	18 Hours after.
1. Ground cells + <i>B. typhosus</i>	672	∞	∞
2. Serum	1040	0	0
3. Do. heated 55° C., half an hour	668	0	∞
4. Do. do. 63° C., half an hour	352	∞	∞
5. 1+3	864	∞	∞
6. 1+4	896	∞	∞

Here, again, the results go to prove that a mixture of microphages and macrophages of the rabbit produces no bactericidal effect on typhoid bacilli.

EXPERIMENT XVI.—It has been noted that in almost every case a considerable proportion of red corpuscles was unavoidably present in the exudate. The ideal experiment is, of course, that in which leucocytes alone are collected and disintegrated. The difficulty does not seem an easy one to overcome. Gengou makes no reference to any admixture of red cells. Weleminsky, in the account of his work, frequently mentions the presence of erythrocytes in the leucocytic deposit, a circumstance which indicates that the complication is incidental to the mode of operating. Although it might not be possible to eliminate this disturbing factor, it seemed advisable to ascertain whether the lack of bactericidal action on the part of the leucocytic products was not traceable to an anti-complement contained in the red cells. On the assumption that the leucocytic alexin is identical with that of the serum, the following experiment was undertaken:—

The red corpuscles of a normal rabbit were thoroughly washed free of serum, and were then ground in Rowland's apparatus for half an hour at -180° C.

TABLE XII.

	Immediately after.	2 Hours after.	18 Hours after.
1. Disintegrated red cells + <i>B. typhosus</i>	320	480	∞
2. Normal serum	176	0	0
3. Do. + disintegrated red cells	256	0	0
4. Typhoid immune serum	45	0	0
5. Do. + disintegrated red cells	308	0	0

No doubt can be entertained that the alexin, both of a normal and an immune serum, is not in the least affected by an admixture of disintegrated red corpuscles in an amount proportional to that present in the leucocytic deposit. One is driven to the conclusion, then, either that the complement of leucocytes is not identical with that of the serum, or that they contain no complement at all. The latter view, taking all the experimental facts into consideration, is to my mind the logical outcome of the present inquiry.

The search for complement in the leucocytes having given negative results, certain fresh organ juices were examined.

EXPERIMENT XVII.—A portion of the liver of a healthy rabbit was excised immediately after death. It was cut into small pieces, and these were further broken up in sterile salt solution by means of a glass rod. The blood was removed as much as possible by repeated spinning in fresh saline. The tissue was finally disintegrated at -180° C. for forty minutes.

- | | |
|---|--|
| 1. Liver extract + <i>B. typhosus</i> . | } A = immediately, B = 3 hours, and
C = 18 hours. |
| 2. Do. + <i>B. enteritidis</i> (Gaertner). | |
| 3. Liver extract, heated half an hour to 60° C. + <i>B. typhosus</i> . | |
| 4. Serum + <i>B. typhosus</i> . | |
| 5. Do. inactive + <i>B. typhosus</i> . | |
| 6. 1 + 5 + <i>B. typhosus</i> . | |
| 7. 1 + 5 + <i>B. enteritidis</i> (Gaertner). | |

The plates showed an increase in the number of colonies except in the case of the serum.

It will be recollected that Ehrlich and Morgenroth, from indirect evidence, were inclined to look upon the liver as a source of complement. Ascher, on the other hand, by direct experiment, could not demonstrate that the liver was specially concerned in its production. This experiment gives evidence that fresh liver juice is not bactericidal, and that no complement is contained in the liver cells.

EXPERIMENT XVIII.—The spleen of a normal rabbit was excised aseptically, cut up into small pieces with sterile scissors, and washed several times in sterile salt solution. The fragments were then ground at liquid air temperature for half an hour.

