# A REVIEW OF CURRENT THEORIES REGARDING IMMUNITY.

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## I.

THE primary object of these papers is to attempt to give a brief review of the present state of opinion regarding the many questions involved in the subject of immunity,—a review intended chiefly for those whose work is concerned with other fields of hygienic research. The carrying out of this aim has necessitated the re-statement of many facts familiar to bacteriologists, in order that the continuity of the discussion might be maintained. Wherever it has been considered advisable the details of the data which have formed the basis of theory have been given, but as a rule the leading results have been alone dealt with, partly because details might obscure the general principles it was intended to emphasise, partly because considerations of space have made an exhaustive treatment of the subject impossible.

For the present point of view we must neglect the microscopic structure of protoplasm, and think of living material as having even in its minutest presentations a molecular constitution of extraordinary chemical complexity. This complexity involves both the substances of which protoplasm is essentially composed and the materials which these substances elaborate in that metabolism, or capacity for activity, which we recognise as a very important manifestation of life. For metabolism, or the manufacture of new combinations, assimilation, or the taking up of extraneous material, is necessary. Now so far as any particular group of active living molecules is concerned, any such material with which these may come into contact, (1) may be capable of assimilation and may be used in metabolism, *i.e.* may be food, or (2) being capable of assimilation may disturb metabolism, *i.e.* may be poison, or (3) may be incapable

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of assimilation, *i.e.* be what is usually called inert matter. In vital action there are also, of course, physical factors which either promote metabolism, interfere with it, or have no effect. It is with the second of the chemical interactions of protoplasm with external matter that we have especially to do, namely, with the process of poisoning. Next to the fact that such a process is possible, is the further fact that, while protoplasm may be seriously affected by a poison, it very frequently develops the capacity of tolerating the presence, and it may be the action, of a poison so that the metabolic activities ordinarily interfered with by the latter go on as usual. When this occurs the protoplasm is said to manifest immunity. This capacity is a vital factor in resistance to and recovery from disease.

Poisons are varied in their nature and mode of action, and in a complex colonial organism, such as an animal's body, an interference to a very small extent with the metabolism of a few cells may give rise to serious effects in the colony. But there appears to be a corresponding variety in the capacities of protoplasm to deal with interferences with its metabolism. Of the means by which tolerance to many kinds of poisons is established we know nothing, but with regard to tolerance against the pathogenic action of the bacteria we are beginning to have some understanding. The term immunity strictly applies to the development of tolerance towards any poison, but at present, on account of the fact that most work has been done on the processes by which tolerance arises against bacteria, the word is often limited to immunity against these bacteria, and it is in this sense that it is used in the present paper.

*The two different types of bacterial action.* There is little doubt that the pathogenic action of bacteria depends on a process of poisoning, but the results of enquiries regarding this process are very complicated. The complication arises from the fact that different bacteria interfere with metabolism in different ways, and in any discussion on immunity this cannot be too strongly insisted on. In the case of certain bacteria, such as those of diphtheria and tetanus, the organisms settle down in one part of the body and produce poisons, which, being absorbed, give rise to changes in the functions of relatively distant organs on which they have a selective action. Such bacteria, further, when grown in artificial media, produce poisons, which, after the actual bodies of the bacteria have been removed by filtration through porcelain, are capable of reproducing the characteristic symptoms of the disease. The actual nature of these poisons is unknown, for they have resisted all attempts at isolation in a pure

form. So far as any chemical reactions which they appear to possess go, they are to be grouped with a class of poisons fairly widely spread in nature, namely, with the snake poisons, the poisons of many other noxious creatures, and with certain vegetable poisons, the best known of which are ricin, derived from the castor-oil bean, and abrin, the active principle of the jequirity seed. In all these poisons the true nature is unknown, but they have this in common that they are precipitated by agents which precipitate those intermediate products of ordinary digestion,—the albumoses. This latter fact is, from our present point of view, not uninteresting and may be of importance, for it is possible that these poisons are in real constitution not far removed from the bodies which normally form the food of certain cells. Bacteria growing locally and producing effects by means of poisons or soluble toxines, as they are called, constitute then the *first* great group of such irritants. In the *second* group the disease effects are in some way or other more closely associated with the actual bodily presence of the bacteria themselves. The understanding of the action of the latter presents, however, many difficulties, for in many cases there is here also a certain tendency for the organisms to be local in their distribution. If we take the case of the pneumococcus we have a bacterium which is, in the usual manifestation of the disease it causes, confined to one organ of the body, and yet there is apparent evidence of effects on distant organs. Other instances of the same sort of action are found in the case of erysipelas and in the various forms of blood-poisoning, and, though to a much less extent, in typhoid fever. In man almost the only representatives of a true septicaemic process, *i.e.*, one where the organisms are found all over the body, are to be found in plague and in relapsing fever. In all the members of this second group of bacterial disorders, whether in their site the organisms are selective, as in the case of pneumonia or typhoid, or not, as in the case of the pyogenic cocci, the main general effect produced in the body has as its outstanding feature the development of fever, that condition whose true significance is not yet understood. Whether the latter is the cause of the other forms of disturbed metabolism which accompany it or whether all are part of one process has still to be ascertained. What we have to recognise in this connection is that the type of disordered metabolism is the same for all the diseases of this second group. Further, in all these diseases the local activity of the associated bacteria tends to be associated with the development of that complex of pathological changes summed up in the term inflammation. If we know little of the significance of the effects caused by such

bacteria, so we require more knowledge of the means by which these effects are produced. If cultures of the bacteria in question are filtered after the manner practised in the case of diphtheria and tetanus, the filtrates are often but little toxic; and even when they are there is sometimes evidence to be obtained that other and more powerful toxic agents have been left behind in the bacterial protoplasm. The proof of this is to be found in such facts as that observed by Metchnikoff, Roux, and Taurelli-Salimbeni [1], namely, that an animal immunised against the filtered toxines of the cholera vibrio was not immune against an injection of the living organisms, and further that the serum of one animal immunised against the latter did not protect another animal against a fatal dose of the filtered toxine. Wassermann [2] found the same to be the case with the *Bacillus pyocyaneus.* These facts are difficult to understand. Whether the bacteria within the body produce toxines different from those produced in cultures, or whether the toxic effects produced in the body are due to the fact that bacteria die in the tissues in great numbers, that their bodies break up and liberate the poisons which they contain, and which do not under ordinary circumstances diffuse out of their protoplasm, or whether the changes in local metabolism produced by the inflammatory reaction which so often occurs are responsible for the changes in general metabolism, we do not know.

*From the standpoint of immunity, in the case of both classes of bacterial disease the animal body requires to be protected both against the bacterial bodies and against their soluble toxines,* but in the case of diseases of the first class protection is chiefly required against the latter, while in the second it is very likely that if the body can deal with the local effects of the bacterium this is what is chiefly necessary. In this latter statement is probably involved the fact that the neutralisation of the local effects usually means that the bacteria must be killed, but we cannot be dogmatic on this point. Certainly in very many, if not in all, cases the bacteria are killed. Here it may be remarked that it is probable, in certain cases, that the process of immunisation experimentally produced in animals may differ in some respects from the kind of immunity required in the disease naturally arising. Thus in cholera in man the disease is almost certainly a toxic one, for the bacteria are confined to the intestine. Animals are, however, not susceptible to infection by this path, though they do succumb to a disease process if the cholera vibrio is introduced into the peritoneal cavity. In the latter case if they are to be made artificially

to acquire immunity the important matter is that they should have immunity against the actual bacterium and not against its soluble poisons.

*The methods of producing immunity against bacterial disease.* While the general facts regarding immunity have been deduced from the observation of the consequences of recovery from disease arising under natural conditions, all our knowledge of what really takes place and all the important therapeutic results which have followed on this knowledge, have come from experimental enquiries conducted on animals. The process of the immunisation of these animals follows slightly different lines according to the group of bacterial noxae against which immunity is required. In the case of such bacteria as those of diphtheria and tetanus small doses of a weakened poison are first given, usually hypodermically, at intervals of a few days; these are succeeded by larger doses, and in a very short time the animal has acquired the capacity of withstanding, without symptoms, a dose of the virulent poison which in its former state would have inevitably killed it. It is now said to possess *active* immunity. But if the process of immunisation be carried further, then the serum of such an animal is found to possess *antitoxic* properties, *i.e.*, if injected into another animal in suitable amount it will prevent this second animal from contracting the disease if it be subsequently infected, and further if infection have already occurred in an animal the antitoxic serum has a therapeutic action. This transference of immunity is called, in the case of the second animal, the possession of *passive* immunity. Thus with regard to the diseases of the first group to which we have alluded, the fact that the chief action of the bacterium is effected by its soluble toxines, is reflected in the corresponding fact that, on the side of the animal, there are produced bodies which are capable of neutralising these toxines, and it may be further remarked that if the toxine and antitoxine be mixed *in vitro* in suitable proportions, and the mixture injected into an animal, nothing happens. In immunising animals against the second group of bacteria the same general procedures are adopted, except that here the actual bodies of the bacteria are injected,—killed cultures being often used in the initial stages. The same results so far as active and passive immunity are also obtained, but the therapeutic effects are not so good, for reasons which will appear later. Here the most important fact to be borne in mind is that the serum of the immune animal has *bactericidal* properties, and we have thus again the substance produced which is necessary for the neutralisation of the essential noxious agent. In the case of immun-

isation against the first group of bacteria, antitoxic sera are produced, in immunisation against the second group of bacteria, bactericidal sera are produced. To what these antitoxic and bactericidal sera owe their powers we cannot say, but in the case of the antitoxines the active material is probably a globulin (Brodie [3], Nencki and Sieber [4]). Active and passive immunity as just described are to be grouped together as examples of *acquired* immunity, but it must also be borne in mind that there exists a *natural* immunity in the case of many species of animals against many diseases. This natural immunity is, however, usually not absolute and in most cases is not sufficient to protect the animal against every form of infection by the morbific agent.

