ON THE PROTECTIVE SUBSTANCES OF IMMUNE SERA.

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The theories of Ehrlich[1] on the Substances and Mechanisms concerned in the production of Immunity have opened up a very wide field for speculation and research. His view on the formation of the Antitoxins forms the most satisfactory and useful hypothesis which has been produced upon this subject, and is constantly receiving fresh support from the accumulating evidence of numerous observers. But of the more recent developments of his theories as applied to antimicrobic agencies some of the details appear to be at present insufficiently established. With certain of these details it is here proposed to deal.

Methods.

In all the experiments to be recorded the cultures used were 48 hour cultures of a given variety of the *B. typhosus* on an agar surface of approximately constant area. For injection they were washed off the agar surface with a known volume of ordinary culture bouillon, and emulsified by thorough shaking. The dose to be administered was then measured volumetrically, accuracy of measurement being sought by increasing the dilution of the emulsion where small doses were required. The injections were made intraperitoneally with a hypodermic needle. Serum when given was mixed and injected with the infecting dose of the bacillus. The weights of the guinea-pigs used were determined before injection to within 5 grammes, and all the injections given were calculated for the actual body-weight. They are stated in the accounts of the experiments in terms of the amount per 100 grms.

of weight. Care was exercised to obtain animals as similar as possible in age and weight. An autopsy was made on every animal which died. The Immune-serum used throughout was the antityphoid serum of Tavel obtained from horses.

The Theory of the Specialism of the Addiment.

It has been pointed out by Ehrlich, Wassermann, and others that if the amount of Immune-serum necessary to protect an animal of given weight against a single M.L.D. (minimum lethal dose) of a given bacterium be determined, and a similar animal be now injected with three times this amount of serum and three times the M.L.D. it will die, not being sufficiently protected by the quantity of Immune-serum given. Moreover Roux and Vaillard[2] had shown for tetanus what has been fully confirmed for other infections also (*e.g.* for cholera by Pfeiffer), that an infected animal treated with Immune-serum may rapidly die of the disease in question although it has been given such an amount of serum as to render its body-fluids capable of protecting other animals against infection by the same bacterium. That is to say, in general, that if an animal receives more than a certain maximum dose of an infecting agent it will die, whatever the quantity of the Immune-serum that is employed in the endeavour to protect it. And seeing that in this second type experiment the Immune-body is obviously present in sufficient quantity—since the fluids of the dead animal can protect another animal—Ehrlich attributed the phenomena observed in these experiments to a deficiency of the addiment. And on this deficiency of addiment he founds the theory which it is our purpose to consider.

Taking first the position of Pfeiffer, that both the substances necessary to protection are present in Immune-serum in an inactive form, and become active after injection into the body of an animal, it is evident that if the addiment injected is rendered available and active by the body-cells of the animal concerned it should be possible to supply this addiment in sufficient quantity by the injection of sufficient Immune-serum. But this is by the experiment quoted found to be impossible. Erhlich therefore came to the conclusion that the foreign addiment is useless to the animal injected; in other words, that an animal can only make use of its own addiment, which is limited in amount, or of that of other animals of its own species: in fact that addiment is special to the species.

It remained possible, however, that exceptions might exist and that

certain species of animals might possess addiments acceptable to certain other species. And this appeared to be the case. These exceptions, therefore, become of great importance, for only by a careful search could there be found for each particular species the addiment acceptable not only to the Immune-body for a given bacterium but also to the animal itself. And more especially for serum-therapy in man Ehrlich regards this search as of prime value and of pressing importance for the immediate future.