- | | |
|--|--|
| 1. Ground spleen + <i>B. typhosus</i> . | } A = immediately, B = 5 hours,
C = $7\frac{1}{2}$ hours. |
| 2. Do. + <i>B. enteritidis</i> (Gaertner). | |
| 3. 1 inactivated + <i>B. typhosus</i> . | |
| 4. Do. + <i>B. enteritidis</i> (Gaertner). | |
| 5. Serum + <i>B. typhosus</i> . | |
| 6. Do. + <i>B. enteritidis</i> (Gaertner). | |
| 7. Inactivated serum + <i>B. typhosus</i> . | |
| 8. Do. + <i>B. enteritidis</i> (Gaertner). | |
| 9. 1 + inactivated serum + <i>B. typhosus</i> . | |
| 10. 1 + inact. serum + <i>B. enteritidis</i> (Gaertner). | |

The results may be briefly stated. There was no proof of bactericidal action in 1, 2, 3, 4, 6, 7, 9, 10. *B. enteritidis* (Gaertner) grew well in the serum. A5 672, B5 0, C5 0.

Ascher, in confirmation of this experiment, could find no relationship to exist between alexin and the spleen. This would doubtless be explained by Metchnikoff and his co-workers, on the ground that

it is a macrophagic organ, and can give origin only to a hæmolytic complement.

EXPERIMENT XIX.—The fresh marrow was removed from the hind-limb bones of a normal rabbit. Since no liquid air happened to be available, this marrow was ground in a small pot with admixture of kieselguhr for three hours—cold water circulating in the outer jacket during the process. An equal amount of broth was added to the mixture of sand and ground substance. The supernatant fluid obtained by spinning was used for the experiment. The marrow, stained by Jenner's stain, had a normal appearance.

TABLE XIII.

	Immediately after.	3½ Hours after.	18 Hours after.
1. Ground bone-marrow + <i>B. typhosus</i> . . .	368	192	0
2. Do. inactiv. + <i>B. typhosus</i> . . .	400	176	0
3. Serum + <i>B. typhosus</i>	170	0	0
4. Do. inactivated	208	0	0
5. 1+4	128	96	1056
6. Serum + <i>V. cholerae</i>	4244	0	0
7. Do. inactivated + <i>V. cholerae</i> . . .	7392	∞	∞
8. 1+7	8448	0	0

It is difficult to ascribe the bactericidal effects noted in this experiment to a definite cause. Account must, of course, be taken of the blood circulating in the bone-marrow. It is possible to attribute the result either to the serum or to the marrow cells themselves. I do not feel justified, from a single experiment, in assigning it to either factor. Unfortunately, I had no opportunity of repeating the experiment. The result, however, is in agreement with the statement of Tarasséwitch, that extract of bone-marrow, a microphagic tissue, contains a bactericidal substance—Metchnikoff's "microcytase."

Since neither a bactericidal substance nor complement could be shown to reside in the leucocytes of whatever kind, it appeared to be a matter of some interest to investigate in a similar fashion the leucocytes of rabbits whose natural resistance towards *B. typhosus* had been heightened by a previous immunisation. The statement generally made with regard to artificial immunity against micro-organisms is, that the characteristic feature of the process concerns the immune body, and does not, to any appreciable extent, affect the complement. Yet it is not unreasonable to suppose, if bactericidal substances be present in the white cells of the untreated animal, that a quantitative or qualitative difference might be established in those of the same animal after a course of immunisation. As I wished to extend the study of the subject in this direction to its final issue, in order to

determine as nearly as possible the rôle of the cells in a state of immunity, the following experiments were carried out:—

EXPERIMENT XX.—A rabbit which had received 10 c.c. of a killed broth culture of typhoid bacilli intraperitoneally, and 10 c.c. subcutaneously, with a week's interval, was subjected eight days after the last inoculation to an intrapleural injection of casein. Eighteen hours after the animal was killed, and a copious exudate, quite unmixed with blood, was found in the pleural cavities. The leucocytes, 95 per cent. of which were polynuclear, were treated as in former experiments, and then disintegrated at -180° C. for three-quarters of an hour. The serum was found to agglutinate in a dilution of one in several hundreds.

TABLE XIV.