We have now to proceed to enquire how the apparently comparatively simple facts detailed in the last paragraph are to be explained. These explanations lead us into discussions of the most complicated character and there are many points which are still obscure. As yet bacteriologists have been only able to deal with broad general principles,—in the case of no one disease have all the successive steps in the process been worked out. With regard to many of the principles involved the explanations at present rest largely on what occurs in circumstances analogous to those of bacterial infection, though there is little doubt that what appear now to be only analogies will be found to be examples of the same laws. There is a great probability that in different cases of immunity the details of the processes differ, though the general principles underlying all are the same. The differences in details are chiefly found to correspond with the two great classes of bacterial disease to which reference has been made. As we have already said probably in the development of immunity against every bacterium there requires to be developed a capacity of resistance to its toxines and also the capacity of actually killing the bacterium itself. As the former is the simpler process we shall first of all study it, and here it is usual to take as the typical diseases diphtheria and tetanus where the general toxic action overshadows altogether the local effects of the bacteria,— none the less, however, must it be remembered that such local effects do occur. We shall next take up the principles which underlie the capacity for killing bacteria in the animal body and especially the increase of these powers which accompanies the development of immunity. The diseases which have been mostly studied in this connection are cholera and typhoid and especially the artificial diseases caused by the bacteria when injected into animals. With regard to these diseases again it must be remembered that the animal body may require the

development of resistance to soluble toxines produced by the bacteria. It will be found that this part of the subject has had much light cast on it by the study of processes within the body analogous to the killing and dissolution of bacteria. The theories which have been advanced to account for the many observations regarding immunity have been varied; but in treating of them the task is somewhat lightened by the fact that at present all others are overshadowed by that associated with the name of Ehrlich, and round a discussion of this all that is essential in others can be taken up. This theory starts from certain researches on the nature of the soluble toxines, it then proceeds to treat of immunity against these toxines and therefore against the bacteria producing them, it then deals in similar fashion with the much more complicated question of immunity against infection by the members of our second group of bacteria. All forms of immunity, natural and acquired, and both the active and passive forms of the latter, are embraced by this theory, and we shall now proceed to enter into it in detail.

### A. THE DEVELOPMENT OF THE CAPACITY OF RESISTING THE SOLUBLE TOXINES OF BACTERIA.

*Ehrlich's views on the nature of soluble toxines.* The first of the papers in which Ehrlich sets forth his views on immunity was published in 1897 and dealt with the constitution of the soluble toxines and the nature of antitoxine action. Since then his theories have been developed in a number of memoirs [5]. At that time he had formed the opinion that the union which, as we have seen, can take place *in vitro* between toxine and antitoxine is of a chemical nature. This was founded on such facts as that the two bodies can be titrated against one another like an acid and alkali, that union is hastened by warmth and is slower in the cold, that it takes place more readily with concentrated solutions of the substances than when these solutions are weak. The analogies with ordinary chemical reactions were thus very striking. On looking, however, more closely into the phenomena which accompany the neutralisation by the corresponding antitoxine of crude diphtheria toxine (*i.e.*, the fluid obtained by the filtration through unglazed porcelain of a bouillon growth of the bacillus) certain discrepancies appeared. The investigation of these led to the discovery of the fundamental facts on which the subsequent framework of research and deduction was based. To clear the ground we must observe that the strength of a toxine is measured by taking as a unit the amount which will kill a

guinea-pig weighing 250 grammes in four days.   This is known as the minimal lethal dose ("M.L.D.").   Theoretically the strength of an antitoxine is measured in terms of the so-called antitoxic unit, and the latter ought, as originally defined, to be the amount of the serum of an animal immunised against the disease under consideration which, when mixed with 100 M.L.D. of the toxine *in vitro* and allowed to stand for half-an-hour, will completely neutralise the poison, so that when the mixture is injected into a guinea-pig of the size just mentioned no symptoms will occur.   In an extended series of observations Ehrlich first of all took one unit of antitoxine and found the amount of each of a number of samples of diphtheria toxine which this quantity exactly neutralised (*i.e.*, taking as the sign of neutralisation the fact that when the mixture was injected into a guinea-pig no symptoms occurred).   He noticed that "of one toxine, perhaps 20, of a second, perhaps 50, and of yet a third, it might be 130 simple lethal doses were saturated by one immunity unit."   From this statement it is evident that, whatever theoretical basis underlay the original standardisation of this antitoxine, the result was the setting up of a purely adventitious standard.   The remark evidently applies to all strains of antitoxine in existence and may explain the unsatisfactory results obtained, especially formerly, in the therapeutic use of some of them.   The next step showed a possible explanation of this anomaly.   If in a mixture of toxine and antitoxine union took place in the way in which, say, a given amount of sodium hydrate in solution unites with the amount of hydrochloric acid calculated exactly to neutralise it, it is evident that the addition to a neutral mixture of toxine and antitoxine of one M.L.D. of toxine ought to cause the death of the test animal just as if one M.L.D. had been injected alone.   This, however, was found not to be the case.   Often many times the simple M.L.D. had to be added to the neutral mixture of one antitoxine unit with diphtheria toxine before, on injection, death was caused.   In one sample of toxine investigated 28 M.L.D. had thus to be added to the neutral mixture before a fatal result was obtained, and the smallest amount observed was 1·7 M.L.D.   For the other nine samples of toxine investigated, and which were either prepared by Ehrlich himself or obtained by him from other bacteriologists, the figures lay between the extremes given.   To explain these results Ehrlich calls attention to the case of a toxine which immediately after filtration had an M.L.D. of ·003 c.c.   Nine months later the M.L.D. was ·009 c.c., but it was found that, even after the lapse of this period, one antitoxine unit neutralised exactly the same quantity of the toxine as

at first. In other words one antitoxine unit when the toxine was freshly filtered neutralised 100·2 M.L.D., and nine months later only 33·4 M.L.D. It may be stated that the greatest care was taken to keep the antitoxine exactly in the same condition. In short, the toxic power of the toxine had decreased while its combining power had remained the same. That a toxine does diminish in strength on being kept had been a fact familiar to all workers, but this further fact that it still may require the same amount of antitoxine for neutralisation had not formerly been observed. For such degenerated toxines Ehrlich proposes the name " toxoids" to distinguish them from the true toxine which may be looked on as the most poisonous material present, and it is to be noted that probably every crude toxine, however fresh, contains both true toxine (hence-forward referred to simply as " toxine ") and toxoids, and Ehrlich considers that the fatal effect in four days, which is taken as the standard, is due to the toxine alone. He proceeds to give a theory to account for this phenomenon of loss of toxicity without loss of com-bining power. Suppose that in the ultimate toxine molecule there are two chemical affinities, such as occur in many bodies known to organic chemistry, and that the function of one,—called by Ehrlich the " haptophorous " group,—is to combine with the corresponding affinity in the antitoxine molecule, and that the function of the other,—the " toxophorous " group,—is to exert a poisonous action, then the difference between toxine and toxoid might be that in the latter these groups had undergone change. It is evident that with a loss of toxicity, such as we have seen occurs (caused on this theory by a degeneration of the toxophorous group), the haptophorous group might either be un-affected or it might also be degenereted ; it is also theoretically possible that the change which inimically affected the toxophorous group might increase the potency of the haptophorous group. Take the case where both the haptophorous and toxophorous groups are degenerated, and consider the bearings of such a supposition on the fact that to a neutral mixture of crude toxine and antitoxine more than one M.L.D. has to be added to produce death in the test animal. In the neutral mixture there was both toxine and toxoid with the haptophorous groups of both satisfied. If, say, one M.L.D. of crude toxine is added this also contains toxine and toxoid, the amount of the former present being just sufficient to cause death. What will happen in the mixture will be that the toxine with its more powerful haptophorous groups will displace some of the toxoid already combined with the antitoxine, will combine with the latter and will thus be prevented from exercising its powerfully toxic

influence. The toxoids thus liberated, acting along with the toxoids in the M.L.D. added, may be insufficient to cause death, which in the case of diphtheria they can do, by causing a lingering illness with paralysis as a chief symptom. As a matter of fact, as we have seen reason to believe, a great many M.L.D. may have to be added before there is sufficient poisonous matter free to cause death in the prescribed time. Such, in outline sufficient for the purpose of being able to appreciate its bearing on the question of immunity, is the theory of Ehrlich regarding the constitution of the soluble toxines.

