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A REVIEW OF CURRENT THEORIES REGARDING IMMUNITY.

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II.

The question of the complexity of immune bodies and of complements can be best illustrated by bringing forward certain experiments of Ehrlich, who has always held to the multiplicity of both substances. These experiments have, like the former, to do with haemolysis.

In immunising two goats with a sheep's blood, Ehrlich found that the serum on being heated for $\frac{3}{4}$ hour at 56° C. lost a capacity which the serum of the normal goat possesses of dissolving rabbit's blood. Only after 3 hours at 56° C., or a $\frac{1}{2}$ hour at 65° C. did the serum lose its effect on sheep's blood. There was thus evidence of a thermolabile and a thermostable complement existing in the same serum. Evidence of multiplicity of immune bodies and complements is also present in the following. Goat's serum dissolves rabbit's or guinea-pig's blood corpuscles. This property is lost by heating to 55° C. but returns on the addition of horse serum, *i.e.* a body exists normally in the serum of the goat which corresponds to an immune body and this finds a sufficient complement in the serum of the horse. It may be said that the horse serum has by itself no haemolytic action on rabbit's blood. Ehrlich proceeds to ask, Are the bodies which dissolve the rabbit's and the guinea-pig's corpuscles the same? First of all are the bodies which correspond to the immune body the same? (When such occur in normal sera Ehrlich calls them "Zwischenkörper," which we shall translate "go-betweens.") Here the amount of inactive goat's serum which by the action of a given amount of horse complement could dissolve a given amount of guinea-pig or rabbit blood corpuscles was determined. The amount of inactive serum was about

the same for the two kinds of blood. The given amount of rabbit's blood was taken, the given amount of goat's serum, inactivated by heat so as to destroy the complement and leave only go-between, was added, and, after standing, was centrifugalised. The clear fluid was now tested for a go-between that would with the aid of horse complement dissolve rabbit's blood. None was present, but on the addition of horse complement to the deposit haemolysis took place. The whole of the go-between between rabbit's blood corpuscles and horse complement was thus attached to the corpuscles. If a second quantity of the rabbit blood was now in the same way treated with inactive goat serum and centrifugalised, and to the clear fluid guinea-pig blood and horse complement was added haemolysis occurred. Haemolysis also occurred when horse complement was added to the deposit. This showed that the rabbit corpuscles, while taking up all the go-between concerned in their own complete solution left something behind which could act as a go-between between guinea-pig corpuscles and horse complement. In other words, in the original amount of inactive goat's serum which was sufficient when supplied with horse complement to dissolve either guinea-pig or rabbit corpuscles there must have been two bodies, one capable of linking the complement on to the one kind of corpuscles, the other capable of linking it to the other corpuscles. There were thus two Zwischenkörper. Were there also two complements, one for each go-between? To determine this the amount of normal serum from an unimmunised goat necessary to dissolve the corpuscles in 2 c.c. of a 5 per cent. solution of guinea-pig blood on the one hand and of rabbit blood on the other was found. Such ordinary fresh serum was filtered through a particular kind of filter; it was now noticed that while the amount required to dissolve the guinea-pig blood was the same as before, so far as rabbit blood was concerned it was much weaker, If, however, to this weak filtrate horse complement was added its former power returned. This showed that the reason of the weakening was that some complement had been removed by the filtration and that there was as much go-between as before. In other words, there was evidence that here there were two complements as well as two go-betweens. Ehrlich found the same to be the case with the go-betweens of dog serum and guinea-pig corpuscles. Such serum can be activated, *i.e.* can be supplied with complement either from guinea-pig serum or horse serum.

The relation of these experiments not only demonstrates Ehrlich's ideas as to the complexity of the haemolytic process, but it serves to

accentuate the probability that the relations between the different bodies involved are of a chemical nature. This is not the view taken by Bordet[52]. According to the latter, while there is no doubt that the immune body is specific for each case of haemolysis, *i.e.* the serum of a goat treated with sheep's corpuscles would not dissolve any corpuscles except those of the sheep, yet there is not evidence of a chemical reaction between the immune body and the appropriate complement. Bordet thinks that the former sensitizes, as it were, the corpuscles and enables a complement to enter into them and cause the actual solution. The main point with this observer is that once a red blood corpuscle is sensitized by an immune body it is liable to a kind of ferment action on the part of different complementary bodies. He brings forward several facts in support of this view. He finds that of a particular haemolytic immune serum from the guinea-pig ·4 c.c. could dissolve ·5 c.c. of a given solution of rabbit's corpuscles, but if, to such a quantity of the serum, the solution of corpuscles was added gradually (say, ·2 c.c. followed in an hour by ·1 c.c.), then no solution took place of the blood added after the first fraction. He compares this phenomenon to the taking up of dye by blotting-paper. In a given solution of dye there may be quite enough to dye a large piece of such paper, but if this be torn up and a small piece be placed in the dye, followed at intervals by other small pieces, it will be found that the pieces of paper added last will not be stained so deeply as those placed earlier in the dye. Ehrlich admits the fact regarding the immune serum, though he has in repeating the experiment rather varied it. He determined the exact amount of goat's serum inactivated by heat, which, with the aid of the minimum amount of complement derived from the goat or sheep (both of which complements happened in this instance to be effective), would cause complete solution of 2 c.c. of a 5 per cent. solution of dog's blood corpuscles. To each of a series of tubes, containing the latter amount of dog's blood, there were added different multiples of this simple dissolving dose of inactivated serum, thus $1\frac{1}{4}$, $1\frac{1}{2}$, $1\frac{3}{4}$, 2, $2\frac{1}{2}$ times the amount were added. These stood for 1 hour at room temperature,—no haemolysis occurred for no complement was present, though of course during this time immune body would be taken up by the corpuscles. They were then centrifugalised, and to the clear fluid there was in each case added the same amount of blood and also sufficient fresh complement. It was found that only in the tube, to which 2 c.c. or more of the inactive serum had been added, did haemolysis take place, *i.e.* these were the only tubes in which there was left free

enough or more than enough immune body for complete solution. Therefore no more immune body than was required for the solution of the given amount of corpuscles had been in the first tubes taken up by the latter; in another similar experiment, however, in which another immune serum was used, it was found that the corpuscles had taken up 100 times the amount of immune body required for solution. In other words, in such an experiment as that last described it was not till 100 times the simple dissolving dose was added to the mixture of immune body and blood corpuscles that evidence of any immune body remaining unattached was found in the fluid from which by centrifugalisation the corpuscles had been removed. Ehrlich explains these divergent results by supposing that there are differences in the capacities of different corpuscles to take up immune body. The rationale of these differences he thinks is, that in certain blood corpuscles there may be receptors, *i.e.*, affinities capable of being satisfied by taking up a haemolytic immune body, other than are concerned in the fixation which results in haemolysis. Bordet, from his point of view, has brought forward other experiments on this subject. In the case of the guinea-pig treated with rabbit blood, a serum is obtained which, when inactivated by heat, can be reactivated not only by the fresh serum of the guinea-pig but also by the similar serum of the rabbit itself. Bordet's interpretation of this observation is that the blood corpuscles sensitized by the same immune body are capable of being dissolved by different complements. Ehrlich has repeated the observation and confirmed the facts, but pursuing investigations along the lines of the experiments given above, by which he proved the presence in one immune serum of two immune bodies each of which was capable of saturation by a special complement, he holds that here also there were present in Bordet's serum two immune bodies, and therefore in his opinion he establishes the presumption that here also these were satisfied by two complements, one derivable from the fresh serum of the guinea-pig, the other from the fresh serum of the rabbit.