	Immediately after.	1½ Hours after.	6 Hours after.	18 Hours after.
1. Ground leucocytes + <i>B. typhosus</i>	1056	800	3200	∞
2. Do. inactivated	400	448	∞	∞
3. Cell-free exudate	768	400	0	0
4. Do. inactivated	432	about 400	512	∞
5. 1 + 4	480	560	∞	∞
6. Serum	1204	192	0	0
7. Do. inactivated	960	432	∞	∞
8. 1 + 7	1600	1120	∞	∞

The results are on a par with those of the normal rabbit's leucocytes. No evidence of microcytase is forthcoming, and no reactivation of the serum and exudate has taken place.

EXPERIMENT XXI.—A rabbit, immunised with typhoid bacilli by means of frequent injections extending over five weeks, was treated in the usual manner for the purpose of obtaining an exudate. Eighteen hours after, a moderate amount was procured, 95 per cent. of the white cells being of the polynuclear variety. Deposit ground at -180° C. for half an hour.

TABLE XV.

	Immediately after.	2 Hours after.	18 Hours after.
1. Ground leucocytes + <i>B. typhosus</i>	288	0	∞
2. Do. inactivated	224	5	726
3. Cell-free exudate (clotted)	640	896	∞
4. Do. inactivated	528	735	∞
5. 1 + 4	320	about 300	1072
6. Serum	325	112	512
7. Do. inactivated	96	110	896
8. 1 + 7	384	416	672

The results substantiate those in the last experiment.

EXPERIMENT XXII.—A rabbit, immunised with typhoid bacilli, yielded, eighteen hours after an injection with casein, a good creamy exudate with 90 per cent. polynuclear cells, on the right side. Left lung greatly congested—probably due to injury at time of injection; exudate, deeply blood-stained, was not used. Cells ground half an hour at -180° C.

TABLE XVI.

	Immediately after.	2 Hours after.	18 Hours after.
1. Ground leucocytes (free from red cells) + <i>B. typhosus</i>	416	128	640
2. Ground leucocytes, heated	38	145	1680
3. Exudate	304	0	0
4. Do. inactivated	448	1008	1232
5. 1+4	496	256	∞
6. Serum	384	0	0
7. Do. inactivated	224	368	668
8. 1+7	300	350	35

This experiment bears out the previous results.

EXPERIMENT XXIII.—This experiment is on the same lines as the previous three. A good exudate, consisting entirely of polynuclear cells, was obtained. A very small proportion of red corpuscles was present. Cells ground for half an hour at -180° C.

TABLE XVII.

	Immediately after.	2 Hours after.	18 Hours after.
1. Ground leucocytes + <i>B. typhosus</i>	704	448	∞
2. Cell-free exudate (clotted)	250	18	∞
3. Do. inactivated	208	496	1280
4. 1+3	1360	1382	∞
5. Serum	400	44	0
6. Do. inactivated	?	176	1720
7. 1+6	144	194	1220

The results are evidently comparable to those of the previous experiments.

EXPERIMENT XXIV.—A rabbit which had received frequent doses of typhoid bacilli was chloroformed to death forty-two hours after a casein injection intrapleurally. A small amount of viscid, blood-stained exudate was obtained. The pleural cavities were washed out with salt solution, and the cells thus recovered were added. It was largely a mononuclear exudate—40 per cent. The cells were ground for half an hour at -180° C.

TABLE XVIII.

	Immediately after.	2 Hours after.	18 Hours after.
1. Ground leucocytes + <i>B. typhosus</i>	415	240	∞
2. Do. inactivated	96	192	∞
3. Serum	208	0	0
4. Do. inactivated	336	352	∞
5. 1+4	176	56	∞
6. Ground leucocytes of last experiment, three days old	44	43	∞
7. Ground leucocytes of last experiment, three days old, inactivated	560	∞	∞

There is a close correspondence between this experiment and Nos. 13, 14, and 15, in which the late exudate was employed. The results show that a previous immunisation leaves both polynuclear and mononuclear leucocytes unaffected, as far as it relates to the formation of an intracellular bactericidal or reactivating substance.

EXPERIMENT XXV.—A rabbit immunised with *B. coli* injected eighteen hours previously with casein; a moderate exudate. Procedure as before. Cells ground at -180° C. for half an hour.