*Method of action of soluble toxines and relation of action to production of antitoxines.* Ehrlich next proceeds to develop from these views a theory of immunity against this class of poisons. The selective action of the morbific agent in diphtheria and tetanus was, of course, familiar to the clinician long before the true pathology of the diseases was known. Ehrlich accounts for this selective action as follows: As there is evidence of the existence of a chemical affinity between toxine and antitoxine, so, probably, there exists the same affinity between the toxine and corresponding affinities in the cells of the body, and the capacity of these affinities being mutually satisfied constitutes the susceptibility of the tissues. In short, the haptophorous group in the toxine fixes the latter in the cells and allows the toxophorous group to act, which it does by disturbing metabolic processes dependent on the activity of other molecules. Further, the production of antitoxine finds a possible explanation on this supposition. It is impossible to conceive that the affinities in the brain cells to which, say, the tetanus toxine becomes attached are ordinarily of no use in the cellular metabolism. In the latter they must bear a part or they would be examples of absolutely useless structures. Now the process of immunisation consists in its initial stages in the administration of small non-fatal doses of the pathogenic agent. Looking at the action of the first of these, though little or no toxic effect is produced, we see that the cells must be robbed of affinities, needed in ordinary metabolism, by the fact of the attachment to these affinities or "side-chains," or "receptors," as Ehrlich calls them[1], of toxine molecules. But it is a

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[1] The term "side-chain" is unfortunate, as when applied to a chemical molecule whose constitution is known it has a definite meaning. Ehrlich has only used the term to express an analogy. It cannot but be wrong to speak, as is sometimes done, of the "side-chains" of a cell, though such side-chains may occur in molecules within the cell. The word "receptor" is much more fitting to express the group within the cells which may carry an affinity capable of saturation by a molecule outside the cell.

general biological law, on which the repair of many kinds of damage to the organism depends, that, if protoplasm be not too seriously injured, it tends to replace the damaged parts, and not only so, but, very frequently, it tends to over-regenerate the lost parts. Thus in the case under consideration, as has been specially pointed out by Weigert [6], the affinities lost to the cell by having toxine anchored to them are reproduced; these new affinities are, however, again lost to the cell by saturation by the further doses of toxine injected in the immunisation process, and here it is to be specially borne in mind that there is always a progressive increase of the amounts of toxines injected. The cell thus has to go on manufacturing the affinities whose use it is constantly losing, and finally the latter are formed in such enormous numbers as to be present in proportions altogether beyond the cellular requirements. They are, therefore, being no longer of any use to the cell, waste material and are excreted accordingly. They thus pass into the serum and form the antitoxic agent in the latter, for they retain the original capacity they possessed within the cell of combining with the toxine molecules. When these cast-off receptors or "side-chains" of the susceptible cell meet the toxine their free affinities saturate the haptophorous group of the latter, which thus loses the means by which it becomes attached to cells, and therefore, as anchoring is impossible, the toxophorous group of the toxine no longer has the opportunity of working a pathogenic action. The toxine is, in fact, bereft of its toxic power. If the toxine is saturated thus with antitoxine and injected into an animal's body nothing occurs,—if the saturation takes place within an animal's body the mixture is again inert, and its fate in either case we do not know.

*Discussion of Ehrlich's theory.* Such are Ehrlich's views on the origin of immunity against bacteria giving rise to soluble poisons and on the nature of the interactions of toxines and antitoxines, and it must at once be admitted that they have opened up entirely new ground. Up to the time of their publication bacteriologists had been aiming at the obtaining by chemical means of pure samples of the substances involved, in order that their properties might be studied. For this method Ehrlich substituted one of analysis by the study of the physiological results. We therefore pass to enquire how this study and the theories based upon it stand in relation to other lines of research on the questions at issue. We shall first here look at the evidence for Ehrlich's fundamental canon that the union between toxine and antitoxine is a chemical one, secondly, at what

evidence there is for his views on the constitution of the toxines, thirdly, at the evidence from other sources of his views on the origin and development of the antitoxines, and, finally, discuss certain difficulties which the adoption of the view may seem to raise.

(1)   *The nature of the antagonism between toxine and antitoxine.* Two theories as to the interaction of these substances have been put forward, one based on chemical, the other on physiological grounds. According to the former view (that adopted by Ehrlich) antitoxine neutralises toxine in the way that an alkali neutralises an acid.   According to the latter, there is no such interaction, but, when both are present in the body of an animal, the antitoxine stimulates the tissues to resist the toxine.   The evidence for the physiological view rests chiefly on three experiments.   Buchner [7] is stated to have taken a mixture of tetanus toxine and antitoxine which was quite neutral to the mouse, and to have found that it was not neutral to the guinea-pig. From this he concluded that no actual combination of the two substances had taken place.   His view was that as, weight for weight, the guinea-pig is a more susceptible animal towards tetanus than the mouse, neutrality for the latter meant that there was present in the mixture named enough of the antitoxine to enable the animal to resist the toxine, while for the guinea-pig more stimulation would be required and enough antitoxine was not present.   Calmette [8] adduced another series of experiments to support a similar view.   The antitoxine to certain serpent poisons is more susceptible to heat than the actual poison, the opposite being the case with tetanus and diphtheria toxines.   This investigator took a mixture of venene and antivenene which he stated was neutral and heated it, and he found that its toxicity was restored, from which he deduced that no interaction *in vitro* had taken place, but that the two bodies simply existed side by side unchanged.   Wassermann [9] obtained a similar result with the antitoxine of the soluble poison of the *Bacillus pyocyaneus*.   The former substance is destroyed by boiling, while the toxine is not.   A mixture of the two bodies said to be neutral regains its toxicity when heated, and further the toxicity was again lost if fresh antitoxine was added.

Let us look more closely at these results.   If Buchner's experiments be carefully studied it will be found that undoubtedly he was dealing with mixtures which were not neutral even for mice.   He first took 10 mice and found that ·0001 gramme of a particular toxine was an unfailing fatal dose.   Whether it was the M.L.D. in the modern sense of the phrase cannot be determined.   In the case of a comparative

experiment on 10 guinea-pigs (whose weight was on an average 20 times that of the mice) the same dose was not fatal, though it gave rise to slight tetanic symptoms. In the test experiment 23 mice received each ·014 gramme of toxine (*i.e.* 140 times the fatal dose) mixed with ·00135 gramme of antitoxine, an amount which was said to be sufficient to neutralise the 140 fatal doses. It is evident that the basis on which the latter amount of antitoxine was calculated was the amount of antitoxine supposed to be capable of neutralising ·0001 gramme of toxine, for in the case of three of the mice death from tetanus occurred, and 11 suffered from slight tetanus. Only in nine cases were no symptoms of the disease observed. The mixture of toxine and antitoxine was, it is thus evident, not neutral for the majority of mice. In the case of 23 guinea-pigs injected with the same mixtures 8 died, 12 suffered from tetanus, and 3 had no symptoms. Thus the greater susceptibility of the latter species of animal was only evidenced by its succumbing more easily to a dose of non-neutralised toxine. Calmette's results have been criticised by C. J. Martin and Cherry [10], who have shown that in them the importance of time as a factor in complete neutralisation had been neglected. If the apparently neutral mixture were allowed to stand long enough *in vitro,* at the end of a given period no return of toxicity on heating could be obtained. Apparently the mixtures used by Calmette were not really neutral, but there was sufficient excess of free antitoxine to prevent the toxine, which at the moment of injection was still unneutralised, from having a toxic effect on the animals. Union in fact here was completed within the animal's body. So far as appears, a similar test has not been applied in the case of Wassermann's results, but this would have to be done before they were finally accepted as evidence in favour of a physiological explanation of the reaction of toxine and antitoxine. The general conclusion to be drawn is that, at present, there is no evidence of the action of antitoxine being a physiological one to which cogent objection cannot be urged.

In favour of the view that the reaction between toxine and antitoxine is really of a chemical nature, several facts can be adduced. That the reaction takes place more readily when the respective solutions are concentrated and when they are warmed has been confirmed by Knorr [11]. Again, C. J. Martin and Cherry have adduced other evidence pointing to the same conclusion. They have investigated the behaviour of various albuminous substances when these are filtered under high pres-

sure through a porcelain filter, the pores of which have been filled with
gelatine. A very strongly supported dialyser is thus, to all intents and
purposes, formed. They found that through such a filter antitoxine
did not pass, while toxine did. Mixtures of toxine and antitoxine were
allowed to stand for varying times and then subjected to filtration.
The longer the mixture was allowed to stand before being filtered the
less toxine passed through, till a time arrived when no toxine appeared
in the filtrate and further what had not passed through the filter
was found to be non-toxic. This indicates that a combination between
the two bodies took place when they were left long enough in contact.
Ehrlich [12] has brought forward other results besides those mentioned
which support his view. A poison, ricin, can be extracted from the
castor-oil bean, and among its other properties is the capacity of
dissolving red blood corpuscles. Animals can be immunised against
its poisonous action by a process identical with that employed in
immunisation against tetanus or diphtheria. The serum of these
animals contains an antiricin corresponding to an antitoxine. By
experiments in test-tubes it can be shown that the blood corpuscles of,
say, the rabbit can be protected against the action of the ricin by means
of this anti-ricin. Now red blood corpuscles are usually looked on as
mere carriers of oxygen and incapable of physiological reaction such as
is presupposed to take place in a cell when it is stimulated to resist an
external noxious agent. Such a response to a stimulus would have to
be supposed to take place in the blood corpuscle, *i.e.* a physiological view
of the antagonism of the two substances would have to be taken, if the
action of anti-ricin on the ricin were not merely a chemical one. Again,
strong support of the chemical view is obtained from a more than
analogous case of another anti-body. As is well known, rennet curdles
milk. Morgenroth [13] injected after the manner of an immunisation
this ferment into a goat, and he found that the serum of the animal
acquired a property of protecting milk against the action of the
ferment. No one will deny that milk is an inert substance quite
incapable of physiological reaction, and therefore there is little doubt
that the reaction of the antirennet on the rennet is of a chemical nature.
Taking into consideration all the facts bearing on the interaction of
toxines and analogous bodies on the corresponding toxines, we must
hold that, in the absence of direct experiment with pure samples of the
substances in question, there is very strong presumptive evidence that
these substances combine after the manner of ordinary chemical bodies
which have affinities for one another.

