IMMUNISATION AGAINST IMMUNE SERUM.

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IN endeavouring to determine the nature of immunity, and the precise elements and factors in the process of immunisation, careful investigation of the substance known as immune body is of the first importance. It appeared to the writer that valuable results might be obtained from experiments directed to the production of immunity against an immune serum. In the following observations the experiments were made with antityphoid serum of the horse, and a variety of the *Bacillus typhosus*, the tests being carried out on guinea-pigs. The results obtained fall into two divisions, namely—(1) The change induced in the bacilli by their growth in immune serum, and incidentally in the immune serum also in which they were grown; and (2) the change in the susceptibility of guinea-pigs to the specific infection consequent on their treatment previously with a succession of increasing doses of that serum. Two series of observations are included, the first of which was made in the Bacteriological Institute in Berne, the second in the Pathological Laboratory of the University of Oxford. The immune serum used was the antityphoid serum of Tavel, and the variety of the *B. typhosus*, one for which this serum was both special (1) and specific.

I. GROWTH OF BACTERIA IN IMMUNE SERUM.

1. *The Bacteria.*

In his original experiments on the growth of bacteria (anthrax) in their immune sera, Metchnikoff (2) found that the virulence appeared to be remarkably diminished, and the observation was confirmed by the results of Charrin (3) and Roger (4) with the *B. pyocyaneus*, the pneumonococcus, and the streptococcus. This failed entirely to accord with the original discovery of Pasteur, that the bacteria undergo increase of virulence by "passage," and led to reinvestigation of the subject. It was now shown that the apparent attenuation previously observed was due entirely to the protective action in the animal of the immune serum culture medium injected with the

bacteria, which themselves exhibited no diminution in virulence when isolated from the protective serum—Metchnikoff (5), Sanarelli (6), Issaeff (7).

The investigation might with advantage have been carried further, for bacteria, like other cells, are capable of being immunised to a remarkable degree—Kossiakoff (8), Nuttall (9), Haffkine (10), Trambusti (11). And by the theory of Ehrlich (12) the production by any organism of an antibody to a given body depends entirely on the possession by its cells of suitable side-chains—receptors—for the body in question. Thus the immune body for a given bacterium is produced under the action of certain atom complexes of the bacterial substance, which, being taken up by cell receptors in the animal, stimulate multiplication and the eventual separation of those cell receptors into the plasma as the immune body. But if the stimulating atom complex of the bacteria be called the I-group, then the I-group is by hypothesis attractive to the haptophore of those cell receptors of which the immune body consists. It follows, therefore, if the Ehrlich doctrine be correct, that on the cultivation of bacteria in their immune serum their I-groups will take up the immune body, for which they offer suitable receptors, and will be thereby stimulated to multiply, with the result of their eventual liberation in the serum as an anti-immune body. Similarly, if the serum be an agglutinative serum, the bacterium should form anti-agglutinins, and should itself become less easily agglutinable, and so on.

Hence immune serum in which bacteria are grown should become continuously less rich in immune body and in free agglutinins; this the experiments recorded later show to be the case. As regards the bacteria themselves, the I-groups of these organisms should become continuously and lastingly increased. Indeed, some such formation of an anti-immune body, or multiplication of bacterial I-groups, alone affords an explanation of the means by which a bacterium is at times enabled to survive within the body of its phagocytic host, and of the fact that the bacteria thus living on within the phagocyte are found to have an undiminished virulence—Metchnikoff (13), Sawtschenko (14), Sanarelli (15), Cantacazène (16). For without such formation of new I-groups, to resist the leucocytic cell receptors,[1] the bacterial groups would become saturated by the receptors of the phagocyte, and addiment would then at once initiate bacteriolysis.

Let us consider what this stimulation of the I-groups must imply, speaking, for the sake of clearness, of the corresponding cell receptor (immune body) as the I-receptor. Then, on the continuous cultivation of bacteria in their immune serum, their I-groups will be continually saturated by the I-receptors present in the serum, and the bacteria will acquire facility in the continuous new formation of these atom

[1] The immune body is a leucocytic product, cf. "On the Protective Substances of Immune Sera: Preliminary Communication," *Lancet*, London.

complexes. And since they multiply by fission, not by procreation, this acquired character will certainly be handed on and gradually increased in each new generation. But this must mean a heightening of the virulence. For if we leave aside the question of the action of bacterial toxins, which is more correctly named toxicity, the virulence of a bacterium depends upon its power of living and multiplying in the body of the animal invaded. This in turn depends on its capacity for resisting lysogenesis; and this again upon ability to neutralise the I-receptors of the phagocytes or the immune body (free I-receptors) that may be present in the plasma. But this concerns solely those atom complexes of the bacterial protoplasm which we have spoken of as I-groups. Hence, increase in the I-groups, and acquired facility in their new formation, must imply an increase in the number of I-receptors or molecules of immune body required to render possible the action of the addiment and the initiation and successful issue of bacteriolysis; that is to say, an increase in the virulence of the bacterium. Accordingly, the proof that virulence is not diminished when a bacterium grows in immune serum, fell far within the probable expectation, it being *a priori* likely to increase.