This brings us to a point of great importance, and one which bids fair to be the cause of considerable discussion, namely, the question of the specificity of immune bodies and of complements. With regard to the former there is, as has already been remarked, very little dispute as to their specificity in relation to any particular haemolytic or bacteriolytic reaction. But in the broad view of the theory of immunity we must clearly bear in mind the limits of this specificity. It must be remembered that the immune body is supposed to be derived from side-chains which

play a part in the normal metabolism of the cell. Therefore we must assume that these side-chains are capable of saturation by other affinities than those possessed by such foreign bodies as bacteria, blood cells, etc. It is quite conceivable that in such normal metabolism these side-chains might be saturated and over-saturated by some material, say, some normal food-material of the cell, and that, through this, there might be cast off into the serum a body which would be identical with the body produced by the saturation of the same side-chains by some foreign body. Such an occurrence would explain the presence of immune bodies in ordinary normal serum, of which we have already seen one example. So far then is the specificity of the immune bodies limited. The main controversy will, however, evidently take place over the specificity of the complements. Is there, as Ehrlich holds, normally existing in the serum of each species of animal a whole series of complements or is there only one? As long ago as 1888 Nuttall, followed in 1892 by Buchner [53], had attributed the property, exhibited by many sera, of killing bacteria to the presence of substances which the latter observer called alexines and which Nuttall found were destroyed by heating at 55° C., and these, if not identical with what are now referred to as complements, belong probably to the same class,—in fact Bordet calls his complementary bodies alexines in recognition of this relation. That one immune serum when inactivated by heating at 55° C. can be activated by different fresh sera is undoubted, but, as we have seen, Ehrlich attributes this to the existence of a series of immune bodies in the same immune serum, each of which acts in conjunction with a corresponding complement. The chief upholder of the non-specificity of complements is as we have seen Bordet [54], who, while admitting that the complements from different species of animals may exhibit differences, yet apparently thinks that the difference is of a very subtle nature, regarding which our total ignorance of the essential structure of the bodies leaves us entirely in the dark. That they are different he sees he must admit because of this fact;—against these bodies as against toxines there exist anti-bodies which can be produced by injecting the sera containing them into animals. Now suppose we have the case where, as we have shown above is possible, we have an immune body which can be made active against the blood corpuscles which stimulated its formation, by two fresh sera, *i.e.*, by complements *A* and *B* each derived from a different species of animal. Suppose, further, that by the injection of *one* of these sera, say that containing *A*, into an animal, after the manner of an immunisation, we obtain an anti-serum which is capable of neutra-

lising the complement *A* present in the serum injected. In other words, this anti-serum will be able to protect the corpuscles against the serum,—will take away from the serum the property of activating the immune body which is ready to attack these corpuscles. The important fact which indicates that the two complements are not the same is that this anti-serum will be found incapable of preventing the immune body being activated by the other complement *B*. Seeing that Bordet is an opponent of the reaction of immune body, complement, and corpuscle being of a chemical nature and looks on the sensitizing of the corpuscle to the action of the complement by the immune body as being more probably of the nature of the preparation of the corpuscle for a ferment action, it would be quite fair to take as an analogy for his views of the nature of the differences between different complements the differences which exist between the peptic and pancreatic ferments, both of which have a proteolytic action, though each is distinct from the other. While according to Bordet some kind of difference must therefore be admitted to exist between different complements they have this common property, namely, that given a blood corpuscle or a bacterium sensitized by its appropriate immune body a whole series of complements is then capable of entering in and causing solution. Thus, Bordet believes that not only can the blood from two species of animals each sensitized by its appropriate immune body be dissolved by the same complement but that the same complement can also dissolve the sensitized bodies of bacteria. Some of the experiments on which these views are based may be given. A haemolytic serum *A* was obtained by treating a guinea-pig with the blood of the rabbit; another haemolytic serum *B* was obtained by treating a rabbit with the blood of the fowl. In each of two tubes *X* and *Y* the following mixture was made. Of *A* ·2 c.c. was taken (this therefore contained immune body capable of dissolving rabbit corpuscles *plus* guinea-pig complement). There was then added 1 c.c. of *B* which had been heated to 56° C. (containing therefore only immune body capable of dissolving fowl corpuscles). To the one such mixture *X* there was added ·6 c.c. of defibrinated fowl blood and haemolysis at once took place. This mixture *X* was then allowed to stand some hours. There was now added to both mixtures—*X* and *Y* (to which latter nothing had been previously done)—two drops of rabbit blood. In *Y* the corpuscles of the latter were soon dissolved while in *X* they remained intact. The deduction from this is that the guinea-pig complement present in *X* had been all consumed in dissolving the fowl blood corpuscles. It further shows, according to Bordet,

that the complement present in the mixture was not united to either immune body, but was ready to enter into whichever corpuscle was prepared for it by one or other of the immune bodies. To take another example, which illustrates the taking up of complement by sensitized bacilli. The materials used here were (1) anti-plague serum from the horse heated to 56° C. (therefore containing anti-plague immune body only), (2) serum from ordinary horse similarly heated (containing therefore no active substance), (3) a 24-hour old culture of plague bacilli mixed up in ·75 per cent. sodium chloride solution (plague emulsion *infra*), (4) some fresh serum from a guinea-pig (containing therefore guinea-pig complement). Mixtures of these were made as follows:—

1.	·2 c.c. complement,	·4 c.c. plague emulsion,	1·2 c.c. anti-plague immune body
2.	·2 c.c. complement,	·4 c.c. plague emulsion,	1·2 c.c. heated horse serum (*i.e.* No. 2 *supra*)
3.	·2 c.c. complement		1·2 c.c. anti-plague immune body
4.	·2 c.c. complement		1·2 c.c. heated horse serum (*i.e.* No. 2 *supra*)
5.		·4 c.c. plague emulsion,	1·2 c.c. anti-plague immune body
6.		·4 c.c. plague emulsion,	1·2 c.c. heated horse serum (*i.e.* No. 2 *supra*)

The tubes were allowed to stand for some hours. A guinea-pig had been prepared by the injection of rabbit blood so as to furnish a serum haemolytic to this animal's corpuscles. 2 c.c. of this serum was now taken, heated for half-an-hour at 56° C., so that it now contained only immune body capable of sensitizing rabbit corpuscles, and 20 drops of rabbit blood were added. No haemolysis took place because no complement was present. Of this mixture ·2 c.c. was added to each of the tubes, in fact to each was added a quantity of sensitized rabbit corpuscles. Haemolysis occurred in tubes 2, 3, and 4, but not in the others. Bordet's deductions from this experiment are, (1) that the plague bacillus mixed with the serum of an ordinary horse did not absorb any complement, (2) that the plague bacillus in the presence of the anti-plague immune body of the horse fixed complement of the normal guinea-pig and caused its disappearance from the mixture, (3) that the anti-plague immune body, when plague bacilli were not present, did not unite with the guinea-pig complement. Broadly speaking, no union took place between immune body and complement when these existed apart from sensitive bacilli or blood corpuscles, and further, the same complement was capable of dissolving sensitized

plague bacilli and sensitized rabbit corpuscles. With regard to the proof afforded by Ehrlich as to the multiplicity of complements, based on supposed differences of susceptibility to heat and differences in filtering reactions, Bordet thinks that physical differences may be originated by such processes in different moieties of a substance which is really single. He further submits that different corpuscles present different degrees of sensitiveness to the action of the complement. Gruber[55], who is an upholder of the non-multiplicity of complements, thinks that Ehrlich's results are to be explained by a failure to take into account different degrees of concentration of complement, and he adduces the fact that while normal ox serum and normal sheep's blood will each dissolve rabbit blood corpuscles, sheep's blood will only do so in very concentrated solution. This criticism has very little point, for in all the researches bearing on the matter the amount of immune body and of complement have been estimated by Ehrlich by the method of von Dungern already alluded to. It may be here noticed that Bordet is of opinion that bacteria when sensitized are more easily affected by a complement than is the case with blood cells. Thus he has found that the cholera vibrio when sensitized by anti-cholera immune body can be dissolved by many different sera. Walker[56], also, has found that anti-typhoid serum derived from an immune horse can be sensitized by the serum of the rabbit, the ox, and the pig. There is no doubt that the question of whether there exist a large number of complements or whether there is only one such body in each animal species, cannot be at present definitely settled. A settlement can only be looked for from the careful application of the method of von Dungern referred to, and it is fair to say that in the work of Ehrlich and Morgenroth on the subject this has been fully realised.

The therapeutic use of immune sera. There is one point which may here be referred to in concluding this part of the subject, and a point which has an important bearing on immunity so far as the latter is concerned in the process of recovery from disease. It has already been pointed out that, when an animal is being treated for the obtaining of a haemolytic serum, there exists according to von Dungern's observations a much greater proportion of immune body than of complement. Whether during immunisation there is always an absolute increase of complement requires further enquiry. It is, further, a fundamental fact, following from what has been already said, that when a given number of bacteria are to be killed these must be completely sensitized by immune body (if we take Bordet's view),

or completely saturated with immune body (if we take Ehrlich's), in order that they may become amenable to the action of the complement. Further, there must be a sufficiency of complement present to act fully on the bacteria thus saturated with immune body. A deficiency either of immune body or of complement will result in an incomplete reaction and the bacteria will not be thoroughly destroyed. Wassermann [57], studying the protection which can be afforded to guinea-pigs against typhoid infection by the injection of anti-typhoid serum, found that a case might arise where an animal had been treated with the anti-serum and where after death there might be in its body enough immune body to protect another individual from a fatal dose of the bacilli. The first animal had died because it had not enough complement to utilise the immune body which existed in its blood. Therefore in the use of an immune serum therapeutically, seeing that the latter probably usually contains an excess of immune body, it depends on whether the animal can furnish enough complement additional to that which may exist in the serum to enable it to utilise the immune body injected in the latter. That deficiency of complement is here the danger is further accentuated by the fact that as Walker has pointed out immune serum rapidly loses its complement on being kept. Herein probably lies one explanation of the comparative want of success that has attended the application of anti-sera in the treatment of diseases belonging to the second class which we are now considering. The question of the specificity or non-specificity of complement thus becomes of the highest practical importance. In helping an animal in its struggle against disease it is not only necessary to supply it with immune body in addition to what it may itself be able to manufacture but it is also necessary to see that it is properly supplied with complement. Apparently, the supplying of complement is not such an easy matter as at first sight it seems. It might be thought that all that would be necessary would be to take some of the fresh serum from the same species of animal, which had been used for the immunisation, and inject it along with the immune serum. On this point there seems to be some difficulty. Wassermann states that, if a guinea-pig be immunised against typhoid and the immune serum produced be used for the protection of another guinea-pig against the typhoid bacilli, the serum of a fresh guinea-pig does not supply a sufficient complement. He thinks that in such circumstances the complement either becomes destroyed or is bound in new combinations. He has found that in protecting guinea-pigs against typhoid infection by means of an anti-