(2) *The confirmation of Ehrlich's views as to the constitution of toxines.* We now pass to enquire if confirmation is forthcoming of Ehrlich's views of the degeneration of toxines and of the existence in the toxine molecule of independent binding and poisonous groups. The most weighty contribution here is the work of Madsen[14] on the poison known as tetanolysin. Ehrlich[15] first noticed that in certain bouillon cultures of the tetanus bacillus, though not in all, there occurred the property of dissolving the red blood corpuscles of certain animals. That this property is probably dependent on a poison distinct from that which gives rise to the spasms of tetanus, is indicated by the fact that it is not possessed by all bouillon cultures, and further that it is very readily lost, as, for example, by heating for 20 minutes to 50° C.—a temperature which will scarcely affect the spasm-producing action. Ehrlich therefore calls the substance tetano-lysin, and the ordinary spasm-producing body tetano-spasmin. With regard to this tetanolysin when an animal is immunised by a bouillon containing it, its serum contains an anti-body which will protect the susceptible blood corpuscles against the dissolving action. Taking advantage of these facts Madsen has investigated the constitution of this poison along the same lines as those pursued by Ehrlich with the diphtheria toxine. The only difference in the method was that the effects of different mixtures of the toxine and antitoxine on the blood corpuscles were observed in test-tubes, instead of as in Ehrlich's experiments by injecting the mixtures into guinea-pigs. In this way not only could many more experiments be done at one time, but the possibility of a physiological action of the antitetano-spasmin was excluded. The results were to show that the crude tetanolysin of the bouillon culture was not a single substance, but contained, besides the most potent body, another which, while requiring the same amount of antitoxine for its neutralisation, had much less haemolytic action. Further the crude substance contained also other bodies which had less combining power associated with less haemolytic action. In fact, the investigation of this substance, under circumstances where only chemical reactions could take place, showed it to have an absolutely analogous constitution to that which Ehrlich had assigned to the diphtheria toxine. It may here be added that Ehrlich's experiments with diphtheria poison have been confirmed by Bulloch[16]. There is thus the strongest ground for believing that in such crude toxines as that of diphtheria there is a mixture of bodies. All of these possess two unsaturated affinities, one associated with the capacity of combining with antitoxine, the other

having a toxic action, and the differences between the different bodies present in the crude toxine are differences chiefly in the toxophorous group, though differences also occur in the haptophorous group. It may be said that Ehrlich has devised experiments by which the relative amount of toxoid and toxine in the crude toxine can be estimated. It would lead us too far afield to go into this matter fully, though the results confirm generally the soundness of his physiological analyses of toxines. Roughly speaking the method consists in first determining the amount of, say, crude diphtheria toxine which will exactly be neutralised by one immunity unit of antitoxine. In a long series of animals the effects are now studied of adding in each case one two-hundredth less of antitoxine to such a dose of toxine and injecting the mixture. Now in one such series the animals up to that which received the toxine *plus* the one-hundred-and-sixty-seven two-hundredths of one antitoxine unit died of paralysis after long illnesses, while in animals which received less than this amount of antitoxine death with acute symptoms occurred in a few days. In other words, up to the point named there was enough antitoxine still present to completely neutralise all the toxine (with its stronger haptophorous group), and the symptoms were produced by the toxoids. Below the point named there was toxine unsaturated and thus rapid death was produced. In such a crude toxine, according to Ehrlich's nomenclature, there would be reckoned to be 33 toxoid equivalents. This experiment is only cited to bring forward another point, namely, the question as to the nature of the relation of the toxines to the toxoids. Dreyer and Madsen [17] (one of whose experiments furnished the figures just quoted) have immunised animals by means of mixtures of toxines and toxoids in which the toxine part was completely saturated by antitoxine. They found that the antitoxine present in the sera of these animals neutralised ordinary crude diphtheria toxine, *i.e.* containing both toxine and toxoid. Therefore there is strong ground for supposing that the haptophorous group in toxoid is the same as that in toxine. This is fresh support to Ehrlich's views.

(3) *The evidence in support of Ehrlich's views on the origin and development of immunity.* This part of the subject divides itself into two parts, firstly, the evidence for the fixation of the toxine in the bodily cells, and, secondly, the evidence for the production and over-regeneration of the antitoxine by these cells. We may first of all here clear the way by qualifying a quasi-popular statement as regards the development of immunity in an animal. It is usually said, and the statement is often quite true, that an attack of a disease, which

has been recovered from, protects the individual from fresh infection. In the study of immunisation experiments, however, it is found that the development of disease symptoms is not necessary to the production of immunity. In immunising animals for the purpose of obtaining from their blood an antitoxine for use as a therapeutic agent, it has long been the custom to commence the process by using a toxine whose toxicity has been impaired by the action of such agents as iodine or terchloride of iodine. In such cases often no symptoms of disease may manifest themselves. The present writer[18] has studied a curious reaction of tetanus toxine bearing on this matter. If the toxine be acted on by hydrochloric acid it gradually loses its toxicity, but, after all toxicity has apparently gone, a certain degree can be made to return if the acid be neutralised by sodium hydrate. It was found, however, that, during this period when the toxicity was only held in check, repeated doses of the acid mixture produced definite immunity in guinea-pigs, no tetanic symptoms being caused. If we accept Ehrlich's view of the constitution of such a toxine we would say that here the toxophorous group had been destroyed while the haptophorous group had still the capacity of combining with susceptible cells and producing immunity. Whether we use this phraseology or not, we must admit that a toxine can lose its toxicity without losing its capacity of producing immunity, and therefore immunity can be produced without an animal suffering from the disease. This is a not unimportant point in support of Ehrlich's theory.

We now come to examine *the evidence for the fixation of such toxines as those of diphtheria and tetanus in the bodies of the animals in whom immunity is capable of being produced.* Here we may first look at certain experiments by Dönitz[19]. This observer determined the amount of tetanus antitoxine which would neutralise 12 M.L.D. of a particular toxine. He then injected the latter amount into the vein of one ear of each of a series of rabbits, and into the vein of the other ear he injected quantities of the antitoxine at intervals after the toxine injection which varied with different animals. He found that while, if the mixture were made before injection the amount of toxine mentioned was neutralised by 1 c.c. of a 1 in 2000 dilution of the antitoxine, if injection of antitoxine took place 4 minutes after the toxine injection 1 c.c. of a 1 in 600 dilution was necessary—otherwise death occurred—if 8 min., 1 c.c. of a 1 in 200 dilution, if 15 min. 1 c.c. of a 1 in 100 solution. He found that similar facts were true of the amount of diphtheria antitoxine required to neutralise diphtheria toxine. From the facts regarding tetanus he concludes that at least

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within 8 min. of the toxine being injected enough is fixed in the animal's body to cause death. Heymans[20] found that, if all the blood were removed from an animal a few minutes after the injection of a M.L.D. of tetanus toxine and the blood of another animal substituted, still the animal died of tetanus. This is still more conclusive evidence in the same direction.

When we come to enquire where the toxine is fixed we are face to face with a very difficult question. It is natural that in attempts at its solution attention should have been largely directed to what takes place in tetanus, for, as has already been remarked, in this disease there is strong clinical evidence of a selective action on the part of the poison for the central nervous system. The experiments usually brought forward here are those of Wassermann and Takaki[21]. These observers took the brain of the guinea-pig, an animal very susceptible to tetanus, and bruising it thoroughly in a mortar mixed it with varying amounts of tetanus toxine. They found that it was capable of neutralising a considerable amount of the poison. Not only so but if an emulsion of the brain were injected within the 24 hours previous to the injection of toxine the latter appeared to be neutralised. This property of the brain was not shared by the other organs of the body. The deduction drawn from these experiments was that the brain substance acted on the toxine in the way that antitoxine does, and they are accepted by Ehrlich as bearing out his view as to the source of the latter substance. This work has given rise to a great deal of controversy, and the view of the authors and of Ehrlich has been combated by many observers. That the neutralisation takes place has been confirmed by Knorr[22], Metchnikoff[23], Roux and Borrel[24], Danysz[25] and Marie[26]. Several objections are, however, raised by these observers. Metchnikoff has been unable to find that the brain possesses any more antitetanic power than the other organs of the body. Danysz has found that if the apparently neutral mixture of brain and toxine be subjected to maceration with ·75 per cent. sodium chloride a certain amount of the toxine again passes into solution—a fact unlike anything which happens in mixtures of toxine and ordinary antitoxine. He has further made the very important observation that if the brain be heated to 100° C. it still retains its neutralising properties. Now, according to the results of Tizzoni and Cattani[27], tetanus antitoxine is destroyed at 68° C., *i.e.* loses its power of neutralising toxine. It would therefore appear as if the body, which, in the emulsion of brain used, neutralises toxine, may differ from true antitoxine. The view