We now proceed to a description of the observations.

SERIES I.—A variety of the *B. typhosus*, which will be known as *A*, was cultivated in the antityphoid serum, diluted with diminishing amounts of normal culture bouillon. It was transferred each day to a fresh tube of dilute serum in successive subculture, and was grown in this way in dilutions 1 to 8, 1 to 4, 1 to 3, 1 to 2, 1 to 1, 2 to 1, and 3 to 1 of immune serum bouillon, four tubes of each dilution being used. From the last tube of 3 to 1 dilution, the bacillus which we now call *B* was subcultured on agar, and only after several transferences to fresh agar was employed in the inoculations. The bacillus grew extremely well after the first two tubes of dilute serum, and underwent the following culture modifications strikingly evident to the naked eye. On its first introduction into 1 to 8 dilution, the growth, though fairly copious at the end of twenty-four hours, was completely precipitated to the foot of the culture tube, or sedimented, the fluid remaining clear; and this occurred at first in every case of transference to a stronger serum bouillon. But during successive culture in the tubes of any given dilution, and continuously through the diminishing dilutions, the sedimenting action of the serum—that is, the agglutinability of the bacillus—became less and less marked, until eventually the growth, having become first flocculent in the clear fluid, then flocculent with some general turbidity, acquired the character of a uniform turbidity without apparent clumping at the end of twenty-four hours, though always with considerable sediment at the foot. Further, in one and the same tube, observed from day to day, the completeness of the sedimentation gradually diminished. The changes witnessed were recorded in daily notes, of which the above supplies a general outline of the gradual modification which occurred. This modification could be demonstrated in a most striking manner by taking two fresh tubes of dilute serum and inoculating one with *A* and one with *B*. At the end of twenty-four hours' growth in the hot chamber, the former was completely sedimented, while the latter showed a uniform turbidity. Bacillus *B*, which was thus modified from *A*, and had become much less agglutinable, was then compared with *A* in animal experiments. Here and throughout the inoculated doses are given in terms of a

forty-eight-hour culture, on an agar surface of approximately constant area, and all doses, both of bacteria and immune serum, are stated at the amount per 100 grms. of guinea-pig.

Determination of the Minimum Lethal Dose.

For *A*.			For *B*.		
Guinea-pig.	Dose.	Result.[1]	Guinea-pig.	Dose.	Result.[1]
1	0·213	Dead in 18 hours.	1	0·08	Dead in 24 hours.
2	0·114	,, 18 ,,	2	0·075	,, 18 ,,
3	0·125	,, 18 ,,	3	0·07	,, 22 ,,
4	0·115	,, 18 ,,	4	0·07	,, 18 ,,
5	0·1	,, 18 ,,	5	0·06	,, 30–36 ,,
6	0·096	,, 20–22 ,,	6	0·06	,, 30–36 ,,
7	0·085	,, 20 ,,	7	0·05	Recovered.
8	0·085	,, 20 ,,	8	0·05	,,
9	0·085	,, 20 ,,	9	0·05	,,
10	0·08	,, 24–36 ,,			
11	0·075	Recovered.			
12	0·075	,,			
13	0·075	,,			
14	0·065	,,			
A. M.L.D. fatal within 24 hours, 0·085. Dose not fatal, 0·075.			*B*. M.L.D. fatal within 24 hours, 0·07. Dose not fatal, 0·05.		

A few observations had also been made with agar cultures of the bacillus at the end of its period of culture in the dilutions 1 to 2, as follows:—

Guinea-pig.	Dose.	Result.
1	0·08	Dead in 24 hours
2	0·075	,, 30–40 ,,
3	0·07	,, 44–48 ,,

The virulence, therefore, was apparently increased by growth in immune serum, and the increase was gradual.

[1] Where only one hour is mentioned, the animal was already dead when first observed that number of hours after inoculation; where limits are given, the animal died between the hours stated.

Serum Protection.

Against *A*.		
Guinea-pig.	Dose.	Result.
1	0·12 and serum 0·09 c.c.	Recovered.
2	0·12 ,, 0·08 ,,	,,
3	0·12 ,, 0·07 ,,	,,
4	0·12 ,, 0·06 ,,	Dead in 24 hours.
Protective serum for 0·12 of *A*, 0·07 c.c.		
Against *B*.		
1	0·12 and serum 0·07 c.c.	Dead in 30–36 hours.
2	0·12 ,, 0·08 ,,	,, 24–28 ,,
3	0·12 ,, 0·09 ,,	,, 30 ,,
4	0·12 ,, 0·1 ,,	Recovered.
Protective serum for 0·12 of *B*, 0·1 c.c.		