serum derived from the dog the best complement is furnished by the serum of the non-immunised horse. Walker has pointed out that some of Wassermann's results depend on a mathematical error, and following this up he has obtained the results regarding the satisfaction of anti-typhoid serum by complements derived from various animals which have been already mentioned. But further investigation is necessary before these results can have a practical application. From what has been said above it will be gathered that Ehrlich does not hold that each immune body has one complement and only one which satisfies it, but he holds that the complements which will satisfy each immune body are probably limited in number, and that the search for such as may be useful in the therapeutic applications of the anti-sera may be attended with considerable difficulty. Even on the supposition that the view which regards the complements as generally interchangeable is correct, the fact that complements derived from different sources may act with different degrees of avidity makes it desirable that the most avid should be used for the purpose under consideration.

There is another matter relating to the therapeutic use of immune sera which is of great importance. It has been shown by Neisser and Wechsberg [101] that under certain circumstances the injection of more than a certain quantity of an immune body derived from another animal may be positively injurious. They have investigated this subject by testing the bactericidal powers *in vitro* of a great variety of sera. In these experiments the amount of complement and the number of bacteria were kept constant while the amount of immune body was varied. It was fonnd that when the latter was present in less than a given amount no bactericidal action was traceable; when it was present in greater amount the bacteria were killed, and again, when there was more than the last amount present, absence of bactericidal action was again noticeable. The explanation which these observers put forward is that if in a bactericidal mixture there be a sufficiency of complement the effect of adding an excess of immune body will depend on whether the effect of the linking of immune body to complement be to diminish or increase the affinity of the immune body for the bacterial cell. If the effect be diminution then the combined immune body and complement will pass the bacteria by, and the latter will therefore remain uninjured. Whether this view be correct or not it is interesting to note that, as has already been shown, Ehrlich found with certain haemolytic sera there was a greater

affinity between the immune body and the blood corpuscle than between the former and the complement. Now in no haemolytic serum has the phenomenon of the injurious effect of excess of immune body been observed. Such facts accentuate not only the difficulty but it may be the danger of the therapeutic use of immune sera in our present incomplete state of knowledge.

To sum up this part of the subject so far as we have gone it is to be observed that the methods by which bacteria are dealt with in the body are similar to those which obtain when many kinds of foreign cells gain an entrance into the latter. The development of artificial immunity against such bacteria depends on the latter being introduced either in a form not strong enough to cause death, or, if virulent, not in sufficient numbers to cause death. In either case, the affected animal probably resists infection because it can develop in its body or already possesses a substance,—immune body,—which attaches itself to the bacterial protoplasm, and in virtue of this attachment permits another body—the complement—which exists normally in the animal's body, to act on the bacteria with a fatal result to the latter. In the case of a further infection with bacteria, such as might occur naturally or as occurs during the process of immunisation, then no illness may result, but a fresh formation of immune body may occur. Whether a fresh formation of complement to any great extent occurs is a question for further investigation, but in an immune serum the complement is always present to a less degree than is the case with the immune body. What the nature of these bodies is is unknown, but the complements are less resistant to heat than the immune bodies. Further, the nature of the reaction which takes place between bacteria, immune bodies, and complement is disputed, and lastly, while the multiplicity of immune bodies is undoubted, it is still an open question whether there are a great number of complements in each animal's body, or whether there is, for each species at least, only one complement which is capable of acting in conjunction with a great variety of immune bodies so as to produce a solvent effect on many different kinds of bacteria.

The sources of the bodies concerned in bactericidal action: the phagocytic theory. It will have been observed that, in what has hitherto been said regarding immunity against bacterial infection, no reference has been made to the source of these bodies, which are found in the serum of immunised animals, and which possess the power of killing and dissolving the offending bacteria, nor has any reference been made to the

site where in the body the death of the latter occurs. It is to be noted that Ehrlich, while holding that the immune bodies are the product of side chains normally present in bodily cells, has never condescended on the cells in which these side chains are situated. Other investigators have put forward theories as to their origin. Chief among these is Metchnikoff, whose phagocytic theory of immunity has been prominently before the world for the past fifteen years. The necessity of co-relating this theory with the facts as to the bacteriolytic properties of sera has now been fully recognised by its founder.

Before taking up this process of co-relation, however, it may be advisable briefly to recapitulate the chief points of the theory in its original form. According to Metchnikoff's view when a bacillus gains an entrance to the body of a susceptible animal, whether it will produce pathogenic effects or not, will depend on whether or not it attracts, does not attract, or repels certain wandering cells present in the body. If such cells come in contact with the bacteria they will englobe, kill and digest the latter, but if this process of phagocytosis does not occur then the bacterium will be free to multiply and will work pathogenic effects. Such a determination of wandering cells towards any foreign material introduced into the body, whatever the nature of such material may be, is of frequent occurrence. The cells chiefly concerned in the process are naturally the wandering cells of the blood, and of these the varieties which have a phagocytic property are the large mononucleated leucocytes and the polymorphonucleate leucocytes, but it is to be borne in mind that these are not the only cells, which, in various parts of the body, are capable of movement and of phagocytosis. In the serous cavities there are many cells, derived in many instances probably from the endothelial lining, which are endowed with these properties, and the same may be true of certain cells, present in connective tissue spaces, which may be derived from the connective tissue corpuscles. In order to clear the ground we may say here that these wandering cells, while the most important, are not the only cells with which Metchnikoff associates a phagocytic property. He recognises a group of what he calls fixed amoeboid cells. Here although the cell is fixed on, say, one side it is free on others and from these can put forth protoplasmic processes and seize on any materials which may be brought into contact with it. Such cells are to be found in the large cells of the splenic pulp and of lymphatic glands, in certain endothelial cells, especially those of small blood-vessels and of serous cavities, in the cells of the neuroglia, and even in certain nerve-cells (in the latter according to Metchnikoff

because of their capacity of taking up leprosy bacilli, which, being non-motile, cannot move into the cells). It is evident, however, that this latter group of phagocytes must play a subordinate part, for such cells can only exercise their functions in this direction when by lymph currents or the blood stream the bacteria are brought in contact with them, or when the latter come into contact with such cells by means of their own movements. It follows from what has been said that the cells taking part in this variety of phagocytosis will depend on the part of the body in which the reaction is taking place.

Of the two groups the free amoeboid cells are thus by far the more important for they can move towards any foreign object. How do they do so? By virtue of this capacity of being attracted or repelled,—the phenomenon of what is known as chemiotaxis. This term was first applied by Pfeffer, and the subject has been studied by many observers, who have dealt with the occurrence as it affects the relations of leucocytes to solid and liquid substances with which they may come in contact. That these cells are attracted by many such bodies there is little room for doubt. The subject has been investigated by Massart and Ch. Bordet [58] and by Gabritchevsky [59] by the method of placing in animals capillary tubes filled with the materials and observing whether the cells did or did not wander in. In this way it has been shown that many pathogenic bacteria attract leucocytes under such circumstances. That negative chemiotaxis in the repulsion of bacteria by cells takes place, or at least that leucocytes can exercise a selective effect on bacteria is indicated by the following observations. Disselhorst [60] studied the effects of applying quinine to the frog's mesentery and found that the leucocytes became round and were rendered immobile and did not migrate as under ordinary circumstances they would have done. On being removed from the vessels it was, however, found that they were not killed but that they now regained their active movements. Jules Bordet [61] stated that in peritoneal infection of guinea-pigs with virulent streptococci no phagocytosis occurred, and the cause of this was not that the leucocytes were paralysed by bacterial action, for if a culture of *Proteus vulgaris* were also injected this bacillus was taken up by the white cells. Zilberberg and Zeliony [62] in similar experiments performed with the fowl-cholera organism state that while the phagocytes of an animal do not take up virulent bacteria they still retain their capacity of englobing individuals from non-virulent cultures. There is no doubt that the phenomenon of actual repulsion has been fully substantiated in the case of some of the lower forms of life, especially for some myxomycetes.