of the four observers last named, is that the neutralisation is not of the nature of a chemical union but that it is a mere entanglement of the toxine by the *débris* of the nerve cells. The reason given for its not giving rise to tetanus when the mixture containing it is injected into an animal is that the cellular *débris* is taken up by leucocytes and that within the latter the toxine is destroyed. This explanation is rather difficult to accept. If there is free toxine present in the mixture then according to the results of Vaillard and Rouget [28] the leucocytes will be repelled, for this is the effect produced by the poison in question—an effect which according to the observers just mentioned is the explanation of the fact that tetanus spores deprived by heat of the small amount of toxine naturally adhering to them are taken up by phagocytes and prevented from causing death. If toxine be present they are not thus taken up and thus tetanus follows. Shortly after the publication of the first paper by Wassermann and Takaki it was stated by Ransom [29] that the brain of the fowl, which is very insusceptible to tetanus, did not fix the toxine. This observation has not been confirmed, for Knorr (*loc. cit.*) found that there was very little difference in the fixative properties of this animal's brain and that of the guinea-pig.

This question of the fixation of toxine has been by many observers confused by the introduction of side issues. Thus Metchnikoff adduces as evidence of the non-fixation of toxine by the central nervous system of the fowl the fact that in an animal treated with tetanus toxine the other organs of the body may be more antitetanic than the brain and spinal cord. He has found the same to be true of the alligator, which is non-susceptible to tetanus but which will develop a fairly strong anti-tetanic serum if treated with tetanus toxine. Such an occurrence does not in the least detract from Ehrlich's theory. There is evidence that in this disease other organs besides the brain can fix the toxine. This is seen from certain experiments of Roux and Borrel (*loc. cit.*). In these it was found that in the rabbit, while one-tenth of a c.c. of toxine produced tetanus of a fatal kind when introduced into the brain, 2·5 c.c. was the fatal dose if introduced under the skin. In the guinea-pig on the contrary one-hundredth of a c.c. introduced subcutaneously caused death in 50 hours, while the same amount given intra-cerebrally caused death in three days. Such results are distinctly in favour of Ehrlich's view, for in the case of the rabbit the extra amount of toxine must have been taken up by tissues other than the nervous system. In the guinea-pig, on the other hand, all the toxine must have gone directly

to the latter. Dönitz (*loc. cit.*) had previously suggested that something of this kind can occur in the rabbit to explain what he calls *tetanus sine tetano*. In this phrase he refers to the fact that when nearly neutral doses of mixtures of toxine and antitoxine are given to this animal sometimes death does not occur from tetanus but from a kind of cachexia. It is quite conceivable that toxine can be fixed by cells, interference with whose function is not of such vital importance to the body as the nerve cells, and that it is only under certain very special circumstances that the pathogenic effects of such cells being affected manifest themselves. It is to be remarked, however, in this connection that from Ehrlich's standpoint all these sites where the toxine is fixed are potential sites of antitoxine formation, and, therefore, in such a disease as tetanus, antitoxine may be formed in a variety of organs. It is possible, again accepting Ehrlich's position, that in such an animal as the alligator the explanation of its insusceptibility to tetanus along with its capacity of forming antitoxine may be that the nervous system cannot fix the toxine while other organs can, and it is in these that antitoxine is produced.

It must be admitted that the evidence with regard to the fixation of toxines is very unsatisfactory, and much further investigation is here necessary before Ehrlich's position can be unreservedly accepted. It is to be remarked, however, that the methods which have hitherto been applied to the solution of this question have been of a somewhat insufficient kind, for the work of Buchner [30] on the sugar-fermenting substance in yeast has shown that the mere bruising of cells in an ordinary way is probably a very uncertain method of obtaining the intracellular juices.

It is evident that according to Ehrlich's theory *the question of the site of fixation of toxine is co-related to that of the site of antitoxine formation*, for if the theory be correct where the toxine is fixed there the antitoxine is formed. Not only are the attempts to determine the place of toxine fixation rather unsatisfactory, but what has been found regarding the relative richness in antitoxine of the different organs of the bodies of immunised animals does not shed any light on the question of the site of formation of this substance. Metchnikoff, in a guinea-pig immune to tetanus, found that all the organs, the brain included, had less antitoxic power than the blood, the kidney being the only organ that had any great antitoxic value. These results are borne out by Dzierzgowski [31], who, in a horse immune to diphtheria, found the kidneys and supra-renals more rich in antitoxine than the other

organs. The chief conclusion the latter observer draws from his work is that the antitoxine is excreted by the urine and, also, it may be incidentally mentioned, by the sweat.

The general conclusion to be drawn as to this branch of the subject is that, while such experiments as those of Dönitz and Heymans leave little doubt that such a toxine as that of tetanus is rapidly taken from the blood into the organs, more research is necessary as to the site of its fixation. More knowledge is also required as to the site of anti-toxine formation.

While the site of antitoxine formation in the body is obscure, certain facts are known regarding what happens in the course of its development which require attention. From time to time it has been suggested that antitoxine might really be a modified toxine. This view was first put forward by Buchner[32] on theoretical grounds. Such an idea has some fascination, or rather had, because up till the time of Ehrlich's theory no definite attempt had been made to co-relate the occurrences of the process of immunity with any normal function of cells. It thus seemed very unintelligible that an animal, which had never been subjected under natural conditions to infection by a toxic agent such as ricin poison, should all the same be at once able not only to develop immunity to it, but should be able to transfer a certain degree of this immunity by means of its serum to another individual. It might thus appear natural to think that the substance, by which this immunity was transferred, was only a modification of the toxic agent. No facts, however, can be advanced to support this view, for the great difficulty here has been that, as we shall see presently, the amount of antitoxine developed in an animal's body is very much greater than the amount of toxine which was used to produce the immunity. To get over this difficulty it has been suggested that the toxine molecule might split up into a series of molecules of less size, each of which might contain a group capable of neutralising a toxine molecule. On theoretical grounds the existence of such molecules is not impossible, but unfortunately all the evidence we have (Brodie, Martin and Cherry, *loc. cit.*) goes to show that the antitoxine molecules are larger than those of the toxines. The following considerations support the view that antitoxine is formed somewhere in the body and during the course of its formation is shed out into the blood. Roux and Vaillard[33] took a rabbit immune against tetanus and possessing a strongly antitetanic serum and in the course of a few days removed from it a quantity of blood equal to the total amount of blood calculated originally to be in its body. This was

not followed by a sensible diminution in the antitoxic value of the serum. Similar and more exact experiments by Salamonsen and Madsen [34] on goats immunised against diphtheria had a similar result. These observers removed large proportions of the animals' blood and at once substituted for it normal saline solution. They noticed that immediately after the bleeding there was a fall in the antitoxic value of the serum, which corresponded in degree to the dilution of the blood by the saline injected. But after a short period of time there was again a rise in the antitoxic value. Both of these sets of experiments show that, when antitoxine is removed from an animal's body by removing its blood, there is after a time a fresh passage of the antitoxic substance into the circulating fluids.

The next point to be considered is the relation of the amount of antitoxine produced to the amount of toxine injected in the immunisation process. Knorr (*loc. cit.*) showed in one experiment that, in a horse already furnishing an antitoxic serum, the injection of as much toxine as could be neutralised by one unit of antitoxine was followed by the production of 100,000 times that amount of antitoxine. This is no doubt an extreme case but it illustrates the capacities of immunisation. I have obtained (*loc. cit.*) similar results in another way. If tetanus toxine be acted on by hydrochloric acid until its toxicity is destroyed, it still retains the capacity of giving rise to immunity. By acting for the same time on different moieties of toxine there is no doubt that on each occasion the state of the modified toxine would be the same. Guinea-pigs were immunised by this modified toxine and instead of gradually increasing doses being used the amount was kept the same. One set of animals received four doses of the modified toxine and another set received eight such doses. Of the serum of the first ·5 gramme was required to protect a guinea-pig against an M.L.D., while of the second ·005 gramme was sufficient. Thus twice the amount of toxine gave rise to a serum 100 times stronger. Such experiments indicate that the process of antitoxine production resembles a process of hypertrophy and bears out the idea that certain cells get into the habit of producing it in greater and greater degree.