The result of growing the bacillus in its immune serum was therefore a diminution of its agglutinability, a heightening of its virulence, and an increase in its resistance to serum protection.

Series II.—After an interval of about ten days, bacillus *B* was again inoculated in successive agar cultures. From these it was transferred to undiluted immune serum, in successive tubes, of which it was allowed to grow for some five weeks (thirty-four days), being, however, transferred to agar for a day or two, on six occasions before cultivation further in the serum. This procedure was adopted after the third, sixth, eighth, thirteenth, twentieth, and thirtieth days of growth in serum tubes; the whole succession of cultures having a duration of some six or seven weeks, in the course of which, as stated, the bacillus passed through thirty-four subcultures in the serum. From the last serum tube the culture was continued upon agar, and the bacillus now denominated *C*.

Just as in Series I., the first effect of culture in the serum (now undiluted) was the appearance of complete sedimentation at the end of twenty-four hours' growth. But with successive subcultures this became less and less complete, till, after some half-dozen transferences, the culture tubes presented growth of uniform turbidity, in striking contrast with the complete precipitation seen if a control was made by inoculating the original *B* in a fresh serum tube.

The virulence of *B* and *C* were now determined. That of *B* was found to be unchanged from the determination made in Berne.

Determination of the Minimum Lethal Dose.

For *B.*			For *C.*		
Guinea-pig.	Dose.	Result.	Guinea-pig.	Dose.	Result.
1	0·07	Dead in 24 hrs.	11	0·06	Dead in 24 hours.
2	0·07	,, ,,	12	0·05	,, ,,
3	0·06	Dead in 28–44 hrs.	13	0·05	,, ,,
4	0·06	,, 30–40 ,,	14	0·05	,, ,,
5	0·055	,, 48–72 ,,	15	0·0475	,, in 48–72 hrs.
6	0·055	(Very ill) recovered.	16	0·045	Recovered.
7	0·0525	(Ill) recovered.	17	0·745	,,
8	0·05	,,			
9	0·05	,,			
10	0·05	,,			
B. M.L.D. fatal within 24 hrs, 0·07. Dose not fatal . . . . 0·05.			*C.* M.L.D. fatal within 24 hrs., 0·05. Dose not fatal . . . . 0·045.		

Determinations with the bacillus, after thirteen and twenty days of growth in immune serum, gave respectively, with the former, guinea-pig 18, dose 0·06; result, died in twenty-four to thirty hours; and with the latter, guinea-pig 19, dose 0·06; result, died within twenty-four hours, showing a gradual increase in the virulence, as in Series I. The following were the results obtained with immune serum in protection against the bacilli *B* and *C*.

Serum Protection.

Against *B.*		
Guinea-pig.	Dose.	Result.
20	0·07 (1 M.L.D.), and serum, 0·03 c.c.	Recovered.
21	0·07 . . . . ,, 0·025 ,,	Dead in 24 hrs.
22	0·14 (2 M.L.D.) ,, 0·135 ,,	Recovered.
23	0·14 . . . . ,, 0·1 ,,	Dead in 24 hrs.
24	0·21 (3 M.L.D.) ,, 0·24 ,,	Recovered.
25	0·28 (4 M.L.D.) ,, 0·345 ,,	Dead in 24–48 hrs.
26	0·28 . . . . ,, 0·375 ,,	Recovered.
Against *C.*		
27	0·07, and serum, 0·03 c.c.	Dead in 24 hrs.
28	0·07 ,, 0·04 ,,	Recovered.
29	0·14 ,, 0·135 ,,	Dead in 24 hrs.
30	0·14 ,, 0·16 ,,	Recovered.
31	0·21 ,, 0·24 ,,	Dead in 24 hrs.
32	0·21 ,, 0·28 ,,	Recovered.
33	0·28 ,, 0·35 ,,	Dead in 24 ,,
34	0·28 ,, 0·375 ,,	,, 24–48 hrs.
35	0·28 ,, 0·4 ,,	Recovered.

Thus the protective dose of serum for 1 M.L.D. of *B* is 0·03 c.c. of serum, while for an equal dose of *C* 0·04 c.c. is necessary. And giving 2, 3,

and 4 M.L.D. of *B* and serum, according to the theoretical requirement[1] for protection, the animals are protected against 2 M.L.D. and 3 M.L.D.; while with equal doses of *C* and the same amounts of serum they have died. Also at 4 M.L.D. of *B* the animal is protected by a dose of serum which does not protect against an equal inoculation of bacillus *C*.