With regard to these a further important fact has been established, namely, that an organism which at first shows a negative chemiotaxis, *i.e.*, which is repelled by a substance, may later manifest a positive chemiotaxis, *i.e.*, may be subsequently attracted by the same substance. This possibility of the transformation of the negative into the positive reaction is of great importance in Metchnikoff's theory. In the taking up of foreign substances by phagocytes there has been noticed according to this author a difference in function among the different classes of cells involved.

Roughly speaking the free phagocytes may be divided into two groups, firstly the macrophages which include the large mononucleate leucocytes of the blood and also the large mononucleate cells derived from endothelium, and, in Metchnikoff's opinion, from the large cells lining the sinuses of lymph glands and the sinuses of the spleen. It may be said, however, that Metchnikoff's views on this point are very indefinite, and it is probable that the content of the term phagocyte varies according to the part of the body where the reaction to which it is applied takes place. Differences also may exist between different groups of phagocytes from the point of view of their function. Thus according to Metchnikoff the macrophages are largely concerned in the ingestion of foreign cells such as blood cells when these gain an entrance into the body, while the microphages are chiefly concerned in the ingestion of bacteria. Numerous exceptions to this rule, however, occur and it is by no means certain that the process of phagocytosis can be classified according to such a simple scheme; for instance, as Durham has pointed out and as many other observers have noticed, the microphages may take up foreign bodies and in turn may be taken up by macrophages. The identity of the various cells in the body which are capable of phagocytosis, the processes followed in the course of phagocytosis, and, as we shall see presently, the fate of cells which have exercised a phagocytic action are all subjects which urgently demand further and, above all, unbiassed investigation, for undoubtedly many statements of a too general character have been made regarding them.

According to the phagocytic theory immunity depends essentially on phagocytosis. The recovery of an animal from bacterial infection depends on whether the cells can take up and destroy a sufficient number of the infecting bacteria. Natural immunity depends on the fact that the phagocytic reaction is very pronounced and so effective that an infecting agent is invariably destroyed. The rise of an artificial immunity in an otherwise susceptible animal also depends

on cellular activity. In such an animal under ordinary conditions when infection takes place the phagocytes may either be attracted in insufficient numbers, or be not sufficiently powerful to kill the bacteria, or be unaffected by the presence of the bacteria, or be repelled by the latter. In the process of immunisation the administration of small doses of bacteria or of bacteria in a state of diminished virulence enables the phagocytes to gradually become accustomed to the presence of the latter so that they can ultimately endure and dispose of what under ordinary conditions would have constituted a fatal dose for the animal to which they belong. In the case where ordinarily the phagocytes are repelled, first of all the negative chemiotaxis becomes converted into a positive chemiotaxis and then the same accustoming of the attracted cells takes place.

The adaptation of the phagocytic theory to Ehrlich's observations. If we analyse the process of phagocytosis we see that it divides itself into two parts,—firstly, the attraction of cells by bacteria, secondly, the killing and digesting of the latter by the attracted cells. On the former of these phenomena Ehrlich's work throws little or no light, but Metchnikoff adapts the results of investigations on immune sera to explain what occurs in the latter. According to his new view the immune body and complement are substances produced in the protoplasm of the phagocytes, and it is by means of them that bacteria are killed and digested after being englobed. These substances therefore normally occur in certain cells and may play a part in the digestive activity of these cells. In the phagocytosis which occurs in natural immunity Metchnikoff holds that they never leave the cells and can thus only come into action when the bacteria are taken up by the cells, but in the process of artificial immunisation there is evidence of the immune body escaping into the plasma. There is, however, according to his view no evidence of complement becoming free in the body, and therefore so far as the immune animal is concerned its escape from the bacteria which are injected in the immunisation process depends on these bacteria being taken up by phagocytes and there meeting the complement which is necessary for their destruction. The fact that in immune sera both bodies are found is to be explained by the latter escaping through the breaking up of dying phagocytes or by the phagocytes giving up substances in the process of dying. The resistance of the artificially immune animal to large doses of the infecting agent, like the resistance of the naturally immune animal to ordinary infection, depends on the bacteria being taken up by cells. It is only in passive immunity that

materials present in the serum of immune animals in consequence of the death and disintegration of phagocytes are utilised for an extra-cellular destruction of bacteria in the bodies of the animals to which such sera may be transferred. In immune animals whether the immunity be artificial or natural there is no such extra-cellular destruction of bacteria.

We must now proceed to discuss the evidence which has been brought forward in support of this hypothesis. First of all here we must say that a purely humoral view of the process of immunity is to be put aside as untenable. Such a view would, it may be supposed, rest on the idea that the presence of bacteria in the fluids of the body could cause such chemical changes in these fluids as would make them antagonistic to the life of bacteria. But all changes in the bodily fluids that we know of are traceable to cellular activity, and no evidence can be brought forward that antibacterial action is any exception to the rule. The only modification of such a theory would be that which attributes antibacterial action to substances formed by the bacteria themselves, but at present there is no evidence of the existence of such bodies. Practically then the question which has to be decided is whether bacteria when they gain an entrance into the body are destroyed only by being actually taken up by cells or whether cells can respond to the stimulus of the presence of bacteria to shed forth substances which can kill and digest these bacteria in the fluids of the body. The idea of the possibility of such substances being the active materials in immunity against infection has long been entertained, having been in the first instance suggested by the discovery of the part played by antitoxines in immunity against intoxication by bacterial products.

Bactericidal properties in normal sera. In speaking of these we must strictly differentiate between the bodies of this kind which have been found to exist in the sera of non-immunised animals and those which as we have seen have been found in the sera of immune animals, and we shall first of all treat of the former. Now in 1888 Nuttall[63] had pointed out that the sera of normal animals possessed bactericidal properties which were destroyed when the serum was heated at 55° C. Such properties have been found to be possessed by a great number of sera, and by many it was thought that in naturally immune animals the immunity was due to the existence of these properties. Those who adopted such a view overlooked the fact that Nuttall had pointed out that the serum of the rabbit was capable of killing *in vitro* the

B. anthracis, though the animal is susceptible to anthrax, and it was not long before it was recognized that the mere possession of bactericidal serum in an animal was not invariably co-related with the existence of natural immunity. About 1892 Buchner, to whom the above discoveries are usually erroneously attributed (for he only confirmed Nuttall's results), gave to the bactericidal substances in sera the name of alexines. An enormous amount of work has been done with regard to the bactericidal properties of sera into which it is unnecessary from the present point of view to enter. Its result generally speaking may be said to indicate that, as in fact Nuttall pointed out in his original paper, very great differences exist in the action of a given bactericidal serum upon different bacteria. Thus in man Wright [64], who has made many valuable contributions to this subject and who has designed a method by which bactericidal power can be quantitatively measured, has shown that the serum while bactericidal towards the cholera vibrio and the typhoid bacillus has little action on the pyogenic cocci or on the plague bacillus. Further it may be said that neither is natural immunity necessarily associated with bactericidal power in the serum, nor is the absence of such immunity associated necessarily with the absence of such bactericidal action. It is difficult to explain such facts, but there is one line of investigation which has still to be followed and which may throw some light on the real nature of these natural bactericidal powers. It has still to be shown that the bactericidal action thus naturally present is of the same nature as that which is present in the sera of immune animals. If it is, then probably it might be found that two substances are involved just as is the case with immune sera, and some light might be thrown on this very difficult question of the relation of bactericidal power to natural immunity. That such a line of enquiry might be profitably followed is indicated by what has resulted from the pursuing of enquiries relating to analogous haemolytic actions. Thus Ehrlich and Morgenroth (*loc. cit.*) have shown that ordinary goat's serum will haemolyse the blood corpuscles of the rabbit, and as in the case of an ordinary immune serum this power is lost by heating the serum for half-an-hour at 55° C. Now it is found that in many horses the serum which has in itself no haemolytic action on the corpuscles in question, will, if added to the inactivated goat's serum, cause the latter to regain its haemolytic action. There is thus reason for believing that in the serum of the ordinary goat there are two bodies capable when acting together of dissolving the corpuscles mentioned. One of these—that

which is resistent to heat—corresponds to the immune body found in the serum of an animal which has been subjected to treatment with blood corpuscles,—the other which corresponds to the complement of such an immune serum. In the serum of the horse, on the other hand, there exists, as far as rabbit's corpuscles are concerned, only a complementary body, which, however, when the normal complement is removed by heat from the goat's serum can supply its place. It is quite possible that similar facts may hold with regard to bactericidal action, though the search for them would be of a laborious description. That it is possible that in the bactericidal action of normal sera the same factors are at work as in immune sera is further indicated by certain results of Neisser and Wechsberg (*loc. cit.*). These observers found that just as the bactericidal effect of an immune serum was inimically affected by an excess of immune body, so when a serum naturally possessed bactericidal properties and when at the same time it was capable of supplying complement to an immune body the addition of an excess of the last robbed the serum of its natural bactericidal action. Thus normal guinea-pig serum is bactericidal towards typhoid bacilli. It also has the capacity of replacing the complement which is present in the serum of a dog and which when the latter animal is immunised against typhoid renders the immune body developed active. But if to a bactericidal mixture of guinea-pig complement and dog immune body an excess of dog immune body be added then the bactericidal action is no longer manifest.