The writer[3] has already elsewhere expressed the opinion that this theory of the extreme specialism of the addiment is both unnecessary and misleading; and that the facts are capable of better explanation on the view that owing to its extremely labile nature the addiment of antibacterial action becomes broken down and degenerated, and rapidly disappears from artificially separated serum. That is to say, that addiment is *absent* from the ordinary antimicrobic sera. That the position of Ehrlich is not by any means satisfactory is shown by the following considerations among others. In every observation of the so-called Pfeiffer phenomenon made by means of the Immune-serum of other animals in the peritoneal cavity of the guinea-pig, the school of Pfeiffer believes that the inactive addiment of this serum is rendered active by the agency of body-cells, that is to say, a foreign addiment is satisfactory to the reaction in the guinea-pig. Again Wassermann[4] found that in infection of guinea-pigs a satisfactory addiment for this animal, and for the Immune-serum of dogs which was used as the protective agent, existed in the fresh serum of the ox—an animal of a species as widely different from the guinea-pig as from the dog; while Ehrlich and Morgenroth discovered similar relations in their work upon the haemolysins. Taking all the facts into consideration I came to the opinion formulated in a previous paper that (stored) antibacterial sera possess in general no addiment, and that in the Pfeiffer phenomenon as in the other experiments in question the animal concerned has to make use of *its own* addiment, which is limited in quantity, and therefore may prove insufficient for the work required of it. And evidence in support of this contention can be adduced from various directions, as I now proceed to show.

In the former of the two type-experiments quoted, in which the fact that three serum-equivalents[1] do not protect against 3 M.L.D. is attributed to lack of addiment; the position held by Wassermann appears to have

[1] By the term serum-equivalent is meant the amount of serum necessary to protect 100 grammes of guinea-pig against a single M.L.D.

originated in a mathematical error. The result is in reality due to a deficiency in the amount of Immune-body injected.

For if d represent the M.L.D. then if the M.L.D. has been determined within 10 %, say, of the total dose $\frac{9}{10}d$ was the nearest dose less than d which proved not fatal. That is to say, an unimmunised animal has natural immunity to destroy a dose of $\frac{9}{10}d$.

Hence in the case of the first injection of one M.L.D. and one serum-equivalent, the latter was only engaged in actively protecting against—that is in providing Immune-body against $\frac{1}{10}d$. And since the least amount of serum necessary for protection against the M.L.D. constitutes the serum-equivalent of that M.L.D. we may say that one serum-equivalent is the protective equivalent of $\frac{1}{10}d$. Now in the second case $3d$. and 3 serum-equivalents are injected. And the 3 serum-equivalents are the protective equivalents of $3 \times \frac{1}{10}d$, that is of $\frac{3}{10}d$ only. Also the animal itself can if the remainder of the dose be neutralised by serum-protection (but only then) deal with an amount of the infective material equal to $\frac{9}{10}d$. There remains however a dose of $(3 - \frac{3}{10} - \frac{9}{10})\,d$, *i.e.* $1\frac{8}{10}d$, against which no protective arrangement whatever is made and obviously the animal must die.

If the M.L.D. had been determined to within nearer limits than the 10 % supposed, the result is all the more favourable to the argument, if within wider limits only, somewhat less so. But in any case the greater the number of M.L.D.'s and the number of serum-equivalents given the larger will be the dose of the bacterium against which no protection whatever is afforded, and the more rapidly and certainly will the animal die. And if in this experiment the serum-equivalent had been determined not for the M.L.D. but for some multiple of this and the experiment continued as before, then by a similar train of reasoning the serum given would fail to be protective when the doses of infective material and serum were progressively multiplied as before. While therefore in the experiment with large infecting doses and unlimited amounts of Immune-serum there is exhibited a deficiency of addiment, in this experiment there is no evidence of deficient addiment but only of deficiency of Immune-body. In proof of this assertion may be quoted the following series of observations.

The M.L.D. of a particular variety of typhoid bacillus, one of the varieties actually employed in the preparation of the serum itself from horses, was found to lie between the limits 0·05 and 0·075 of a 48 hour culture on an agar surface of approximately constant area for each 100 grms. of guinea-pig. The serum-equivalent of this M.L.D. (taken always as 0·075

of a culture) was 0·025 c.c. of serum per 100 grms. of guinea-pig, and 0·02 c.c. per 100 grms. was not protective. The animals themselves could therefore deal with a dose of 0·05 of a culture per 100 grms. Hence the serum-equivalent of the M.L.D. was engaged in protection against 0·025 of the culture only: it was therefore the protective equivalent or true serum-equivalent of this amount of culture. I shall speak of this volume of serum as the serum-unit. A simple calculation now showed that if the view maintained be justified 2 M.L.D. would require serum protection against $[(2 \times \cdot 075) - \cdot 05]$, *i.e.* 0·1 culture and this would be afforded by $\frac{0 \cdot 1}{0 \cdot 025}$, *i.e.* 4 serum-units,