Hence, as in Series I., the bacillus has again, by growth in immune serum, become less easily agglutinable, and has become more virulent and increasedly resistant to protection by the immune serum. And the total result of the two series of experiments is that the M.L.D. of the bacillus has been diminished from its original amount of 0·085 to one of 0·05, a diminution of some 40 per cent.; that is, the virulence has been increased by this amount.

This increase may, perhaps, appear not very striking nor convincing in its magnitude, but it is at any rate suggestive and quite definite. It is, indeed, considerably greater than the differences which I have found existing between a series of varieties of the *B. typhosus* ([17]), which corresponded to large differences in their agglutinative reactions; yet it is out of all comparison with the increase of virulence obtainable by "passage," and this discrepancy demands an explanation. First, we must clearly bear in mind that, in the heightened virulence produced by passage we are dealing with an excellent example of the production of a variation, by the survival of the fittest only in the struggle for existence in the invaded organism, since the bacteria which propagate and live and are removed for further passage are evidently those which, by possession of excess of I-groups and facility in their formation, have survived where weaklings have been rapidly exterminated. But in the case of growth in immune serum there is no selective influence in action, since there is no bactericidal activity engaged against the growth of the bacteria concerned, addiment being absent ([18]); and in the result we have only the average of the educative process as applied to the sum total of the bacteria produced in culture. This in itself is probably sufficient to explain the difference in the results obtained.

But, secondly, we have here determined virulence upon the guinea-pig, while the bacteria have been immunised against the immune serum of the horse; and it is possible that the virulence for the latter animal may have been increased to a much higher degree than is apparent from the tests with guinea-pigs. Accordingly, it is desirable to carry the investigation further by the preparation of an immune serum in which to grow the bacteria from animals of a species which can be conveniently used in subsequent examinations of

[1] *Lancet, loc. cit.* If d be a fatal dose of a bacillus, c the largest dose not fatal, and s the amount of immune serum required for protection against d, then the amount of immune body necessary for a dose of nd is contained in a volume of the immune serum given by the formula—

$$\frac{nd-c}{d-c}=s$$

bacterial virulence; for it may ultimately prove to be the case that with the same bacterium, but in different animals, the immune body presents important differences, or appears in forms allied but not identical; so that immunisation to a high degree against a special immune body may not confer a high and special immunity against another but only one of low non-special character. That is to say, that immune body is special to the species.

However this may be, it is apparent that the virulence of a bacterium is increased by immunising it against its immune serum; that is, by causing it to multiply its I-groups; and the relative virulence of the bacterium is dependent on the number and the facility in the new formation of those I-groups. From this it follows that, in regard to living bacteria, viewed apart from any toxic action, natural immunity implies the possession by the leucocytes of the animal in question of sufficient I-receptors to completely fix or neutralise the I-groups, and provide the necessary condition for addimentary activity. And similarly the acquirement of immunity, whether of active or of only passive character, implies the acquirement, either by natural formation under the action of the stimulus employed, or artificially by the injection of an immune serum, of a suitable supply of the I-receptors previously present in an insufficient quantity.

There is another factor or phenomenon of importance in immunity to which we must refer, namely, the events observed in chemiotaxis. And it has been most definitely established that the possession or acquirement of immunity is invariably associated with the exhibition of a positive chemiotactic influence by the bacterium in question on the leucocytes, while negative chemiotaxis is exerted in the unimmune. Moreover, the change from a susceptible to an immune condition is accompanied by a change of chemiotaxis from negative to positive. But we have seen that the possession or acquirement of immunity depends entirely on the possession or acquirement of a sufficiency of I-receptors. And the change occurring in the production of acquired immunity is an active change, consisting in the multiplication of receptors by the leucocytes. But from the complete analogy throughout the animal series from amœba and plasmodia to man, we know that the reversal of the chemiotaxis in the acquirement of immunity or of tolerance to previously harmful substances must also be an active change, resulting from an alteration in the organism itself. The altered chemiotaxis on the leucocytes is therefore due to active changes in the leucocytes themselves. Hence there is evidently a close relation between production of the immune body and the exhibition of a positive chemiotaxis.

Now the basis of the chemiotactic influence is the same, whether the expression of its action be attraction or repulsion. The latter is a question merely of degree, since the same substance which repels in strong solution acts in the reverse direction, and becomes attractive

when diluted. And the phenomena observed depend on an affinity of the body attracted for the attracting substance, such as implies a power of entering into some combination with the attracting molecules. The phase is found to vary also, in agreement with the laws which regulate diffusion, according to the distance of the cell in question from the chemiotactic centre. In what consists the chemical basis of these physical events? What are the molecules of the bacterial protoplasm concerned, and what the leucocytic molecules affected? We have seen that the I-groups of the bacterium have a strong affinity for I-receptors of the leucocyte, and that the specific process of immunisation consists in the multiplying of these latter groups, and thus increasing the activity of the leucocyte against the corresponding micro-organism. It may be, therefore, that on these particular groups of the bacterium and the phagocyte, respectively, also depend the phases and phenomena of chemiotaxis.