In connection with the bactericidal action of normal sera and the relation of the fact to immunity some observations have not long ago been made which are of great importance. Is it certain that the properties of the shed blood of an animal are the same as those of the circulating fluid? This question has been attacked by Gengou [65] who received the blood of normal (*i.e.*, non-immunised) animals into paraffined tubes. In these no coagulation or only the least degree of coagulation took place, and therefore the fluid which was separated by the centrifugalising process to which Gengou next submitted these tubes was almost unaltered *liquor sanguinis*. Other moieties of the blood were, at the same time, received into ordinary tubes in which coagulation took place as usual and therefore the properties of the ordinary serum could be compared with those of the blood plasma. He found in the bloods he examined (rabbit, dog, rat), that while in many cases the serum showed very definite bactericidal action, the plasma showed sometimes very little, sometimes none

at all. This must be looked on as constituting the most important contribution to recent enquiries as to the nature of bactericidal action. The method must be further applied before the full significance of the results already obtained can be realised and before it can be seen if any light is thrown on the nature of natural immunity. That this is necessary may be judged of by the fact that in Gengou's experiments with the serum and plasma of the dog no attempt was made to investigate the action of these on the *Bacillus anthracis*, to which this animal shows considerable natural immunity, whereas this organism was used with fluids derived from the susceptible rabbit. If the result of an extension of the method be to substantiate the view that under ordinary circumstances no bactericidal power is possessed by the plasma of animals it must be admitted that strong support will be given to the idea that the actual taking up of bacteria by cells is necessary to immunity. On this point, however, judgment must meantime be suspended, for another series of experiments must now be referred to which seem to point to the extra-cellular presence in the *liquor sanguinis* of an ordinary animal of a substance corresponding to a complement and which may play a part in the struggle of such an animal against bacteria to which it is susceptible.

Can complement and immune body occur free in the liquor sanguinis? It has already been pointed out that when an immune serum is injected into an animal after the manner of an immunisation there are produced bodies which are capable of neutralising its action. Immune serum contains both immune body and complement so that an antiserum to such might contain an anti-immune body and also an anti-complement. It will be remembered that an immune body inactivated by heat can be reactivated by normal serum from a non-immunised animal. Now if the latter, which contains only complement, be introduced into the body of an animal a serum containing only anti-complement will be obtained. Wassermann [66] obtained such an anti-complement by injecting the serum of an ordinary guinea-pig into a rabbit. Now the guinea-pig is naturally susceptible to virulent races of typhoid bacilli, and it can be protected against the pathogenic action of these if along with the bacteria some serum from a previously immunised animal be injected. Such an immune serum may or may not contain much complement, for as we have seen an immune serum rapidly loses its complement. If there be any present, its action will be reinforced, and if there be none its place must, if recovery is to take place, be supplied by complement which ordinarily exists somewhere

in the guinea-pig's body. Now if to a dose of bacilli sufficient to cause death there be added enough immune serum to enable the animal under ordinary circumstances to resist such dose, and there be also added sufficient of the anti-complement described above to neutralise the complement which would ordinarily act through the immune body present, then as the complement is neutralised by the anti-complement and as therefore the immune body is in such a mixture of no use, the animal ought to die, and this Wassermann found to be the case. He therefore deduces that complement is not confined within cells but naturally exists free in the guinea-pig's blood. Besredka [67] criticises these results and holds that what the anti-complement actually does is to paralyse the functions of the phagocytes. This view is based on a comparison hour by hour of what takes place in the peritoneal cavity of an animal injected as above and that of one which received bacilli plus immune serum alone. Unfortunately apparently no comparison was made of the condition in the former with the condition in a case where the bacilli were alone administered and gave rise to the ordinary fatal illness. It might thus have been observed if there was any less evidence of negative chemiotaxis in the last case. Without this the experiments lose much of their significance.

We have already said that Metchnikoff admits that in the struggle between an immunised animal and infecting bacteria two substances play a part—the immune body and complement of authors who have investigated the properties of immune sera,—but he holds that while in the living animal the former can escape from the cells where it is formed and be free in the bodily fluids, the complement, on the other hand, always is confined within cells. He apparently is further of opinion that both bodies are formed in the same class of cells, and under ordinary circumstances bacteria are destroyed only when they are taken up by these cells. Thus in a case where a particular species of bacterium is taken up by the microphage cells these would elaborate both the immune body and complement necessary for the manifestation of bactericidal action. The evidence alleged in favour of this view rests very much on a discussion of the phenomena of Pfeiffer's reaction to which attention has already in part been given. In this reaction, as we have seen, when immune serum heated so as to destroy the complement is injected into the peritoneal cavity along with active cholera vibrios the latter are killed and dissolved in consequence of the immune body being activated by complement derived from the plasma of the infected animal. How, if Metchnikoff's view is correct, does this complement

come to be free? This leads us to consider his view of the incidents of a peritoneal infection. When any foreign material,—bacteria, bouillon, etc. —is injected into the peritoneum there is stated to occur within a few minutes of the introduction an apparent almost complete disappearance of the cells,—phagocytes, wandering cells generally,—from the cavity. This Metchnikoff called the stage of leucopenia, but now rather prefers the term phagolysis, and he attributes the scarcity of cells to the fact that they are broken up from being injured by the operation of the injection. This breaking up of the phagocytes, in the case when immune body and cholera bacilli are injected, liberates the complement or "cytase" as he calls it, which acting through the immune body causes the destruction of the bacteria. Considerable attention and great controversy has arisen regarding the precise march of events in the very commonly practised peritoneal infection, and various interpretations have been put on the phase of phagolysis. The general sequence of events is that when bacteria are injected into an animal of moderate susceptibility towards them they very soon begin to be taken up by cells, but within a short time the apparent disappearance of cells from the serous cavity begins. This stage is succeeded by one in which a great influx of fresh cells occurs, which are at first of the order of macrophages, but later microphages predominate. If the reaction is successful free bacteria gradually disappear and the hyperleucocytosis subsides. Durham[68] holds that the phagolysis is almost entirely accounted for by the fact that within a few minutes of injection there occurs a gathering of the leucocytes into balls which adhere to the peritoneum, especially to the great omentum. Here it may be said that, especially by French investigators (cf. Roger[69]), a much more active function is assigned to this part of the peritoneum than it is usually credited with in this country. It is looked on as a great opened-out gland (*un ganglion lymphatique étalé*) whose cells have proliferative and protective powers, and whose removal renders an animal more susceptible to bacterial infection than usual. This, however, by the way. To resume, Durham denies that there is any evidence of much, if there is of any, disintegration of cells during this period. Pierallini[70] finds evidence of the shedding of materials by the leucocytes during leucopenia in that, by Weigert's stain for fibrin, strands of this material can be seen on the omentum removed at this stage, but apparently he has not made control preparations of the normal omentum, from which it could be judged if this fibrin formation did not take place during the time occupied in putting up the specimen. He shows, however, that the

mere clumping of the leucocytes has not robbed them of life, for though if removed from the omentum they appear immobile, in the course of a few hours they regain this power and also can be proved capable of phagocytosis. It is evident that the actual disintegration of white cells is not absolutely necessary to the appearance in the blood of substances derived from their protoplasm. Pfeiffer[71] brings forward as evidence against the setting free of material by phagolysis a fact observed by him, namely, that in an exudation very rich in leucocytes his phenomenon takes place rather more slowly than in one poor in these cells. Bordet[72] on the contrary states that Pfeiffer's reaction does not take place when the vibrios are introduced into parts of the body of an immune animal where phagolysis cannot take place on account of the poorness of cells. Such parts are the anterior chamber of the eye, the subcutaneous tissues, and the fluids of passive oedema. He says that in the latter the fluid can be made active if along with the bacteria there is introduced some serum from a fresh unimmunised animal. This last experiment is what leads Metchnikoff to admit that in the immune animal immune body can exist free in the plasma. Pfeiffer states that the reaction does occur in the subcutaneous tissue, though more slowly than in the peritoneum. Metchnikoff retorts that probably in Pfeiffer's experiments there was a little subcutaneous bleeding, and that in the process of clotting destruction of some leucocytes would occur and thus complement be liberated. A further proof brought forward by Metchnikoff in this connection is that when the stage of leucopenia is suppressed then Pfeiffer's phenomenon does not occur or only does so to a slight degree. If some bouillon be introduced into the peritoneal cavity of an animal the stages above described take place, but if on the following day the operation is repeated then the stage of leucopenia is suppressed, and if at this time bouillon containing the cholera vibrio and immune serum be injected, then Pfeiffer's phenomenon does not take place, *i.e.*, there is no extracellular destruction of the bacteria, such destruction now taking place entirely within the cells. All these considerations lead Metchnikoff to the conclusion that under ordinary circumstances whether or not the bacteria are combined with the immune body within or without the cells it is only within the cells that the final step—the action of the complement—necessary to the bacteriolysis can take place. It must be remembered that the phenomena occurring in a peritoneal infection are as yet by no means clear, for so much depends on the technique practised. This is especially the case when the question of the diminu-

tion or increase in the number of free cells is concerned, for there is no means of estimating the total number of cells present, nor of measuring variations in the amount of fluid exudation. Great caution is therefore called for in forming any opinion from such data as are available. It is evident that the general question of the free existence of immune bodies and of complement demands that further enquiries be made along the lines of the experiments of Gengou on the one hand and of Wassermann on the other.