3 M.L.D. would similarly require $\frac{(3 \times 0 \cdot 075) - 0 \cdot 05}{0 \cdot 025}$ serum-units, *i.e.* 7 serum-units,

4 M.L.D. would similarly require $\frac{(4 \times 0 \cdot 075) - 0 \cdot 05}{0 \cdot 025}$, *i.e.* 10 serum-units, and generally n M.L.D. require $\{1 + 3(n-1)\}$ serum-units.

And in general if d be the M.L.D. and e the largest dose not fatal, then the number of serum-units required to protect 100 grms. of guinea-pig against n M.L.D. of the bacterium will be given by the formula $\frac{nd - e}{d - e}$, that is $\left[1 + (n-1)\frac{d}{d-e}\right]$ serum-units.

The following were the experimental results obtained with a series of guinea-pigs.

Guinea-pig	1	received	1	M. L. D.	and	1	serum-unit,	the animal	recovered
„	2	„	1	„	„	$\frac{4}{5}$	„	„	died in 16–18 hrs.
„	3	„	2	„	„	4	„	„	recovered
„	4	„	2	„	„	3	„	„	died in 18 hrs.
„	5	„	3	„	„	7	„	„	recovered
„	6	„	3	„	„	6	„	„	died in 14–16 hrs.
„	7	„	4	„	„	10	„	„	recovered
„	8	„	4	„	„	9	„	„	died in 18–20 hrs.
[1] „	9	„	5	„	„	13	„	„	died within 14 hrs.
„	10	„	5	„	„	12	„	„	„ „ „
[1] „	11	„	6	„	„	16	„	„	„ „ „
„	12	„	6	„	„	15	„	„	„ „ „

[1] Deficiency of addiment.

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And these results have been confirmed[1]. Hence up to and with four M.L.D. and the corresponding number of serum-units theoretically required on our hypothesis the animals are completely protected, but not by any less amounts of serum. I submit therefore that the view which has been here put forward is sufficiently established; and have in all the subsequent experiments calculated the amount of serum which contains a quantity of the Immune-body sufficient for protection by this formula. This quantity I have called the *theoretical serum requirement* of the animal for the given dose of the infective agent.

It was apparent in the above series of experiments that a deficiency of addiment first began to occur on the exhibition of 5 M.L.D. together with the appropriate number of serum-units. It seemed quite certain therefore that by working with doses of ten times the M.L.D. inaccurate results from accidental coincidence could be avoided in an investigation of the addiment. I therefore proceeded to examine the theory of its specialism as follows.

Experiments were made,

(*a*) with the *fresh* blood-serum of certain unimmunised animals;

(*b*) with the same sera when they had been kept for some days after separation in an ice-chest, at a temperature approaching zero;

(*c*) with the fluids obtained by digesting such old sera with *fresh* blood-clot which was broken up and added to them, the whole being kept for one or two hours in the incubative chamber, and the fluid afterwards centrifugalised from the fragmented clot as described more fully later.

The results obtained are illustrated in the subjoined series of experiments.

Experiment (*a*).	Result
Guinea-pig 1 received 10 M.L.D. and 28 serum-units[2] of Immune-serum	The animal died within 16 hrs.
Guinea-pig 2 received 10 M.L.D. and 28 serum-units of Immune-serum and 1 c.c. *fresh*[3] Rabbit serum per 100 grms.	died within 16 hrs.
Guinea-pig 3 received 10 M.L.D. and 28 serum-units of Immune-serum and 2 c.c. *fresh* Rabbit serum per 100 grms.	recovered

[1] In another series of experiments with a more virulent variety of the *B. typhosus* the deficiency of addiment appeared at the fourth M.L.D.

[2] The theoretical serum requirement according to the formula obtained above $\frac{nd-e}{d-e}$.

[3] Fresh serum here means serum used within 10 hours of the bleeding of the animal from which it was obtained.

EXPERIMENT (*a*).