That this is actually the case will follow from the following considerations. In the production of a passive immunity, the injection of a quantity of immune serum enables the phagocytes to exhibit the events of positive chemiotaxis. But positive chemiotaxis is induced in the acquirement of active immunity by a chemical modification in the phagocyte. And the only phagocytic product in the immune serum special to immunity is the immune body which evidence has shown to be produced by phagocytes—for addiment is absent from stored serum ([17]). Therefore the change from negative to positive chemiotaxis in immunisation is determined by the increase of the leucocytic I-receptors, under the stimulating influence of bacterial I-groups. For, suppose some other group of the bacterium, which we will call the K-group, and some other receptor of the leucocyte, the K-receptor, were concerned in chemiotaxis, and not the I-group and the I-receptor, then the production of the immune body would be dependent only on the number of I-groups taken up by leucocytes, and the development of positive chemiotaxis only on the number of K-groups similarly taken up. This would depend, again, not only on the number of the I- and K-groups present respectively in the bacteria, but also on the number of I- and K-receptors in the leucocytes. Accordingly, in different animals, and with different micro-organisms, the production of the immune body and the development of positive chemiotaxis would not vary jointly and together, but independently and along divergent lines. There would, in consequence, appear in different animals, and with different bacteria, all possible relations between the degree and phase of chemiotaxis and the degree of immunity possessed, from universal phagocytosis (strong positive chemiotaxis) with a low immunity to complete immunity, with absence of phagocytosis and a negative chemiotaxis. But this is not the case, for, as the school of Metchnikoff has shown, phagocytosis and immunity vary conjointly, and the higher the immunity obtained the more marked and active is

the phagocytic process. The groups concerned in chemiotactic influence, therefore, are not other than the groups affecting the production of the immune body. That is, they are the I-groups and the I-receptors.

The relation may be roughly pictured in the following manner, by imagining a bacterium and a leucocyte in contact, to avoid discussion of the physical effect of distance on the chemiotactic influence. Suppose the bacterium to have five I-groups, but the leucocyte only four I-receptors. The latter can only take up four I-groups, and the remaining unfixed I-group causes negative chemiotaxis. If, now, the leucocyte, under the stimulation of the I-groups taken up, produce another and another I-receptor, the chemiotaxis passing through zero will become positive on the appearance of the sixth receptor. The leucocyte is also then, by the possession of sufficient I-receptors, able to bring its addiment to work on the bacterium, which, under positive chemiotactic influence, it has now ingested.

This conception also enables us to understand why, as was pointed out by Wassermann ([18]) especially, while immunisation with the living bacteria only protects against bacteria and not against their toxins, immunisation with bacterial products will protect both against these and against the bacteria themselves. For the substance of the bacteria concerned in chemiotaxis, being of necessity diffusible and diffused, is present also in their culture media, and will be included in an injection of bacterial products. But this substance is the I-group, which gives rise to the formation of the immune body. Hence, on injecting the bacterial products, we inject among the rest a sample of that substance which gives rise to immune body, and the result will be the acquisition of immunity, not only against products but against bacteria also. But immunisation with the living microbes only results in the habituation of the animal to procure the rapid and complete destruction of the invading agents, which are, therefore, not permitted time for the formation and excretion in the living body of their toxic products in amounts sufficient to provoke development of corresponding antitoxic substances.

And as regards passive immunity, we have the following position:— On the injection into an infected animal of an immune serum, the immune body introduced will tend, from its affinity for the I-groups, to rapidly become attached to the bacteria. So soon, then, as it has in this way fixed sufficient of the I-groups of the micro-organisms to bring their numbers into due relation to the pre-existing I-receptors of the leucocytes, chemiotaxis becomes positive and phagocytosis and bacteriolysis are brought about.