The sites of formation of complement and immune body. It is evident that in this connection it is important to enquire if any other light can be thrown on the sites of formation of the immune body and complement. The earlier work on the bactericidal actions of normal sera had indicated that the leucocytes were the cells responsible for the formation of the bodies concerned in this process. Thus Denys and Havet[73] found that while the whole blood of the dog manifested considerable bactericidal power toward the *B. anthracis* the serum of the same animal had very little action; further, that when from the blood the white cells were removed by filtration through filter-paper the action also disappeared. As showing, however, what care is necessary in making generalisations on the subject, it may be remarked that in testing the blood of man by the same method with the *B. coli* it was found that very little difference existed between the serum and the whole blood. Havet[74] found that, if *Staphylococcus pyogenes aureus* was injected intravenously in dogs, within a few minutes there was a great disappearance of leucocytes from the blood, and along with this there was a diminution of bactericidal power in the shed blood. After a few hours there was a great increase of these cells above the normal, and there was a corresponding increase of the bactericidal action. In connection with the subsequent discoveries of immune body and complement, some facts observed by Denys and Leclef[75] are of interest. They immunised rabbits against *Streptococcus pyogenes* and compared the properties of the blood with those of the blood of unimmunised rabbits. They found that the serum of fresh rabbits exercised no bactericidal action on the bacterium in question. The serum of immune animals showed a degree of potency, but not very much. The leucocytes of fresh rabbits mixed with the serum of fresh rabbits had a very feeble action and died before they naturally would have done if no bacteria had been present. The leucocytes of fresh rabbits transported to the serum of immune rabbits destroyed the bacteria and showed a normal degree of vitality. The leucocytes of the immune rabbit when

added either to the serum of immune rabbits or to the serum of fresh rabbits behaved exactly like the leucocytes of fresh rabbits. These results pointed to the fact that for a maximum bactericidal effect two factors were necessary, one present in the immune serum, and the other existing in the leucocytes either of the immune animal or of an ordinary animal, and they indicate that in some way the leucocytes are concerned in the formation of the active bactericidal bodies. Deutsch [76] states that the anti-body of typhoid serum is specially developed in immune animals in the bone marrow, and that there is little evidence of its presence in peritoneal exudations or in the great omentum. His method consisted in comparing the effects of emulsions of given weights of different organs. As to what weight is to be attached to results thus obtained constitutes a difficult question. In the cases of these bactericidal and protective bodies just cited no experiments have been performed which indicate the source or sources of the two necessary constituents of the reaction. Bulloch [77] has brought forward more precise observations to show that the development of a haemolytic complement is in the rabbit to a certain extent associated with a rise in the number of certain polynuclear leucocytes in the blood, while on the other hand a development of immune body is associated with the activity of the mononucleate corpuscles. My friend Dr Walker informs me that in the case of anti-bacterial sera he has found that in the first few hours after the removal of blood from the body there is a gradual rise in the amount of complement—this it is certain must come from the white cells present. As has been remarked, the view of Metchnikoff is that both the immune body and complement are formed within the same cells, but that the former is not so intimately bound to (*lié*) the cells as the latter. This would not accord with the results of Bulloch, and the question evidently demands further enquiry. Tarassévitch [78] has found that emulsions of the organs of the body associated with the formation of the large mononucleate leucocytes (which constitute such a large proportion of the total number of macrophages),—such organs, namely, as the great omentum, the lymphatic glands, the spleen, have a marked haemolytic action on the red blood corpuscles of birds, and that this property is destroyed by heating to 55° C., while it is greatly augmented by the addition of corresponding immune bodies. From a few experiments done it was further observed that the emulsions named had no bacteriolytic effect. On the other hand exudates containing many polymorphs (*i.e.*, microphages) manifested no such haemolytic action even

when abundant immune body was furnished. From this the author deduces that the complement (macrocytase, as he and Metchnikoff call it) for the solution of corpuscles is produced by the macrocytes, and is different from the complement manufactured by the microcytes which is probably concerned in the bacteriolytic manifestations and which is called microcytase.

To speak generally from the rather fragmentary state of our present knowledge, the complement and probably also immune body are produced by the white blood corpuscles, but when we think of the very varied cells which may take on a phagocytic action, we must be careful not to exclude the possibility that they are not the only cells of the body capable of manufacturing these substances. We must also bear in mind that the reaction of the body against infection may not be confined merely to cells which act as actual phagocytes. There are many cells of which the eosinophile leucocytes may be taken as the type which enter into the struggle against an invading agent, and which may discharge from their protoplasm substances having a bactericidal or bacteriolytic function.

The relation of agglutination to immunity. It was long after the promulgation of the phagocytic theory that the very great complexity of the questions involved was realized, and it must now be recognized that very many different processes are involved in the reaction of the body against infection. A very good example of this is found in the process of agglutination of the invading bacteria which is often observed, and to which great attention has been paid from its diagnostic importance in such a disease as typhoid fever. If in a droplet of the serum of a typhoid patient diluted with bouillon there be mixed some typhoid bacilli, these rapidly lose their mobility and gathering together in large clumps gradually also lose their characteristic shape. This clumping occurs under similar circumstances with many other bacilli, and an identical condition often occurs when red blood corpuscles are placed in an immune serum capable of haemolysing them. Further, the reaction is generally speaking specific for a given species of bacterium in relation to a particular serum produced by immunisation with that species, and a certain degree of specificity also exists among races of a particular species. Thus a serum produced by one race of typhoid bacillus will clump that race better than other races (Walker[79]). Sometimes, however, agglutinating properties are possessed by normal sera. The real significance of the occurrence and its relation to immunity are

still obscure, but it is known not to be an essential factor in the action of an immune serum. Thus Fraenkel and Otto[80] fed young dogs on typhoid bacilli and found their serum was agglutinative, but not bacteriolytic. Pfeiffer and Kolle[81] produced an immune serum which was bacteriolytic but not agglutinative, and Widal and Nobecourt[82] often obtained a similar result with mice immunised against typhoid by injecting urine containing the bacilli. Facts of a similar kind have been observed with sera derived from unimmunized animals. Thus the rat, which is susceptible to anthrax, has a serum bactericidal but not agglutinative towards anthrax bacilli; the insusceptible dog has a serum agglutinative but not bactericidal. Further, Trumpp[83] showed that while the bactericidal properties of a serum were lost by heating at 55° C. the agglutinative action still remained. It has been observed that several chemical substances such as saffranine if added to ordinary non-agglutinating serum will confer on it agglutinating properties towards the typhoid bacillus. From the fact that the fluids in which anthrax bacilli have been grown have an agglutinating action on fresh cultures the theory has been advanced by Malvoz[84] that agglutinating substances (often referred to as agglutinins) are really formed by bacteria themselves. Conforming to such a view is the observation of Macrae[85] that if a collodion capsule containing a fluid culture of typhoid bacilli be placed in a guinea-pig's peritoneal cavity the serum of the animal acquires agglutinative action. Such a phenomenon and also the appearance of agglutination in an immune serum would on this view be explained by the diffusion out of the bacilli of the agglutinating substance. Two other theories presuppose the existence of a special substance in an agglutinating serum. One is that of Gruber, to the effect that the membrane of the bacterium is rendered viscous and this favours adhesion together in masses. This view is also in effect that of Nicolle[86], who, however, leans to agglutination being the result of two substances, one present in the serum, the other in the bacterium. He finds evidence of the latter having dissolved out in old cultures of—among other organisms—*B. coli*, and observed the remarkable fact that when such a culture was filtered through porcelain and the appropriate serum added to the filtrate an agglutinating process occurred. This agglutinable material in such a filtrate further could be entangled by typhoid bacilli, or even by talc powder, and on addition of the serum to such mixtures agglutination occurred. On the other hand is the view of Bordet[87], which is that "serum in acting on microbes changes the relations of molecular attraction be-