	Result
Guinea-pig 4 received 10 M.L.D. and 28 serum-units of Immune-serum and 1 c.c. *fresh* Ox serum per 100 grms.	died in 20–24 hrs.
Guinea-pig 5 received 10 M.L.D. and 28 serum-units of Immune-serum and 2 c.c. *fresh* Ox serum per 100 grms.	recovered
Guinea-pig 6 received 10 M.L.D. and 28 serum-units of Immune-serum and 1 c.c. *fresh* Pig serum per 100 grms.	died in 26–28 hrs.
Guinea-pig 7 received 10 M.L.D. and 28 serum-units of Immune-serum and 2 c.c. *fresh* Pig serum per 100 grms.	recovered

Hence the freshly won serum of the three different animals examined—the rabbit, ox, and pig—can all supply the missing addiment for guinea-pigs, and this addiment is 'satisfactory' to the Immune-body of the immune-serum of the horse. We have here five distinct species of animals concerned, and it seems reasonable to conclude that Ehrlich's theory of the extreme specialism of the addiment to its own species of animal is untenable.

I have suggested on the other hand that owing to its extreme *lability* this addiment is absent from *stored* serum, and this I found to be the case. Thus I examined serum of the rabbit, ox and pig after it had been kept for some days in the ice-chest. In most cases it was a week to ten days old, but in the case of the ox serum only two to three days old, when thus made use of in the experiments from which the following are taken.

EXPERIMENT (*b*).

Guinea-pig 1 received 10 M.L.D. and 28 serum-units[1] of Immune-serum	
Guinea-pig 2 received 10 M.L.D. and 28 serum-units of Immune-serum and 2 c.c. *old* Rabbit serum per 100 grms.	
Guinea-pig 3 received 10 M.L.D. and 28 serum-units of Immune-serum and 3 c.c. *old* Rabbit serum per 100 grms.	
Guinea-pig 4 received 10 M.L.D. and 28 serum-units of Immune-serum and 2 c.c. *old* Ox serum per 100 grms.	all dead within 18 hrs.
Guinea-pig 5 received 10 M.L.D. and 28 serum-units of Immune-serum and 3 c.c. *old* Ox serum per 100 grms.	
Guinea-pig 6 received 10 M.L.D. and 28 serum-units of Immune-serum and 2 c.c. *old* Pig serum per 100 grms.	
Guinea-pig 7 received 10 M.L.D. and 28 serum-units of Immune-serum and 3 c.c. *old* Pig serum per 100 grms.	

[1] The theoretical serum requirement of 10 M.L.D.

The sera therefore which had been kept for a few days had lost the power they previously possessed of supplying the deficient addiment. That is, they had lost their addiment. Hence anti-bacterial addiment rapidly disappears from a stored serum.

Further, the addiment of the fresh sera was found to be destroyed by heating for half-an-hour to a temperature of from 52°—53° C. (nos. 1 and 2 below), though heating the Immune-serum for an hour to this temperature or to 56° C. produced no apparent alteration in its protective power (nos. 3 and 4 below). Thus

Guinea-pig 1 received 10 M.L.D. and 28 serum-units of Immune-serum and 2 c.c. per 100 grms. *fresh* Ox serum	recovered
Guinea-pig 2 received 10 M.L.D. and 28 serum-units of Immune-serum and 2 c.c. per 100 grms. *fresh* Ox serum heated for 1 hour at 53° C.	died within 20 hrs.
Guinea-pig 3 received 10 M.L.D. and 28 serum-units of Immune-serum heated 1 hour at 56° C. and 2 c.c. per 100 grms. *fresh* Ox serum	recovered
Guinea-pig 4 received 4 M.L.D. and 10 serum-units of Immune-serum heated 1 hour at 56° C.	recovered

Thus, while the addiment of fresh serum was destroyed by heating to this temperature, the action of the Immune-serum was unaltered. Hence I conclude that the latter contains no addiment to be destroyed. The addiment which is concerned in anti-bacterial action on the *B. typhosus* is therefore *not* particularly 'special to the species,' but is of an extreme lability, and hence is *absent* from any but fresh serum whether the latter be obtained from immunised or unimmunised animals.