If, then, it be admitted, as we have urged elsewhere ([19]), that both addiment and immune body are exclusively produced by the protective cells—the phagocytes—it follows that immunity to living bacteria is a condition effected solely by the leucocytic tissues. And we are now in

a position to maintain that just as in the production of toxin immunity there is concerned a stimulating action of the toxic substance on certain body cells (for example, of the central nervous system for tetanus) for which it has a special affinity, and by its influence on which it produces its primary and specific pathological effects; and just as in reply to this stimulation an antitoxin is produced, which fixes the toxin and thus shields the susceptible cell from its injurious influence, by a reaction which has its basis in the normal metabolic functions of those cells (Ehrlich)—so also in the development of antibacterial immunity there is concerned a stimulating action of the chemiotactic substance on certain body cells (the phagocytic system) for which it has a special affinity, and by its influence on which it produces its primary and specific pathological effects (negative chemiotaxis and destruction of phagocytes), and in reply to this stimulation an anti-substance is produced by the protective cell (the phagocyte)—the immune body—which enables the phagocytic addiment to destroy the bacteria, and thus protects the susceptible cell from their injurious influence, by a reaction which has its basis in the normal metabolic functions of those cells. That is to say, that the two processes are exactly parallel, with the addition in the latter of the ingestion and digestion of the little mass of protoplasm, the bacterium concerned. Where the animal is highly susceptible and the phagocytic process in abeyance, chemiotaxis is entirely negative, and the destruction of leucocytes, due to the infection, sets free both I-receptors and the addiment in the plasma, which then, by extracellular bacteriolysis, initiate and aid the production of immunity.

2. *The Immune Serum.*

The immune serum in which bacteria had grown was next examined.

A quantity of this had been inoculated in a flask (flask 1) with the bacillus, after it had grown for thirteen days in a succession of serum cultures, as described above in Series II., and had been grown continuously for some five weeks. As a control, another flask (flask 2), with similar serum not inoculated, was submitted to the same conditions as regards temperature, access of air, and so on. After the expiration of the period stated, the contents of flask 1 were filtered through a Chamberland bougie,[1] to free them from bacteria, and were then tested for agglutinative and protective power against the serum of the second flask. Since even old cultures of the *B. typhosus* have no toxic action ([20]), there was no question of the culture serum being toxic and thus giving unreliable results. The observations were as follows:—

Agglutinative Action.

FLASK 1.—Diluted 1 to 2: a sample inoculated with bacillus *B* gave growth with uniform turbidity.

[1] The difficulty of filtering the serum was considerable. Dilution 1 to 2 with normal bouillon made the process practicable. The evaporation in the filter vacuum was estimated and allowed for. It amounted to between $\frac{1}{10}$ and $\frac{1}{17}$ of the total volume.

FLASK 2.—Diluted 1 to 2 : a sample inoculated with bacillus *B* gave growth with complete sedimentation.

Hence the serum in flask 1 had been considerably deteriorated in agglutinative power. This observation has an important bearing on the still debated question of the formation of agglutinins. If these were formed, whether within the body or elsewhere, by the bacteria themselves, as has been stated by Malvoz, they should have been increased, and could not possibly have been diminished in the above experiment. From the result obtained, it follows definitely that the agglutinins must be true antibodies, formed like other antibodies in and by the animal, and probably, as Deutsch has shown, within the lungs.

Protective power.—To examine any alteration in protective power which had occurred, the following experiments were conducted:—

Guinea-pig.	Dose.	Result.
36	0·07 of *B* and 0·03 c.c. serum of flask 2.	Recovered.
37	0·07 of *B* and 0·03 c.c. ,, ,,	,,

Therefore the serum of flask 2 has not suffered appreciable alteration from exposure to the air in the plugged flask. Its power is altogether comparable for protection with that of the serum used in the experiments already quoted. But with the serum of flask 1, in which the *B. typhosus* had been grown, the case is different: thus—

Guinea-pig.	Dose.	Result.
38	0·07 of *B* and 0·03 c.c. of serum of flask 1.	Dead in 24 hours.
39	0·07 of *B* and 0·035 ,, ,, ,,	Died in 30–40 hours.
40	0·14 of *B* and 0·135 ,, ,, ,,	,, within 24 ,,
41	0·21 of *B* and 0·28 ,, ,, ,,	,, ,, 24 ,,

And by comparing these results with those recorded for the guinea-pigs 20, 23, and 24 (see Table, p. 39), we see that immune serum in which *B. typhosus* has been grown has been diminished in protecting power against that organism. That is to say, that it has lost some portion of its immune body content.

II. IMMUNISATION OF GUINEA-PIGS AGAINST IMMUNE SERUM.

In their experiments upon the hæmolysins, Ehrlich and Morgenroth ([21]) have demonstrated that it is possible by injecting lysins to

induce the formation of antilysins which are capable of counteracting the effect of hæmolysins, or of neutralising them. Kossel and Camus and Gley had also obtained an antilysin to the powerful lysin of eel serum. Now, following a line of argument exactly similar to that pursued by Ehrlich [22] in his discussion of the facts and possibilities with regard to the formation of *isolysins*, *autolysins*, *anti-isolysins*, and anti-autolysins, it appears that if an animal be treated by successive injections of specific immune serum, several possibilities arise. Thus, if the immune serum of horses for the *B. typhosus* be injected into guinea-pigs, there are the following possibilities:—

1. The guinea-pig may contain no cell receptor to which the I-receptors (immune body) can attach themselves. In this case, no antibody can be formed, and the I-receptors will be gradually eliminated in the secretions.