tween the bacteria and the surrounding liquid." This observer rejects Nicolle's theory on the ground of the following experiment. Cholera vibrios were clumped by the immune serum and the vibrios separated from the supernatant liquid by centrifugalisation and after washing divided into two parts. One of these was treated with ·7 % sodium chloride solution and the other with distilled water and the deposit shaken. In the former fluid the clumps were re-formed, in the latter the vibrios remained separate. Bordet considers that agglutination may be of the same nature as coagulation chiefly because some haemolytic sera which agglutinate red blood corpuscles also cause a precipitation of the serum to which the corpuscles belong. It must be pointed out however that the haemolytic sera in question were produced by the injection of defibrinated blood, *i.e.*, of corpuscles *plus* serum, and it is now known as will be noticed later that by itself the serum of one animal if injected into another species of animal after the manner of an immunisation will cause that animal's serum to assume the property of precipitating the serum of the first animal. This fact has later been recognized by Nolf[88]. There is no doubt however that there are many facts relating to coagulation, and even to the formation of flocculent precipitates in the chemical reactions of simple inorganic bodies, which must be explained on the lines of changes in molecular attraction such as Bordet lays down with regard to agglutination. On the whole the evidence is rather in favour of some such view as that of Nicolle that for the occurrence of agglutination two substances are necessary, one in the bacterium and one in the immune serum. For one thing, from Nicolle's results, the agglutinable substance of the bacterial cell is apparently more resistant to heat than agglutinins are, and also it is soluble in alcohol, which the latter probably are not. The views of Bordet and Nicolle are not absolutely irreconcilable; the former deals with a process, the latter with the substances concerned in the process. It may here be said that Ehrlich apparently holds agglutination to be due to the formation of special substances in an immune serum, analogous but distinct from those concerned in bactericidal action. This latter fact is the important one in connection with the subject.

The possible part played by ferments in bactericidal action. The question of agglutination has been entered into at some length in order to indicate how complex the problem of immunity is. It illustrates further the possibility of there being many different factors at work in the relation of a phagocyte to the bacteria which it englobes. Metchnikoff seems to attribute both the killing of the bacteria and their

solution to the presence in the phagocyte of a ferment—the cytase—which roughly speaking corresponds to the complement of other authors. If the conception of fermentation has any definite meaning, the term is applicable to the process in which a body possesses the power of originating changes in other bodies while remaining itself unchanged. Of the existence of such bodies there is evidence in the fact that the action of given amounts, of known ferments is indefinite so long as the products of the fermentation are removed. For this idea of ferment action there is often too great a tendency to substitute a proof resting on another attribute of known ferments, namely, susceptibility to moderate degrees of temperature. Very many ferments such as those concerned in peptic and pancreatic digestion lose their fermentative properties at a temperature of 55° C., but this is just about the point when changes begin to take place in albuminous molecules generally under the influence of heat. And very profound changes may be originated in such molecules by exposure to this temperature. Thus Ramsden [89] has shown that if egg-albumin be kept long enough at 55° C. almost the whole of it undergoes coagulation. This criterion cannot thus be applied too rigorously to the substances concerned in immunity, though most of them would fulfil it. If we seek to enquire whether the fundamental quality of ferments is recognisable in the case of the bodies under consideration we find the evidence is scanty, if not entirely non-existent. In the case of a toxine such as that of tetanus, as we have seen, all evidence of the presence of the poisonous substance being present during the duration of the disease is absent. In the guinea-pig the poison is anchored in the sensitive part of the body. Again, the definite relations which exist between a definite amount of immune body acting along with a definite amount of complement to produce a certain definite effect, coupled with the facts discovered by Ehrlich of definite linkings taking place in the interaction, is rather against the idea of a body being concerned which originates change without itself being changed. It is further, however, an assumption on the part of Metchnikoff to suppose that the bactericidal and digestive properties of phagocytes are necessarily due to the same substances. That ferments can be formed by leucocytes there is little doubt. Delezenne is stated by Metchnikoff [90] to have shown that a ferment called enterokynase (which is said to materially assist the pancreatic ferment in digesting proteid) is the product of the lymphoid tissue of the intestine, and the work of Hedin, who has extracted proteolytic ferments from the spleen—the great normal site of leucocyte

destruction—confirms this view. But a ferment action on dead proteid is entirely different from such an action on living protoplasm. The typhoid bacillus will live and multiply in a solution of pancreatic ferment which will digest fibrin. It is thus quite possible that by virtue of one set of powers a phagocyte may kill a bacterium, by virtue of another set of powers it may digest it, and the latter process may be the same as ordinary proteolysis as it occurs in connection with the intestinal glands of an animal.

Fischer[91] has attempted to explain the phenomena of bacteriolysis from a physical standpoint by supposing them sufficiently accounted for by processes of dialysis. Such a view however meets a difficulty in the specificity of immune sera. Further, Bordet[92] has brought forward an experiment which seems to indicate that in the case at any rate of blood corpuscles undergoing haemolysis a change occurs which makes the corpuscular protoplasm no longer capable of dialysis. If red blood corpuscles be treated with distilled water the process of "laking" takes place by which they swell up enormously and lose to the surrounding fluid a very considerable part of their haemoglobin. If however some common salt be added, the corpuscles regain to a certain extent their form, and a certain amount of the haemoglobin is re-entangled in the stroma. In the case of red blood corpuscles haemolysed by an immune serum the appearances in the first instance closely resemble those of the corpuscles of laked blood, but the addition of salt has no effect in restoring the structure of the corpuscles. "It appears," to quote Bordet's words, "as if the alexine of the active serum has destroyed, has digested 'a something' in the corpuscle which controls the operations of plasmolysis,—the phenomena of osmosis." In this connection it may be observed that if haemolysis by toxic sera and bacteriolysis are precisely parallel processes it is fair to ask what in the solution of red blood cells corresponds to the killing stage in the case of bacteria. Our general conclusions here must only be that it is advisable not to take refuge behind such an indefinite term as fermentation when in reality nothing is known of the essential nature of the processes concerned.

The nature of chemiotaxis. What we have said hitherto regarding the phagocytic theory concerns only the process by which the phagocytes once attracted to the bacteria accomplish their death, and we have seen that so far Metchnikoff has accepted Ehrlich's explanation of two bodies being concerned in the bactericidal action. The point of difference here is whether this action is entirely extracellular or whether bacteria may be saturated with immune body extracellularly and meet the complement

only intracellularly. But another and an essential aspect of the phagocytic process requires explanation, namely, the facts of positive and negative chemiotaxis,—the attraction which is necessary for recovery from infection, and the indifference or repulsion which accompanies susceptibility. In his earlier work Metchnikoff seemed to look on these phenomena as due to what can only be described as a vital activity of cells. In this connection much harm was done to the theory by the unguarded language used by many of its adherents regarding this manifestation of cellular function. And even yet leucocytes are sometimes spoken of as if they possessed a sentient intelligence. In his latest work Metchnikoff adopts the language of a disciple who remarks that immunisation effects an education of the leucocytes. At the same time, however, he is tending to attempt a physical explanation of the phenomena concerned. Hitherto such an explanation has been applied chiefly to the case of active and passive acquired immunity. It rests on the idea that a bactericidal serum produced by repeated injections of a bacterium contains substances which stimulate the phagocytes to move towards, englobe and digest that bacterium when opportunity occurs. Why this stimulation of phagocytes leads them to move in a particular direction does not transpire. The evidence for the existence of these stimulines as they have been called is as follows. Gengou [93] injected mice with the bacillus of swine-fever mixed with its bactericidal serum (heated to 55° C. to destroy any complement) and with the serum of the normal guinea-pig (this contained an adequate complement). The animals did not die. Previous experiments had indicated that when an immune body comes in contact with its appropriate bacilli it becomes fixed to them. Bacilli of swine-fever were now treated with the immune serum and, after the supposed fixation of the immune body, were washed so as to free them of the other constituents of the serum. The experiment just detailed was then repeated, these "sensitized" bacilli being used instead of a mixture of ordinary bacilli and immune serum. The animals died notwithstanding that immune body and complement were present. From this Metchnikoff deduces the presence in the immune serum besides the immune body of some substance stimulating the phagocytes. With regard to this experiment no deduction can be drawn. The details are not given, it rests on the assumption derived from analogy that immune body was sufficiently fixed by the bacilli (no investigation of whether or no immune body was present in the washings of the bacilli is described), and its results are in direct contradiction to

similar experiments performed by Savtchenko[94]. This observer impregnated the red blood corpuscles of the guinea-pig with a haemolytic serum derived from the rabbit and allowing time for the immune body to become fixed he washed away all the other constituents of the serum. These were introduced into the peritoneal cavity of a fresh guinea-pig in which by the previous injection of bouillon an active hyperleucocytosis had been set up. Savtchenko states that when red blood corpuscles (and the contention was borne out here by control experiments) are injected into the peritoneal cavity of an animal of the species from which they were derived, no taking up by leucocytes occurs even when hyperleucocytosis has been caused. He found however that the corpuscles sensitised as described, were quickly taken up, in other words a negative chemiotaxis had been changed into a positive.