The Nature and Origin of Addiment.

In one and the same serum the addiment is not the same for all the anti-cellular reactions possible to that serum, but different. Thus as regards the haemolytic action of goats' serum Ehrlich has shown the existence of two kinds of addiment at least, the one destroyed rapidly at a temperature of 56° C., the other only slowly and still resistant at a temperature equal to 65° C. And we have seen that the addiment of anti-bacterial action here examined is still more labile.

Now Ehrlich has put forward the following view of the position and function of addiment on the normal body. It is, he claims, a ferment produced and discharged into the blood by certain cells—which are not

further specified—to serve the purposes of general cell-metabolism. It is taken up from the plasma by means of an addimentophil group of the side-chains of the body-cells, and thereby enables them to carry on their normal cycle of nutrition by its power of splitting up the large molecules of nutritive material extracted from the blood and rendering their assimilation possible.

But anti-microbic serum has *in vitro* no bacteriolytic action. It contains Immune-body, but offers no evidence of the possession of any such ferment as Ehrlich presupposes, for as we have seen the bacteriolytic addiment is absent from stored sera. Such serum may be rendered active by cellular action, as for example by a sojourn in the peritoneal cavity (Pfeiffer), by the addition of fresh normal serum, which contains leucocytes and their products—Bordet's[5] phenomenon,—or by the addition of other leucocytic fluids (Hahn[6]). The activity thus obtained is removed by heating at 56° C., a process which as has been shown destroys the addiment; it may be again restored by the addition of fluid containing leucocytic products (Bordet, Hahn.) Moreover a definite relationship exists between the mass of the leucocytes added and the degree of bactericidal addimentary power obtained (Bordet). Again, a bacteriolytic pleural exudate has been made entirely inactive by the removal of its leucocytes,—active again on their replacement (Denys and Havet[7]). Additional evidence in the same direction was obtained in a continuation of the experiments already quoted, in the observations now to be recorded.

In these experiments I took normal sera of the rabbit and the pig, which had been found to have lost their addiment by keeping, and used them for extracting *fresh* blood-clot of their own, or other species of animals. The procedure was as follows: fresh clot was broken up and rapidly centrifugalised, and the resulting fluid decanted. The amount of serum then remaining in the clot was insufficient to affect the accuracy of the subsequent experiment, in view of the comparatively large amount of fluid next employed. This consisted of a volume of the old serum calculated to be greater than the volume of serum normally corresponding to the quantity of clot which had been centrifugalised. This volume of old serum was now added to the clot and thoroughly mixed with it in a mortar. The mixture was then placed in the incubator for an hour or two, and the fluid subsequently separated by centrifugalisation and tested for protective power, that is for the possession of addimentary action. It was then found that the fresh clot had yielded addiment to the previously addiment-free old serum of

animals, even of a different species from that of the animal which supplied the clot. I quote the following experiments.

Experiment (*c*).	Result
Guinea-pig 1 received 10 M.L.D. and 28 serum-units[1] of Immune-serum and 3 c.c. per 100 grms. of *old* Rabbit serum ...	The animal died within 18 hrs.
Guinea-pig 2 received 10 M.L.D. and 28 serum-units of Immune-serum and 3 c.c. per 100 grms. {of *old* Rabbit serum, extract of fresh Ox clot}	recovered
Guinea-pig 3 received 10 M.L.D. and 28 serum-units of Immune-serum and 3 c.c. per 100 grms. {of *old* Pig serum, extract of fresh Pig clot}	recovered

That is to say the deficient addiment can be supplied not only by *fresh serum* as already shown, but also by an extract of *fresh clot.* This points distinctly to a close relation of addiment to the leucocytes and their products of disintegration which are the only bodies we at present know to be obtainable in quantity both from the clot and from the separated serum of shed blood. It follows therefore from the results of all the different observations quoted, that leucocytes contain addiment, and this not only in the living body but even *in vitro,* and that they yield it on requirement to a serum from which it was previously altogether absent. And that this addiment is a ferment *proper* to the leucocytes, and not one extracted by them from the plasma and stored for future use, is clear from the observations that exudates rapidly freed from their leucocytes contain no such ferment, though if the leucocytes remain its presence can be proved—a condition impossible if the addiment were not a veritable leucocytic ferment but one circulating freely in the blood-plasma to be taken up by cells in general according to their various requirements, as supposed by Ehrlich. It seems therefore also evident that the process which occurs in the peritoneal cavity, by which previously inactive antimicrobic serum becomes active, consists not in the modification under cellular influence of stable but inactive protective bodies into unstable but highly active agents, as maintained by Pfeiffer, but in the addition to that serum of the previously absent addiment.