(4) *We now come to look at certain difficulties which may arise in a careful consideration of Ehrlich's theory.* The chief may be stated as follows. Suppose an animal is being immunised to a very high degree, for the purpose of obtaining a very strong antitoxic serum such as is used in the therapeutic applications of this substance. Suppose that it has reached a stage when its serum contains an enormous number of

antitoxic units. The process of immunisation is being proceeded with by injecting a fresh dose of toxine. As often occurs in actual practice, this amount of toxine could be neutralised by a very small fraction of the antitoxine already free in the animal's blood. If it be injected hypodermically, then, as it is slowly absorbed into the blood, it must meet an enormous overplus of antitoxine by which it must be neutralised. How then does it ever reach the site where it is to stimulate the receptive cells to fresh activity, in, for example, the case of tetanus, where, according to Ehrlich, the brain seems to be the chief site of fixation ? It is true that, in certain animals, a portion of it might be fixed locally at the point of injection and stimulate antitoxine formation there, but to produce the high effects of a long immunisation the toxine would always require to be injected into the same place, and it is found that this is not necessary. But, supposing that it has to reach, say, the brain, in order to effect the purpose of its injection and that it does do so. It is fixed there, but the cells in which it is fixed are constantly bathed with fluids extremely rich in antitoxine. Why then is it not turned out of the cells ? That such an eviction ought to take place we must admit if we consider the rationale of the antitoxic treatment of such a disease as diphtheria. We have seen, from the experiments of Dönitz, that if toxine be injected, followed by the injection of antitoxine, the longer the interval between the injection of the two substances, the greater has the amount of antitoxine to be, if death is to be prevented. To save life many thousand times the amount of antitoxine sufficient to neutralise *in vitro* the amount of toxine in the body has to be administered. All this points to the therapeutic action depending on what is called mass action although how the matter can be put into the language of physical chemistry might be difficult to say. At any rate, the therapeutic action of antitoxine seems to depend on the toxine being turned out of its combinations in cells by an overwhelming amount of the anti-body. Now why should the same action not occur during immunisation, in the circumstances we have cited, and, if it did, would not all possibility of fresh antitoxine formation come to an end ? We have to face the fact that it does not. There is one possible explanation which is still consonant with Ehrlich's theory. Assuming that, in the case we are supposing, the toxine must reach the brain and be fixed there. Let us consider what would be the effect of the affinity of the brain cells for the toxine being very slightly greater than the affinity of the free antitoxine of the blood for the toxine. The antitoxine in the blood might saturate the toxine which had been

hypodermically injected, and, the combined substance, circulating in the blood, would probably come into contact with brain cells. The more powerful affinities of the latter would break up the weak compound and retain the toxine, which would then work its action within the cells. But how would this affect the therapeutic action of antitoxine in disease? In this case we would have the toxine firmly fixed in the brain cells which are bathed in an enormous quantity of fluid having a less affinity for the toxine. Now, under certain circumstances known to physical chemistry[1], in such a case a small quantity of a compound of antitoxine with toxine might be formed. Thus, carbon monoxide has a greater affinity for haemoglobin than oxygen has; but, if a mixture of carbon monoxide and oxygen, in which the latter is relatively in excess, be brought into contact with CO-haemoglobin then a very appreciable amount of O-haemoglobin is formed. In the case of the diseases in question, it is probable that the detachment of a very minute amount of the poison from the cells in which it is fixed would be sufficient to turn the balance in favour of the sick individual. But it might be said that by the same process toxine might be detached from the brain of the animal undergoing immunisation. Here, however, the amount of toxine fixed usually amounts to many thousand times the M.L.D. for the animal under natural conditions, and the detachment of a small amount of it would not be likely to interfere with the essence of its effect. Thus, though the mechanism of the development of high degrees of immunity associated with strongly antitoxic sera is very complex, an explanation is not impossible.

There is, however, one aspect of the question which is very perplexing and which may be now raised. We must look more closely into what is meant by active and passive immunity. In the early stages of the immunisation of an animal against a toxine, to what does it owe its immunity? The following experiment opens up this question. In the work on immunisation by means of tetanus toxine modified by hydrochloric acid it has been stated (*vide supra*) that, of the serum derived from some of the members of one series of guinea-pigs, it was found that ·5 gramme was necessary to neutralise one M.L.D. of ordinary toxine. A careful calculation[35] showed that, in the whole of the blood of the body of such a guinea-pig as that from which the serum was obtained, there could not have been more than enough antitoxine to neutralise two M.L.D. Now a number

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[1] For a knowledge of the bearings of physical chemistry on this subject I am indebted to the kindness of my friend, Mr D. Nagel, Fellow of Trinity College, Oxford.

of the other animals of this series were tested by the injection of large doses of unaltered toxine to find out what amount of resistance they showed to the latter. In fact immunity was now judged of not by the antitoxic quality of the serum the animals produced but by the actual amount of toxine the latter were capable of resisting. It was found that they could resist the injection of about 110 M.L.D., though some tetanic symptoms appeared. One, however, succumbed to the injection of 122 M.L.D. The resistance may thus be said to have been somewhere just under 110 M.L.D. It is evident that the animals thus tested cannot have owed their power of resistance to the amount of antitoxine present in the fluids of the body. This observation has been confirmed by other similar experiments. The conclusion is that resistance to a toxine is not necessarily co-related to the possession of antitoxic power in the serum. This is borne out by other facts, such as those brought forward by Behring, to the effect that sometimes an animal will show great power of resistance to toxine without having much antitoxine in its serum. In fact the experience of serum institutes seems to be that sometimes animals are met with which, though easily immunised, appear incapable of producing a powerful antitoxine. On Ehrlich's theory the experiments given above might appear to be explained by supposing that, while the cells had developed the capacity of producing side-chains in great numbers, these side-chains were not yet cast off, and therefore were capable of fixing toxine within the cells. But if this is the case what power is preventing the toxophorous groups of the toxine from having a pathogenic influence? Yet in the series of animals referred to above certainly 66 M.L.D., and probably more, could be tolerated without the slightest symptom of tetanus. The same difficulty as to what becomes of the toxophorous groups is suggested if we consider the later stages of immunisation for the obtaining of therapeutic sera as that immunisation is practised. We have seen there are difficulties, not, however, insuperable, in the way of the toxine getting to the sensitive cells, but new difficulties arise if we have fresh unaltered toxine (such as is usually employed) coming into contact with sensitive cells. In order to stimulate the production of fresh side-chains the toxine must rob the cell of the normal function of those already formed. It can only do so by saturating the latter with its haptophorous affinity. If it does so then according to Ehrlich the toxophorous affinity can work its toxic action. Seeing that, in the injections of late immunisation, thousands of M.L.D.'s may be introduced, it is difficult to understand what becomes

of the many toxophorous affinities which must be fixed in the cells, and fixed in the cells in far greater number than when, say, only one M.L.D. is thus fixed in an unprepared animal. The sequence of events in the development of active immunity is thus far from clear. Such considerations as these just advanced suggest the possibility that the process of active immunisation may be different from the process in passive immunity. This idea had been mooted by Behring, who considers that the immunity of the cell is a thing by itself; for it he suggests the name isopathic immunity, while the other he would denominate antitoxine immunity.

There is another set of facts which must be taken into consideration in this connection. Sometimes in the course of an immunisation, when an animal has developed a serum of considerable antitoxic power, on a fresh injection of toxine being practised, acute symptoms of poisoning occur and death may supervene. This is usually referred to as oversensibility. No explanation of this accident has been offered. It would appear as if the immunity of the cell to the toxophorous groups was lost, and that the fixation of these by some such event as we have just spoken of led to the toxic action suddenly becoming effective.

To sum up our conclusions as regards the sufficiency of Ehrlich's theory to account for the development of immunity against the soluble poisons produced by bacteria, we would say that his views as to the chemical antagonism between toxine and antitoxine, as to the constitution of toxines, and as to the methods by which these produce disease effects, have very great support from the facts known. Further, the fixation of toxines in the cells of the body and the genesis of antitoxine from an over-production of some product of cellular activity, are very probable, but the theory does not give a complete account of what takes place in the course of the rise of active immunity. It however accounts completely for the events of passive immunity and for the therapeutic applications of antitoxic sera. It may be here said that what will be the event in a case of disease, such as diphtheria or tetanus, arising under natural conditions will probably entirely depend on the amount of a toxine which becomes absorbed, and this last may depend on the capacities of the body to kill the bacteria producing it, in fact on properties which play a leading part in the resistance of the body to the members of the second group of bacterial maladies. Apart from the therapeutic application of antitoxines it is questionable whether recovery from natural disease depends either on active immunity arising or on the development of antitoxine.

## B. THE NATURE OF THE CAPACITY OF KILLING BACTERIA AND ITS RELATION TO THE DEVELOPMENT OF IMMUNITY.

We now pass to the consideration of immunity from the second group of bacterial diseases,—that in which the actual bodily presence of the bacterial cell is necessary for the production of the characteristic pathogenic effects. It must be remembered that we merely take these as types of the general process of bacterial destruction because in them apparently the direct presence of the bacteria is more responsible for the pathogenic effects than the development of soluble toxines. Immunity occurs against such diseases, and as has been said its establishment appears to involve the killing of the bacterium. In the case of many animals a natural immunity exists against many such bacteria, though it can usually be overcome by increasing the virulence of the bacterium, as by passing the latter through the bodies of a series of animals, etc. Acquired immunity can also be developed, both active, by the injection of non-fatal or modified forms of the organism (and it may be said that a very frequent method of modifying the virulence is to kill the microbe by heat), and passive, by the injection of the serum of an animal actively immunised. The latter method is, however, limited in its applications. There are two chief theories that have been advanced to account for the development of this immunity. One, of which the great originator and upholder is Metchnikoff [36], attributes recovery from such diseases to the fact that when the bacteria gain an entrance to the body they attract the phagocytic cells of the latter and are englobed, killed and digested by them. In cases of great susceptibility to such microbic action, either the phagocytes are repelled, or the reaction takes place to an insufficient extent,—some bacteria, not being taken up, multiply and cause the death of the animal. Immunity consists in the conversion of a repellent action of the bacteria on the phagocytes into an attractive one, and the gradual strengthening of the latter so that the phagocytic action is able to meet large degrees of infection without the animal suffering. The development of sera, capable of transferring immunity to other animals, has always been a source of great controversy under such a conception, and has constituted the mainstay of a humoral theory, which had rather a nebulous character until Ehrlich extended the observations already described to antimicrobic immunity also. In looking at the question it will be convenient to take first of all certain facts relating to the sera of immune animals, to give Ehrlich's interpretation of these, and then to consider what

relation the phagocytic theory bears to the production of these sera, and, generally, to immunity from the diseases under consideration.