These results find support in the experiments of Mesnil[95] on the bacillus of swine-fever though Metchnikoff quotes the latter in support of his own view. According to Mesnil the effect of injecting immune serum before infection with the bacilli is that these are quickly taken up and digested by phagocytes, both mononuclear and polynuclear. This phenomenon does not happen in the case of an ordinary inoculation followed by a fatal result. That the effect here was due to stimulation of the phagocytes and not to a direct effect of the serum on the bacteria was deduced from the observation that the immune serum *in vitro* had no bactericidal action. This statement however is only partially true. There is no doubt that when an immune serum is quite fresh it has bactericidal properties in many cases, if not all (as Bordet long ago pointed out). When it is kept the delicate complement disappears and old sera have no bactericidal properties, but these can be revived by the addition of fresh complement in the manner already so often alluded to. Thus all that may have happened here may have been that within the animal's body the immune body may have found an efficient complement,—which circumstance gave rise to the death of the bacteria without the immune serum having had any direct chemiotactic action whatever on the phagocytes. While, however chemiotaxis in immune animals might be explicable on the supposition of a stimulation of phagocytes by substances developed in the serum during the process of immunisation, the question of the occurrence of chemiotaxis in naturally immune animals presents a difficulty on this hypothesis. Metchnikoff[97] drew attention to the fact that the sera of some men could protect the guinea-pig against peritoneal infection with the cholera vibrio. He attributes this to the existence of stimulines in

ordinary sera as well as in immune sera. In the former case they act alone in giving rise to phagocytosis, in the latter their action is reinforced by the immune bodies and also perhaps by the agglutinines. On the other hand, according to Savtchenko's results already quoted, the stimulation of the phagocytes is due to the impregnation of bacteria by the immune body of the immune serum. This view has the merit of being definite. What bodies precisely Metchnikoff refers to under the name of stimulines is very difficult to make out, but probably he considers that the cytases to which allusion has already been made possess this stimulating function in addition to their other powers. Whether when the cytases act alone (as he supposes is the case with normal sera which possess bactericidal action) a different group of these ferments acts from what is involved when their action is reinforced by immune sera does not transpire, but from Metchnikoff's adopting Bordet's view of the singleness of complement one is inclined to the belief that he holds this existence of only one set of ferments within the phagocytes. The fact that he does not believe in the extracellular existence of cytases in any case would lead to the idea that in natural immunity a phagocyte is stimulated to move in a particular direction by something inside its own protoplasm.

Taking all the facts into consideration it must be held that the evidence for the existence of a separate group of bodies having the particular effect of stimulating phagocytosis is of a very nebulous character. At present the observations on the subject are so closely connected with observations on the bactericidal effect which may follow phagocytosis that it is at present difficult to differentiate between a stimulating effect on phagocytes and a bactericidal action. We shall allude later to a certain aspect of the question which arises out of Ehrlich's theory. Meantime it may be remarked that the process may not depend on a chemical but on a physical stimulus. Jennings[98] for instance has shown that in certain cases chemiotaxis can be influenced by the passage of electrical currents.

The process of which phagocytosis is a part. There is one aspect of phagocytosis which has not received Metchnikoff's attention, and this may best be approached by consideration of what process actually can underlie the so-called education of the leucocytes. Take the case of an immunization against peritoneal infection with cholera. A few bacteria are introduced and the animal does not suffer from a fatal illness. Next a larger dose of bacteria is introduced with the same result. The tolerance of this larger dose (which in the first instance

might have been fatal) is due to the leucocytes having acquired greater phagocytic power. Now is this greater bactericidal power possessed by all the leucocytes of the animal's body or only by those leucocytes which exercised phagocytic action on the bacteria previously injected? It is not outside the regions of possibility that it might only be the leucocytes previously involved which acted on the second occasion. The sensitiveness of protoplasm is of a very exquisite kind, for example in many species of butterfly a male will become aware of the presence of a female though the latter be hundreds of yards away and out of sight. And similarly sensitized leucocytes might be so powerfully attracted by bacteria of a species they had formerly englobed as to pass from distant regions of the body to which in the interval between injections they might have been transported. But here another consideration comes into notice. We know little of the duration of the life of a leucocyte before it is broken up, but it is likely that this period may be measured only by days. On what then does the immunisation process exercise its lasting effect?

The hyperleucocytosis which occurs in many infectious diseases in man often rapidly subsides, and the swelling of the spleen which often occurs at the same time may be an indication of over-activity in that great organ of leucocytic destruction. An active immunisation against infection may persist for long periods of time, but the relation of hyperleucocytosis to such immunisation and to the development of a bactericidal serum and especially the relation, if any, of the subsidence of leucocytosis and of the subsidence of the further accompanying phenomena presently to be alluded to, has not hitherto been the subject of sufficient investigation. In fact the aggregation of phagocytes locally at the seat of infection has drawn attention away from the part played by the phagocyte producing tissues. Within recent years these have been studied by Roger[99] in France, and independently by Muir[100] in this country. The fact has long been known that in many infectious conditions the number of leucocytes in the circulating blood is increased, but it was left to these observers to demonstrate the extremely pronounced germinative activity which occurs in any severe infection in the precursors of these cells. With regard to the leucocytic phagocytes Muir has shown both experimentally in animals and by observations on man that in infections where there is a polymorphonuclear hyperleucocytosis not only is there evidence of active division of the parent cells in the bone-marrow, but so active is this process that the red marrow increases in amount and encroaches on the yellow. In a case

of pneumonia for instance a few days after the commencement of the disease the red marrow may have increased so as to occupy a seventh part of the whole medullary cavity of the femur. Not only, however, does proliferation occur in the site of formation of such an important class of cells as the polymorphonucleate leucocyte but Muir has also shown that proliferation occurs during some infections in such fixed cells as those lining the sinuses of lymphatic glands and also in the hyaline cells lying free in the lymph sinuses, which latter may be connected with some at least of the large mononucleate hyaline cells of the blood. He further points out that similar hyaline cells,—endothelial cells, connective tissue cells,—proliferate during infection, as can be shown from mitotic figures being found. It is no doubt the case that in different infections different groups of cells thus proliferate; in typhoid fever for instance there is no polymorphonucleate reaction, but here the proliferation of endothelial cells and hyaline cells in lymphatic glands has been observed. Thus while Metchnikoff has insisted with justice on the importance of the local reaction and of the wandering cells of the body in infection, and has noted the occurrence of phagocytosis in other cells (his "fixed amoeboid cells") he has missed the fact of the great proliferative changes in various parts of the body which may be described as the reaction of the body generally against infection. It must be insisted that there are not only local chemiotactive effects, but in the case of the wandering cells there is the general chemiotactic effect which draws the polymorphonucleate leucocytes from the marrow, and in all cases of severe infection there is the further stimulative effect which leads cells in various parts to divide. Either this stimulation is part of a reparative process or it is to be looked on as the result of injury due, say, to circulating poisons. The fact that, in relation to one aspect of the process, namely, the polymorphonucleate reaction, the effect often is to increase at a given point the available number of cells capable of acting as phagocytes, leads us to think that all these tissue changes may be of the nature of an exaggeration of normal functions, the general effect of which exaggeration is to have a beneficial effect.

It is to be noted as a very important point in this process that most of the distant effects must be due, even in the case of bacteria which *in vitro* do not secrete soluble poisons, to the circulation of soluble toxines unless, which is possible, we consider bacteria capable of emitting purely physical influences. Connected with these is the other very important fact that embryonic activity may be dissociated

from any actual phagocytosis on the part of the proliferating cells, and this taken along with such facts as the proliferation in certain infections of the non-phagocytic eosinophile leucocytes raises anew the question of the possible secretion of chemical substances into the serum which may be concerned in the complicated process by which bacteria are destroyed within the animal body. Here it may be observed that Muir has noted an increase in size and distinctness of the granules in the young polymorphs which occur in the marrow during a severe infection. This might indicate the preparation of material to be secreted. From what has been said *it is thus possible that on the fixed cells of the body and the fixed precursors of the wandering cells are impressed qualities which perpetuate immunity in an animal which has survived an infection.*

These observations and deductions of Roger and Muir open up quite a new field of enquiry, the exploration of which must throw most important light on the whole question of immunity.

(*To be continued.*)