In this connection I would further call attention to the increasing evidence that the Immune-body itself is also a leucocytic product. Thus Deutsch[(8)] has shown that it is formed in the leucocytic tissues,

[1] The theoretical serum requirement.

and Bulloch proved more recently a very marked and definite relation in his haemolytic serum between the increase of the Immune-body, as well as the variations in its quantity during the process of immunisation, and the varying degree of leucocytosis which was present or had been produced in the animal immunised. Moreover the phenomena of natural immunity, in which the serum certainly contains no appreciable amount of Immune-body, come into harmony with the facts of the acquired variety, if it be admitted that the Immune-body-Receptors are pre-existent in the leucocytes. And this admission equally explains the intimate relation of the Immune-body to the addiment, which I have urged consists of leucocytic ferment.

Bacteriolytic addiments, as we have seen, rapidly undergo decomposition and disappear from Immune-serum. Those of haemolysis, on the other hand, apparently remain. This fact may probably be correlated with the great thermolability of the former as against the relatively thermostabile nature of the latter; and with the observation of Metschnikoff that, while in haemolytic action the macrophages are the chief performers, in bacteriolysis, on the contrary, phagocytosis is primarily and chiefly the concern of microphages. This points definitely to the source of origin for the addiment in the two cases being traceable to different varieties of leucocytes.

Increase of Addiment during Immunisation.

If addiment is a leucocytic ferment it would be expected that with the appearance of the leucocytosis which accompanies the artificial production of immunity there should appear an increase in the addiment-content of the blood. Yet von Dungern [9] was unable to determine any such increase during the process of immunisation against red-blood-cells; and Bulloch [10] in his recently published experiments comes to a similar conclusion. These observations however prove only that the addiment quantum of the separated serum is not markedly increased, and do not necessarily give an indication of the conditions in the living animal itself. Incidentally it may be pointed out also that while von Dungern considers that the addiment quantum of normal rabbits' serum, with which he compared that of the immune-sera of similar animals which had been immunised, is fairly constant, yet his results show that the volume of such serum required to complete a given definite reaction varied from $\frac{1}{40}$ c.c. to $\frac{1}{20}$ c.c., in other words, varied 100 %. This would tend to show that the addiment content of serum

is an inconstant quantity, unless this variability prove to have been dependent on varying methods of preparation or durations of storage of von Dungern's sera. But, if the statement of the absence of an increase of the addiment in the serum by immunisation be accepted, as confirmed by Bulloch's observations, there still remains a simple explanation consonant with an actual increase of addiment in the living animal. For the amount of addiment found in the haemolytic serum depends not on the amount of addiment present in the blood—that is, on our view, upon its leucocytic richness—but solely on the amount set free into the serum after bleeding, that is, upon the number of leucocytes destroyed by the procedures and in the process of coagulation. And this is in no way necessarily affected by the leucocytic richness of the blood. It is therefore not to be expected that the serum should exhibit any remarkable increase in its addiment even though there were a marked increase of addimentary ferment in the blood.