2. There may be present suitable receptors for these I-receptors; let us call them X-receptors. Then an antibody to the I-receptor will be formed by the splitting off of X-receptors from the cells in which they become multiplied. Such an antibody may, however, possess one of two relations; it may be related to the same haptophore of the I-receptor as attaches the I-group, or may take its hold upon some other haptophore of this receptor, which we know possesses, at least, two such links — bacteriophil and addimentophil — and may quite well have others. In the former case this antibody will, on meeting with an I-receptor, fix it by that haptophore which may be called bacteriophil, *i.e.* that haptophore which normally attaches the I-group of the bacterium. Accordingly, this anti-immune body will tend to hinder the protective action of an injection of the immune serum, if the animal be now infected with a dose of the bacteria in question. But if the X-receptors anchor the I-receptors by some other haptophore than the bacteriophil, then their existence in the plasma of the animal can have no hindering action on protection by the latter against bacterial infection, even though they enter into combination with them.

Further, the X-receptor may be of the kind which Ehrlich has named *singular* [22], or it may be what we shall call *reciprocal*. In the latter case the foreign group (the I-receptor), which becomes anchored by the X-receptor, is present also in the body of the animal used for experiment, *i.e.* in one and the same organism groups are present which are by nature dependent on each other. A good example of this is seen in the coexistence in the same organism of the lab and anti-lab groups, which occurs, as shown by Morgenroth [23]. And Ehrlich [22] thinks that such a simultaneous occurrence of corresponding groups is very frequent in the economy of the organism, and that it more especially presents itself in cases where a certain group of cells depend for nourishment upon the products of another kind of cell. Singular receptors, on the other hand, are those designed to

extract exogenous molecules of nutritive material from the plasma. If singular receptors are present, as appears to be the case where ordinary toxins are concerned, an antibody may be formed in extraordinary amounts; but if the reciprocal groups are also present in the animal, then a regulative new production of these groups occurs, under the influence of the excess formation of its opposite, and the amount of the free antibody present in the plasma is thus limited. Thus are explained the facts that anti-lab formation cannot be raised to any great amount, and that in normal animals we find at one time free lab in the urinary secretion, at another free anti-lab existing in its serum.

Now the X-receptor of the guinea-pig is the receptor which takes up the immune body of the horses' immune serum; and hence it follows that if the X-receptor is reciprocal, then the immune body of the horse and of the guinea-pig are identical, that of the latter being the opposite of the X-receptor. But if the X-receptor is of the singular variety, and has no opposite in the body of the guinea-pig, the immune body of this animal must be different from that formed by horses. For the immune body of the guinea-pig is formed from groups which certainly are pre-existing in its leucocytes, and must be different from the I-receptors of the horse if the X-receptors of the guinea-pig are singular. And this would mean that immune body is special to the species.

If this be so, it follows that on immunising guinea-pigs to horses' immune serum (and on the supposition that the X-receptor anchors the I-receptor by its bacteriophil haptophore), the antibody formed can be produced in great profusion (singular receptor), and will tend to neutralise a quantity of immune body, subsequently injected for protection. That is to say, it will diminish the protective action of a given volume of the immune serum, if the animal be now submitted to infection by the given bacillus. But it will not affect the M.L.D. of that bacillus for the animal in question, the X-receptor being singular and *not* the opposite of the animal's own immune body receptors. But, on the other hand, if the immune bodies of the horse and guinea-pig be identical, and the X-receptor therefore of the reciprocal variety, the formation of the anti-immune body (free X-receptors) will be limited, as in the case of anti-lab; but, in so far as it is formed, it will diminish both serum protection of the animal by the horses' serum, and the M.L.D. of the bacillus. We have, then, here, three possibilities under consideration, which may be tabulated thus:—

Case.	Effect of Immunising Guinea-pigs against the Immune Serum of the Horse.
1. X-receptors totally absent from the guinea-pig.	1. No antibody formed. Susceptibility of guinea-pigs to the given bacillus unaltered.
2. X-receptor present, but anchors I-receptor by other than its bacteriophil haptophore.	2. An antibody formed, which does not alter guinea-pigs' susceptibility to the infection.
3. X-receptor present and anchors I-receptor by its bacteriophil haptophore.	3.
(*a*) X-receptor singular.	(*a*) Anti-horse-immune body formed, serum protection diminished, but M.L.D. of the bacillus unaltered.
(*b*) X-receptor reciprocal.	(*b*) Anti-immune body formed, serum protection diminished, and the M.L.D. also diminished.