*Pfeiffer's reaction and the results of its study.* The starting point for all recent work on this "immunity from infection,"—as the action of bacteria, apart from the action of their poisons, is often called,—was the discovery of what is known as Pfeiffer's [37] reaction. A guinea-pig can be immunised against the vibrio of cholera by the intra-peritoneal injection of a small quantity of a culture of this microbe which has been killed by the vapour of chloroform, followed at intervals of a few days by injections of similar quantities of living cultures. If now some living vibrios be introduced into the peritoneal cavity and small amounts of the peritoneal fluid be withdrawn by means of capillary pipettes every few minutes, it can be found by microscopic observation that almost immediately after injection the naturally highly motile bacteria become motionless, and that, a little later, they lose their characteristic comma shape, swell up into round granules, and finally within twenty minutes break up and disappear. This is Pfeiffer's reaction, and it can also be observed *in vitro*, when the bacteria are mixed with the serum of an animal so immunised. This discovery gave rise to much controversy, and its essential significance in relation to immunity is still somewhat doubtful. Out of the controversies, however, there emerged several facts which have contributed to the progress of knowledge. It had been long known from the work of C. Fraenkel and Sobernheim [38] that the bactericidal action of the serum of a guinea-pig immunised against cholera was destroyed by heating for one hour at $70°$ C. Pfeiffer noticed that if the heated immune serum along with cholera vibrios were introduced into a guinea-pig's peritoneum the usual reaction took place, and from this he deduced that the immunising material was not altogether destroyed by heat but that in some way it affected the animal's organisation and helped it to dissolve the bacteria. Bordet [39], investigating Pfeiffer's reaction, found that the latter returned if to the heated immune serum a little of the serum of an unimmunised guinea-pig were added (such a serum as the latter, to which it will be necessary to make frequent reference, is usually called "fresh serum").

*Analogous investigations regarding haemolytic sera.* The real significance of these facts was not appreciated until three years later, when a new line of research was followed by Bordet [40] which has been of the greatest service in elucidating the whole subject of

immunity against infection. This consisted in the study of the fact first observed by Belfanti and Carbone that if the blood corpuscles of a rabbit be injected into a horse, after the fashion of an immunisation experiment, the serum of the latter develops poisonous properties towards rabbits, and these properties consist in the fact that on the serum being injected a dissolution of the red blood corpuscles of the rabbit takes place. Further, this phenomenon occurs when the serum is brought into contact with the corpuscles in a test-tube. The method of carrying out the latter experiment is to bleed a rabbit, whip the blood so as to defibrinate it, make up a 5 per cent. solution of the defibrinated blood in ·75 per cent. sodium chloride solution, and treat small quantities of this solution (which of course contains the red corpuscles) with the serum. The mixture is allowed to stand a few hours at 37° C. and the occurrence of the haemolysis is indicated by the whole fluid becoming stained by the dissolved-out haemoglobin. Bordet, applying the fact already observed by him regarding Pfeiffer's reaction, found that the haemolytic serum lost its properties by heating for half-an-hour at 55° C., but that, on adding to this heated serum some serum from an unimmunised guinea-pig, the haemolytic action was once more evident, though the fresh serum by itself had no haemolytic properties. On these facts and on others obtained by himself and Morgenroth, Ehrlich[41] based an extension of the antitoxine theory to account for the facts of immunity against infection. According to this view there are two bodies concerned in the process of haemolysis just described. One of these, that which is susceptible to heat being destroyed by half-an-hour's exposure at 55° C., is present in fresh guinea-pig serum. This he calls the "complement." The other is a body which is developed in the guinea-pig serum by the process of immunisation which the animal has undergone and withstands heating for half-an-hour at 75° C. without being entirely destroyed. This he calls the "immune body[1]." In the latter there are two haptophorous groups, one of which is satisfied by a receptor in the red blood cell analogous to the receptor which fixes such a body as tetanus toxine in the brain cells of a susceptible animal. The other

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[1] Considerable confusion arises through the variety of terms applied to the "complement" and "immune body." Complement is often called by Ehrlich "addiment" and by the French school, constantly, "alexine." By the latter the immune body is called "*la substance sensibilatrice.*" All through this paper we have used the terms complement and immune body. As we shall see substances analogous to the latter sometimes occur in ordinary sera. These Ehrlich calls "Zwischenkörper," which we have translated "go-betweens."

haptophorous group of the immune body is satisfied by being linked to a corresponding group in the complement. In the latter there is a group analogous to the toxophorous group of the tetanus toxine and this is the active haemolytic agent. The complement can thus only act when, through the intermediary of the immune body, it is anchored to the red blood cell. The experimental evidence on which this theory rests is as follows: If a goat be treated with repeated doses of sheep's blood there develops in its serum the capacity of dissolving sheep's red blood corpuscles (it may be here said that a very great number of similar haemolytic sera can be obtained by treating one species of animal with the blood of another species). Ehrlich took 4 c.c. of 5 per cent. defibrinated sheep's blood in ·75 per cent. salt solution, added 1 c.c. of immune goat's serum which had been heated half-an-hour at 55° C. (and which thus contained only immune body), and placed the mixture for 15 minutes at 40° C. The question of where the immune body was he now investigated in the following ingenious way. The mixture was centrifugalised till all the corpuscles were deposited at the bottom of the tube. The supernatant clear fluid was decanted and there was added to it ·2 c.c. of ordinary sheep's blood (containing, therefore, susceptible red corpuscles) and ·8 c.c. of " fresh " goat's serum (containing, therefore, goat's complement). The mixture was placed at 37° C. for two hours without any trace of haemolysis occurring. Now, if the immune body had been left in the fluid after centrifugalisation, the complement from the fresh goat's serum ought by it to have been linked to the sheep's corpuscles added, and haemolysis of the latter ought to have occurred. The immune body was therefore not here. The sheep's corpuscles of the original mixture were, of course, in the deposit separated by the centrifugalisation. This was now taken, stirred up with 4 c.c. of ·75 per cent. salt solution, and there was added ·8 c.c. of fresh goat's serum (containing, of course, complement). The mixture was placed at 37° C. for two hours and at the end of this time there was found to have occurred haemolysis of the corpuscles. During the 15 minutes that the original mixture was kept at 40° C., therefore, the immune body in the immune goat's serum had united itself to the sheep's red corpuscles, as was evidenced by the fact that when the latter were exposed to fresh complement haemolysis occurred. As remarked above, complement cannot cause haemolysis by itself. The method of the above experiment is that three factors are necessary to the occurrence of a given haemolysis,—the red blood cells to be acted on, a body resistant to heat occurring in

the serum of the immune animal (immune body), a body susceptible to heat occurring in the serum of an unimmunised animal (complement). When the presence of any one of these substances is suspected, it can be traced by adding the other two and observing whether haemolysis takes place. To proceed,—in the investigation, sheep's blood was next taken, fresh goat's serum was added, the mixture centrifugalised, and the fluid on the one hand and the deposit on the other investigated for complement. None was found in the deposit but it was found in the clear fluid, so that no combination had taken place between it and the blood corpuscles. Next it was found that there was a greater affinity between the immune body and the blood corpuscles than there was between it and the complement. The proof was as follows:—It was observed that in a mixture of 5 c.c. of 5 per cent. sheep's blood, 1 to 1·3 c.c. of heated immune goat's serum, and ·5 c.c. of fresh goat's serum, there was just enough of all the constituents to satisfy all the affinities and leave none over. If all these substances were mixed at 0° C., and the mixture centrifugalised as before, it was found that the complement present was still free in the supernatant fluid. Therefore the affinity of the immune body for the blood corpuscles was greater than its affinity for the complement. This last experiment also shows that at 0° C. the complement and immune body must have existed free, side by side.