But on the other hand direct experimental evidence can be obtained of the association of a definite increase of addiment with the establishment of resistance to infection. This may be sought in the phenomena observed when an excess of Immune-serum is given in cases of deficiency of addiment. For suppose a certain organism to possess two active phagocytes and that one new phagocyte can be produced on requirement at the expiration of an hour from the demand. Suppose also that the bacteria injected, multiplying if not destroyed, double in number every twenty minutes. Grant further that each phagocyte contains only sufficient Immune-body-receptors to enable its addiment to destroy two bacteria, but that a given quantity of Immune-serum when injected suffices to employ all the addiment of the phagocytes which then destroy a maximum of the bacteria apiece. Now inject into this organism twenty-one bacteria and the same dose of Immune-serum as will enable the destruction of twenty. It follows that twenty bacteria are destroyed, the Immune-serum injected is used up, and the phagocytes are exhausted. Of the bacteria one remains, and this multiplying as supposed is represented after the expiration of an hour by eight. There now appears a new-formed phagocyte whose addiment if supplied with Immune-body from without can destroy ten bacteria, but owing to the absence of a sufficiency of this body it actually destroys two only and the invasion continues to advance. If however additional Immune-body had been injected the infection would now have terminated in consequence of the timely formation of fresh addiment.

Hence by the exhibition of an amount of infective material greater than can be dealt with by the addiment content of the animal, together with an excess of Immune-serum above the theoretical requirement of the infecting dose, we can determine, by the resulting recovery or death of the animal concerned, the presence or absence of the formation of fresh addiment in the reaction to infection. Investigation along these lines was undertaken and experiments were made, starting from the dose of *B. typhosus* at which deficiency of addiment appears (namely in the present instance at 5 M.L.D.), and giving at the same time Immune-serum in excess. The following were the results obtained.

	Result
Guinea-pig 1 received 5 M.L.D. and the theoretical serum requirement[1] viz. 13 serum-units (*control*)	died in 12–15 hrs.
Guinea-pig 2 received 5 M.L.D. and the theoretical serum requirement of 6 M.L.D.[2] viz. 16 serum units	recovered
Guinea-pig 3 received 6 M.L.D. and the theoretical serum requirement of 8 M.L.D.[2] viz. 22 serum units	recovered
Guinea-pig 4 received 10 M.L.D. and the theoretical serum requirement of 13 M.L.D.[2] viz. 37 serum units	recovered

From this it appears that while animal 1 died from deficiency of addiment, animals 2, 3 and 4, in which there was equal or greater deficiency of addiment (in animal 4 much greater), were enabled by the sheltering effect of the serum given to gain time for the formation of fresh addiment which then with the excess of Immune-body supplied completed the destruction of the microbes. That is to say, fresh addiment can be formed in the animal under suitable conditions, and is so formed in the reaction to infection. And indeed the leucocytosis which admittedly occurs would be meaningless if the newly formed and active phagocytes were deficient in their essential ferments, as would follow if the theory of von Dungern were correct.

Fresh addiment being formed in the reaction to a single infection it must evidently be similarly formed throughout immunisation. If further evidence of such formation be required, it may be found in the consideration that since deficiency of addiment appears when a comparatively small multiple of the M.L.D. is given (in the experiments here quoted with 5 M.L.D.) it should be impossible, if new addiment is not so formed, to immunise an animal to withstand more than this number of M.L.D. And this is clearly not the case.

[1] Theoretical serum requirement of n M.L.D. is $\frac{nd-e}{d-e}$, cf. p. 89.

[2] Excess of serum; considerably more than the theoretical requirement.

In those instances, however, where, as has been mentioned, an animal may die although its fluids have been saturated with a great excess of Immune-serum it is evident that we have passed away from the case which has been just discussed to one in which the bacteria are so numerous and active as to be beyond the reach of any possible addiment-production by the animal. Moreover in these cases leucocytosis is inhibited and fails to appear, so that the natural formation of fresh addiment does not occur.

Relation of 'Agglutinins' to the Protective Substances.

Agglutination has been shown to bear a close relation to protection. In an endeavour to throw light on this relation I have made certain experiments with dead typhoid cultures.

The toxins of the *B. typhosus* are undoubtedly intracellular toxins, for even old cultures freed from bacilli by filtration possess no toxic action. The action of dead typhoid cultures is the action of these intrabacillary toxins. And Funck [11], who has worked upon this subject, states that his antityphoid serum had no greater antitoxic power against dead cultures than had a normal serum, though his figures might be thought to bear a different interpretation.