We may now consider the experiments performed, in the light of the above discussion.

SERIES I. (Berne).—Five guinea-pigs received inoculations of 1, 2, 3, 4, and 5 c.c. of horses' immune serum, subcutaneously, upon alternate days, and at the expiration of a week from the last serum injection were examined as regards serum protection against M.L.D. of the bacillus *A*.

In the result they were found to be protected by a *less* amount of immune serum than required for normal animals. This was explainable only on the supposition that sufficient time was not allowed for the removal of the immune serum injected, in the six inoculations, by a delay of only a week before examination.

SERIES II.—A further series of eight animals was therefore taken in Oxford. These received inoculations of 1, 2, 4, and 5 c.c. of immune serum on alternate days, and were kept under observation for a longer period subsequently before being tested. The results of their examination are given below:—

Guinea-pig.	Dose.	Results.
Dose of *B*.		
42	0·0525 ...	Recovered.
43	0·055 ...	,,
44	0·055 ...	Died in 50 to 60 hours.
45	0·07 ...	,, within 24 ,,
46	0·21 and serum 0·24 c.c.	,, in 24 to 30 ,,
47	0·21 ,, 0·32 ,,	Recovered.
Dose of *C*.		
48	0·14 and serum 0·18 c.c.	Died within 24 hours.
49	0·21 ,, 0·32 ,,	,, ,, 24 ,,

From those results, comparing guinea-pigs 42 to 45 with guinea-pigs 1 to 7, it follows that the M.L.D. remains unchanged from that for normal animals. But on comparing 46 to 47 with animal 24, and 48 to 49 with 30 to 32 above recorded, it is evident that the protective power of immune serum for those guinea-pigs is less than that obtained in normal animals. Here, then, we have an instance of the case 3 (*a*) above. An antibody has been formed to the immune body of the horses' serum, which is not an antibody to the immune body of the guinea-pig. That is to say, the X-receptors here are singular, and the immune body of the guinea-pig for *B. typhosus* differs from the immune body of the horse for the same micro-organism.

It follows that the injection into animals of immune serum, while not increasing their susceptibility to infection, as has been suggested, does diminish the protective value of the serum for those animals, if they acquire the infection later, and hence it is a matter of importance in the use of antisera in disease in man, not to give greater doses than are necessary to ensure recovery; while the injection of an antiserum, as a measure of preventive medicine, is unscientific and injurious, since in a brief period, when the injected serum has been eliminated, the subject is not only not protected, but will be less easily protected by the antiserum, should he eventually acquire infection.

And if it prove to be the case with other animals also, and with other immune sera, that the cell groups which we have spoken of as X-receptors are usually or invariably singular, we shall arrive at the conclusion that immune body is in a manner special to the species. But this implies in the bacillus the possession of a variety of different kinds of I-groups (such as I, I_1, I_2 . . . I_n, corresponding to the various immune bodies formed by different animals for the given bacillus (namely, I.B, $I.B_1$, $I.B_2$ . . . $I.B_n$). And seeing that all these immune bodies can be used protectively for any given animal, it follows that they all possess the same addiment-haptophore — a further evidence ([24]) how little special to the species is an addiment which can act with immune body from so many different sources.

The number of the different kinds of I-groups in a given bacterium must, however, be limited, and similarly the number of possible immune bodies corresponding. Hence it is probable that in the animal series it will prove that the same immune body is produced as a coincidence in two or several different kinds of animals. And it is not impossible that, among all the various immune bodies which can be produced by different animals against different micro-organisms, those for two different bacteria might in two different animals be identical, always allowing that an immune body has a variety of haptophores and not merely two. Evidence is required on all these points. Meanwhile it may be pointed out how closely the result accords with Ehrlich's observations on the non-identity of isolysins.

Conclusions.

1. A bacterium may be immunised against its immune serum, and thereby becomes more virulent, and less agglutinable if it be of an agglutinable variety.

2. The immune serum in which its own bacillus has been grown is rendered less agglutinative and less protective against that bacillus.

3. Agglutinins are true antibodies.

4. The basis of bacterial virulence and of chemiotactic influence is identical, and constitutes that atom group which causes the production of the immune body.

5. An animal may be immunised against an immune serum. It is then less capable of being protected by that serum, but its susceptibility to the bacterium is not increased.

6. The immune body is not identical in different animals for the same bacterium, but exhibits a specialism to the species.

I take this opportunity of again recording my deep indebtedness both to Prof. Tavel of Berne, and to Dr. Ritchie, Lecturer in Pathology in the University of Oxford, for their kindness in facilitating the experiments which I have carried out in their laboratories.

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