TABLE XIX.

	Immediately after.	3 Hours after.	18 Hours after.
1. Ground cells + <i>B. coli</i>	816	480	∞
2. Cell-free exudate	384	464	1648
3. Do. inactivated	304	864	∞
4. 1+3	256	912	∞
5. Serum	176	256	6590
6. Do. inactivated	272	320	∞
7. 1+6	336	496	∞

It will be noted how much more resistant *B. coli* is to rabbit serum and exudate than *B. typhosus*.

EXPERIMENT XXVI.—A rabbit immunised with cholera cultures, injected eighteen hours previously with casein intrapleurally. Killed by chloroform. Pleural cavities opened, and creamy exudate pipetted off into salt solution. 90 per cent. polynuclear cells; the remainder being large vacuolated mononuclear cells. Cells washed as before, and ground at -180° C. for half an hour.

TABLE XX.

	Immediately after.	2 Hours after.	18 Hours after.
1. Ground leucocytes + <i>B. cholerae</i>	2	4	4
2. Do. inactivated	22	0	0
3. Cell-free exudate	66	1	0
4. Do. inactivated	4	72	624
5. 1+4	29	9	480
6. Serum	26	0	0
7. Do. inactivated	10	11	688
8. 1+7	9	6	928

This experiment recalls those of Ascher which led to similar results.

The immunity of the dog to anthrax involves many interesting questions. Metchnikoff, in the absence of direct proof, has endeavoured to find an explanation of the phenomenon which will square with the principles of his phagocyte theory. Thus, he gets over the difficulty inherent in the circumstance that dog's serum is not bactericidal towards anthrax bacilli, by suggesting that the cytase

does not easily escape from the cells, and that it may be modified after it is set free in the surrounding fluid. He also rather vaguely hints that it may be aided by other soluble ferments. Recently Malvoz, in the work shortly outlined in the preliminary sketch, offers a tangible contribution to the subject, in his discovery of specific fixatives for anthrax bacilli in the serum. The statement is rendered doubly interesting by the additional allegation that no such substances are to be found in the serum of the newly born dog which happens to be susceptible to anthrax. Malvoz does not proceed further into the matter; he apparently leaves it open to the assumption that the fixatives act in conjunction with the microcytase. Gengou has found that the extract of the dog's polynuclear leucocytes is bactericidal towards anthrax bacilli. In a series of experiments, about to be described, I examined the problem from the same standpoint as in those already set forth.

EXPERIMENT XXVII.—A dog was injected with 20 c.c. casein emulsion in each pleural cavity. Twenty-four hours afterwards it was killed by chloroform. The thoracic cavity contained about 80 c.c. of somewhat blood-stained exudate, the leucocytes of which were almost exclusively polynuclear cells. The usual procedure was carried out. The deposit of cells was ground at -180° C. for three-quarters of an hour. A non-sporing eighteen hours' old broth culture of anthrax was used in the experiment.

TABLE XXI.

	Immediately after.	2½ Hours after.	18 Hours after.
1. Ground cells + <i>B. anthracis</i>	22	64	∞
2. Do. inactivated	18	89	∞
3. Cell-free exudate	26	192	∞
4. Do. inactivated	14	96	∞
5. 1+4	contaminated	73	∞
6. Serum	17	45	∞
7. Do. inactivated	50	17	∞
8. 1+7	50	33	∞
9. 1+3	22	95	∞

No evidence of a bacteriolytic or complementary substance within the polynuclear leucocytes is obtainable.

EXPERIMENT XXVIII.—A portion of the cells of the previous experiment kept twenty-four hours was used in this.

TABLE XXII.

	Immediately after.	5 Hours after.	18 Hours after.
1. Ground cells + <i>B. anthracis</i>	30	560	∞
2. Do. + <i>B. typhosus</i>	∞	∞	∞
3. Exudate + <i>B. anthracis</i>	10	∞	∞
4. Do. + <i>B. typhosus</i>	1920	15	108
5. Serum + <i>B. anthracis</i>	16	8	∞
6. Do. + <i>B. typhosus</i>	2400	0	0

The numbers of the colonies in the above table are self-explanatory.