In the following experiments cultures were killed by heat, and a dose of the dead cultures was employed of nearly twice the M.L.D. of this material. This corresponded to the bacterial content of 20 M.L.D. of living culture, whose theoretical serum requirement was 58 serum-units. The control animal received a volume of normal serum equal to the volume of this number of units of Immune-serum. The results are given below.

	Result
Guinea-pig 1 received 20 M.L.D. living typhoid and 58 serum-units of Immune-serum	died in 16 hrs.
Guinea-pig 2 received 20 M.L.D. killed typhoid and 58 serum-units of Immune-serum	recovered
Guinea-pig 3 (control) received 20 M.L.D. killed typhoid and volume of normal Rabbit serum equal to volume of 58 serum-units ...	died in 14 hrs.

Accordingly the serum possessed *specific antitoxic action* not possessed by normal serum.

Now Gruber showed that if the agglutinative action of an Immune-serum be diminished by the application of heat, the protective power also undergoes a corresponding diminution. And this has been confirmed by Trumpp. I accordingly next heated a quantity of Immune-serum at 67° C. until its protective power against the living bacilli had been considerably reduced—as shown by direct trial and also by the fact

that its agglutinative action was diminished to about one-half its former value, namely from the limits

$$\begin{cases} 1 \text{ to } 550{,}000+ \\ 1 \text{ to } 600{,}000- \end{cases}$$

to the limits

$$\begin{cases} 1 \text{ to } 300{,}000+ \\ 1 \text{ to } 350{,}000- \end{cases}$$

it nevertheless still protected against the dead injections, though no longer against the living typhoid bacillus, even when given in excess of the theoretical requirement. Thus

	Result
Guinea-pig 1 received 2 M.L.D. living typhoid and 4 serum-units[1] of the heated Immune-serum	died in 16 hrs.
Guinea-pig 2 received 2 M.L.D. living typhoid and 5 serum-units of the heated Immune-serum	died in 16 hrs.
Guinea-pig 3 received 20 M.L.D. killed typhoid and 58[2] serum-units of the heated Immune-serum	recovered

that is to say, the antitoxic power remained although the agglutinating action and the protective action against living cultures had been markedly diminished. The antitoxic action of the serum against the intracellular toxins, which can only come to act upon the animal infected after the solution of the micro-organisms themselves, has therefore no intimate relation with the agglutinative action which proceeds equally with the living as with dead bacteria.

Hence may be now deduced an explanation of the lack of complete parallelism between the phenomena of agglutination and protection. For the protection in the cases here considered must be a function of combined antimicrobic and antitoxic action, while the agglutinative process can be related only to the former of these two components. The parallelism therefore between agglutination and protection cannot be complete. It may be so between agglutination and bacteriolytic action.

Agglutination however is not concerned directly in the events of lysogenesis which is effected by the coordinated action of the Immune-body and addiment, and which can occur, and does with many microorganisms invariably take place, in the absence of agglutinative action. It must therefore be connected with the only other event with which we are acquainted in antimicrobic action, namely the phagocytic process. And I suggest that the "agglutinins" assist this process by their faculty of bringing the microorganisms together (and

[1] 4 serum-units is the theoretical serum requirement of 2 M.L.D.

[2] Theoretical requirement of 20 M.L.D. of living typhoid.

at rest if previously motile) in larger or smaller masses, and by the alteration in the bacterial envelope which they produce—changes which clearly simplify the process of ingestion.

Conclusions.

1. The amount of Immune-body needed for protection against n M.L.D of a bacterium is contained in $\frac{nd-e}{d-e}$ c.c. of its Immune-serum, where d is the M.L.D. e the largest dose invariably not fatal and the serum-equivalent of one M.L.D.
2. Addiment is not extremely special to the species.
3. Addiment is a leucocytic ferment.
4. Addiment is increased during and by immunisation.
5. Agglutinins assist the phagocytic process of ingestion.

The experiments here brought forward were made in the Bacteriological Institute of the University of Berne during the early months of the present year. Many of them have since been repeated and confirmed either there or in the Pathological Laboratory of the University of Oxford. My best thanks are due both to Professor Tavel, of Berne, and to Dr James Ritchie, Reader in Pathology in the University of Oxford, for the facilities of their laboratories, and for their constant kindness and encouragement in the present work.

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