*Application of these facts to the explanation of immunity against infection.* We must now look at the relation of these facts to a theory of immunity from bacterial infection. First of all here, with regard to the meaning of Pfeiffer's reaction, we have to observe that perhaps its most significant presentation lies in what happens when the heated serum of an immune animal is injected along with cholera vibrios into the peritoneum of an ordinary guinea-pig. If these vibrios were injected alone they would cause the death of the animal, but when they are accompanied by immune serum nothing happens except their own death and solution. Taking this along with the parallel experiment of Bordet conducted *in vitro* we would suspect that, in the intraperitoneal killing of the bacteria that occurs, two substances are at work, one developed in the body of the animal which has been immunised, and the other existing normally in the body of every guinea-pig, and that these by their union effect the death of the organisms. This is in fact Ehrlich's theory and the inference just drawn as to the mechanism of the process is borne out by all his experiments on haemolysis. In the latter, to which so much attention

has been paid because they are more convenient to perform and on the whole are more likely to give accurate results, we have only to read " solution and death of bacteria " for " solution of red blood corpuscles," and the altered conclusions, so far as we know, would be perfectly justified, for all the numerous researches on the subject lead to the opinion that the bodily capacities at work in haemolysis are identical with those concerned in bacteriolysis and bactericidal action. It must, however, be clearly understood that, while the processes are the same, it does not follow that the substances which cause haemolysis are identical with those which give rise to bacteriolysis. This is a point which will be considered later on. With regard to the details of the processes it will be convenient to look at present simply at what Ehrlich would say takes place when blood corpuscles are injected after the manner of an immunisation for the purpose of obtaining a haemolytic serum. The corpuscles first injected are foreign bodies. They have in them affinities which are satisfied by receptors in some cells of the body. The question of the cells in which these receptors occur and of the receptors being set free in the serum will be discussed later. These receptors are of a more complicated character than the receptors of which we have hitherto spoken, for they contain two unsatisfied affinities. One of these can be satisfied by the group in the blood corpuscle. The other is satisfied by a group belonging to a substance which exists in the serum of the animal and which is the complement already referred to. The latter thus becomes fixed to the blood corpuscle, and then through its free affinity,—that which would correspond to the toxophorous group of the diphtheria toxine,—it has a haemolytic action. The side-chains or receptors of the cell which act as a go-between between the corpuscles and the complement have a normal function in the body, and therefore the process of immunisation robs the cell of what it requires for its normal metabolism, just as in the case of the other diseases we have considered ; these receptors are replaced by the cell, and by and by, as in the previous case, are over-reproduced beyond the requirements of the body. They are then cast off into the blood stream and form the immune body present in the serum of the immunised animal. If we substitute the word bacterium for blood corpuscle in the above description we have the theory as it applies to bacterial immunity. A question which we shall at present leave over is how the theory applies to the case of natural immunity and to recovery from disease. We may say at once, however, that all are agreed that the two substances named are the essential

factors in the killing of bacteria within the body. The questions at issue are, Are they the only factors? In how far do they exist naturally in the body and where? Where, in any case are they formed? and, Where do the processes of solution take place?

Before we proceed to the consideration of the answers to these questions it is convenient to look at certain facts which have been observed relating to the capacity of the body to affect, and it may be dissolve foreign bodies. Though the fate of red blood cells when introduced into the body of another animal had been long known and in fact had been made one basis for the giving up of the transfusion of actual blood in surgical practice, it was not till after the work of Bordet and Ehrlich that much attention was paid to the occurrence. It was then suggested to various observers to enquire what was the fate of other kinds of cells when these were injected into the bodies of animals other than those from which they were derived. Such researches have been widely carried out under Metchnikoff's instigation. As loss of motility in bacteria, when these were injected into an animal, was a very prominent feature, it was natural to enquire what happens in the case of such motile cells as spermatozoa, and this has been investigated by Landsteiner[42], Moxter[43], Metchnikoff[44], and Metalnikoff[45]. The general result may be said to be that if emulsions of the testicle, or if spermatic fluid, be injected into the peritoneal cavity of such an animal as the guinea-pig after the manner of an immunisation, the serum of the animal develops the capacity of immobilising fresh spermatozoa. Metalnikoff has shown that this property is lost if the serum be heated, but such inactivated serum can be reactivated if the serum of a fresh unimmunised guinea-pig be added to it. Generally speaking, however, there is no solution of the actual protoplasm of the spermatozoon. Similar experiments have been conducted with leucocytes by Metchnikoff[46], Funk[47], and Besredka[48]. In these, emulsions of spleen, bone-marrow, and mesenteric glands have been injected into guinea-pigs and rabbits and sera have been obtained which have had the power of dissolving white blood corpuscles both *in vivo* and *in vitro*. When injected into the living animal these have the power of giving rise to very pronounced toxic symptoms, due to the great destruction of the cells which takes place, and which, in the case of the peritoneum, can be watched occurring by withdrawing small portions of the exudation by capillary tubes. Such sera are, however, according to Besredka, much more susceptible to heat than the haemolytic sera and entirely lose their toxic actions at 55° C.

According to this observer also, if small non-fatal doses be administered an increase of the number of leucocytes can be observed, and he thinks that a stimulation of the blood-forming mechanism occurs. It is said that a serum produced by the injection of spleen emulsion has a solvent action on the mononucleated and also on the poly-morpho-nucleate leucocytes, while a serum produced by the injection of the bone-marrow has a special action on the latter only. This would be largely explained by the modern view that the marrow is the chief site of the formation of these poly-morpho-nucleate cells; the material used in the immunisation would thus be specially rich in such cells. Von Dungern [49] has studied the effects of injecting into the peritoneal cavity of the guinea-pig ciliated epithelium derived from the trachea of the ox, and has found that the cells, while being preserved for days in this situation, gradually lose their motility. If now a second injection of the cells from the same source be practised, the latter lose their motility sooner, and this is due to the development of an immobilising serum, which also has a similar action *in vitro* though to a less extent. Again Delezenne [50] has investigated the effects of injecting into rabbits and dogs emulsions of liver cells and has found that in such animals there develops a serum which possesses a highly toxic action. When it was injected into the animals of the species from which it was derived it produced a condition allied to what occurs in phosphorus poisoning, *i.e.* an acute fatty degeneration of the hepatic cells. Similar poisonous sera have been obtained by the injection of kidney cells and cells from the central nervous system,—the sera in each case acting on the cells of the organs which stimulated their formation. Moxter, working with spermatozoa, and von Dungern in his experiments with tracheal epithelium both noticed the remarkable fact that the anti-sera obtained possessed haemolytic properties.

Such facts, taken along with what we have said regarding haemolysis and bacteriolysis, point towards the conclusion that in the latter phenomenon, with which the subject of immunity has to do, we are dealing with only one example of some great general process which may represent one aspect of normal metabolism. The feature of this process which first arrests attention is the extraordinary complexity of the substances which play a part in it. Evidently the breaking up of foreign cells in the body very usually is dependent on the development of two substances. In one group there is a capacity of resistance to moderate heat, in the other there is not, for at a temperature of 55° C. or thereabout they are destroyed. While these substances have such

features in common, the question arises whether every one is not specific in its action. With regard to the immune bodies all are agreed that this is the case. That which acts on cholera vibrios will not act on typhoid bacilli, and so on. From the standpoint of how these bodies according to Ehrlich's theory originate it must therefore be supposed that, corresponding to each, there was originally a side-chain in a cell capable of being saturated in a particular way and in no other, and from one such group of side-chains each immune body originates. But as we shall see presently the question may be even more complicated, for it is a question whether in many examples of one lysogenic effect several immune bodies may not be concerned. In addition to the complexity of the immune bodies there may also be a complexity of complements, though this is a matter under dispute.

With regard to this last question it may be remarked that while, where an animal is treated with the blood of another, there appears in its serum both immune body and complement adequate to dissolve the blood corpuscles of the species of animal whose blood was used in the immunisation, it does not follow that the immune body developed cannot link on to the susceptible blood cells a complement present in the serum of another species of animal. It is a very common experience when an immune serum has been inactivated by heat that it can be reactivated, not only by the addition of serum from an unimmunised animal of the same species, but by fresh serum derived from another species. This fact as we shall see may have an important bearing on the therapeutic uses of bactericidal sera. There is another point regarding immune sera which may be mentioned, namely, that in them the immune body and the complement are not formed in equivalent proportions. This is shown by the work of von Dungern[51]. In rabbits immunised with ox blood a certain quantity of the immune serum dissolved a certain amount of ox blood, but if to this quantity there was added fresh blood from an unimmunised rabbit, i.e. which contained only complement, 32 times the original amount of ox blood could be dissolved by the same amount of serum. There was thus present in the immune serum much more immune body than could be utilised by the animal on account of the fact that there was a deficiency of complement. This we shall see is a fact of very great importance. Following up the observation, von Dungern notes that, by taking advantage of it, the amount of complement and of immune body in a serum can be measured. For estimating the amount of complement he takes as a standard the amount of immune serum inactivated by heat which can,

when saturated with complement, dissolve the corpuscles in 8 c.c. of a 5 per cent. solution of ox blood in ·8 per cent. sodium chloride solution. Applying this method he states that nearly all rabbits have the same amount of complement, though, as Walker [56] has pointed out, von Dungern's figures showed that sometimes there might be twice as much as at others. The complement content of immune sera can be obtained by comparing the haemolytic action of the serum in the fresh condition with its action after being heated for 20 minutes at 56° C. Von Dungern states that in rabbits no difference in the amount of complement present in the serum could be detected during the 11 days succeeding the injection of blood. Apparently, however, only the effect of one immunising dose was studied. Thus, though von Dungern's results are not above criticism, there is no doubt that they open up the way to what, as we shall see, is a very important field of research in this subject. Of course for each serum studied in this way an artificial standard of reference would require to be set up.

(*To be continued.*)

NOTE. The bibliographical references will be given at the conclusion of the second article.