EXPERIMENT XXIX.—Dog; intrapleural injection of casein eighteen hours previously. Killed by chloroform. Large exudate; almost entirely polynuclear cells. Cellular deposit after usual treatment ground at -180° C. half an hour.

TABLE XXIII.

	Immediately after.	3 Hours after.	18 Hours after.
1. Ground cells + <i>B. anthracis</i>	46	2	∞
2. Cell-free exudate	29	1	∞
3. Do inactivated	46	∞
4. 1+3	39	92	∞
5. Serum	45	23	∞
6. Do. inactivated	16	0 ?	82
7. 1+6	18	336	∞

The results of the action of the cell extract on anthrax bacilli are directly opposed to those of Gengou. The attempt in this and in the last experiment to reactivate the heated serum—a step which presumably leaves the fixatives untouched—has led to no positive result. The indication is then that the polynuclear leucocytes of the dog do not enclose a complement capable of reactivating an amboceptor in the serum in the case of *B. anthracis*.

EXPERIMENT XXX.—A portion of the spleen, liver, and bone-marrow were removed aseptically from a healthy dog, immediately after its death by chloroform. The tissues were freed as far as possible from blood and then disintegrated separately for half an hour at liquid air temperature.

TABLE XXIV.

	Immediately after.	2 Hours after.	18 Hours after.
1. Ground bone-marrow + <i>B. anthracis</i>	19	640	∞
2. Do. spleen	29	98	∞
3. Do. liver	35	57	∞
4. Serum	12	132	∞
5. Do. inactivated	56	336	∞
6. 1+5	37	92	∞
7. 2+5	55	976	∞
8. 3+5	37	1360	∞

The experiment tends to show that these fresh tissues have no bactericidal action on anthrax bacilli, and that they do not possess any complementary powers when added to the inactivated serum.

CONCLUSIONS.

The following abstract of the results represents the general conclusions which may be legitimately drawn from the experiments I have described:—

1. No bactericidal substance for *B. typhosus*, *B. coli*, or *B. enteritidis* (Gaertner) exists in any of the varieties of leucocytes obtained from the normal adult rabbit.

2. No complement capable of reactivating cell-free exudate or serum of the normal rabbit can be shown to be present within the polynuclear leucocytes.

3. A mixture of polynuclear and mononuclear leucocytes has proved equally inactive.

4. No substances reactivating a typhoid immune serum of the rabbit can be demonstrated in the polynuclear leucocytes of the normal rabbit.

5. Even in the case of rabbits which have been previously immunised with typhoid cultures, the leucocytes are similarly found to be devoid of any bacteriolytic or reactivating power; this also holds good in animals immunised against coli and cholera cultures.

6. The cell-free exudate of normal rabbits is bactericidal towards *B. typhosus*, but this action is not as marked as in the case of the corresponding serum.

7. Rabbit serum is invariably powerfully bactericidal towards typhoid bacilli, but not towards *B. enteritidis* (Gaertner).

8. The cells of the spleen and liver of the normal rabbit are incapable of acting as a substitute for the bacteriolytic complement of its serum.

9. The polynuclear leucocytes of the dog, examined for the presence of a bactericidal substance for anthrax bacilli, give negative results.

10. These cells are not even able to furnish a complement to the serum of the dog, which will render it bactericidal towards anthrax bacilli.

11. The intracellular products of the spleen, liver, and bone-marrow of the normal dog, when added to its serum, fail to impart to it a bacteriolytic action.

The general bearing of these experiments on recent investigations into the same subject may be shortly discussed. References to the literature of the complement and its relation to the leucocytes show that the most conflicting views are expressed by workers in this province of the immunity question. The principal results with which my experiments are at variance are to be found in Gengou's article on the "Origin of the Alexin of Normal Sera." This author's statement, accepted as correct by Metchnikoff, is that an extract of the polynuclear leucocytes of the rabbit and dog contains proportionately more complement than the corresponding serum. The proof of the existence of complement (cytase) in Gengou's work lies in its bacteriolytic

action on various micro-organisms. If this be inquired into, one is forced to the conclusion that the complement alone is sufficient for the bacteriolysis. It is needless to point out that this mode of action is essentially different from that of an immune serum. Moreover, accumulating evidence favours the view that the activity of the alexin in a normal serum is conditioned by the intervention of a complement, and of an amboceptor; the section on the action of normal bacteriolytic sera gives examples of such cases. Gengou certainly does not suggest that a normal amboceptor resides in the polynuclear leucocytes. Levaditi has submitted this matter to a special research, and finds, at least in the case of the normal guinea-pig, that the polynuclear cells do not enclose a "substance sensibilisatrice." To put the subject to further proof, the plan of adding the leucocytic contents to an inactivated serum was adopted in my experiments, as it was considered a more direct method than Gengou's of determining the presence of complement in the leucocytes; it at least obviated the risk of mistaking a bactericidal substance in the leucocytic extract for cytase, simply on the ground of its bacteriolytic action. The necessity for this was accentuated by the contradictory results obtained previously by several observers. Laschsenko added washed leucocytes to inactivated serum, and was able thereby to reactivate it. On the contrary, Denys, Hahn, and Leclef quite failed to do so. As Wechsberg describes a normal amboceptor in rabbit's blood for typhoid bacilli, the attempt at reactivation by disintegrated leucocytes seemed particularly appropriate. Even if the view be preferred that the mode of action is dependent upon a single body—Buchner's alexin—the interpretation to be put upon the results need not be materially modified, since they ought to show whether the alexin of polynuclear leucocytes can take the place of the serum alexin. As in my experiments, differing therein from Gengou's, the cell-free exudate is bactericidal for *B. typhosus*, though, to a less extent than the serum, the same method was applied to it. In the case of the serum and exudate of the dog, the statement of Malvoz, that normal fixatives for anthrax bacilli are present lends additional significance to my reactivation results. A possible fallacy that has occurred to me in connection with my own work is, that the negative results may be due to "complement-ablenkung." This is not, however, likely, because the enormous numbers of leucocytes taken would presumably ensure a quantity of complement sufficient for the reactivation of the small amount of serum employed.

It is worthy of note that in a very few of my experiments there was a comparative diminution in the colonies, apparently indicative of a vicarious complementary function on the part of the intracellular products. It does not seem to me that these exceptional results overturn the general trend of the experiments, even although it may be granted that they are due to a small amount of complement—

possibly set free from the cells. This admission is none the less compatible with the conclusions already arrived at, and does not, in my judgment, refute the main contention that the leucocytes do not enclose any appreciable quantity of complement. In this connection, I may refer to the special method adopted in the foregoing experiments. It is, I submit, eminently one which should reveal the existence of an alexin, if it is present to any extent in the cellular contents. It may be claimed that the view of the matter expressed above is sustained by the recent work of a number of observers, amongst whom are Ascher, Wright and Windsor, Guseff, Sweet, and Briscoe. It is significant also that Pfeiffer, Abel, and Ascher have absolutely failed, by repetition of Metchnikoff's experiments, to support his belief in the necessary association of leucocytes with bactericidal action *in vivo*; these authors, on the contrary, insist on the extracellular destruction of bacteria, injected subcutaneously or intraperitoneally.

Although, on the evidence, the facts seem to justify this conclusion, I wish, finally, to put forward my opinion that the experiments I have described do not exclude the possibility of the leucocytes acting as *secretors* of alexin or complement; they do, however, as I interpret them, distinctly negative Metchnikoff's view, that the cytases are preformed within the cells, and do not escape from them unless they have received an injury or are actually destroyed.

The pleasant duty is incumbent upon me, in conclusion, of recording my sense of indebtedness to Dr. Macfadyen and to Mr. Sydney Rowland, for the exceptional facilities generously placed at my disposal, and for the pains they personally took to secure for me accurate and reliable results.

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