STUDIES ON THE SIGNIFICANCE OF "VI ANTIGEN" IN THE MECHANISM OF TYPHOID INFECTION IN MICE

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IN a previous paper one of us (F. K.) corroborated all the facts of great interest brought forward by Felix and his collaborators concerning the new so-called virulence antigen. A few years ago Krogh-Lund investigated typhoid in mice after infection by mouth, and found that they were infected with difficulty by this route, and that the disease produced seldom extended beyond the regional glands of the intestine, where, however, the bacilli were often demonstrable for many weeks. The animals never died of the infection. Since in all Felix's experiments life or death of the animals was used as a criterion of the severity of infection and of the value of vaccination and prophylactic serum administration, we decided to attempt to elucidate some of the processes going on in the animals more clearly—in the same way as we have done in our experiments on the infection mechanism of many different infectious diseases in animals during recent years. (For a review of these investigations, see Ørskov (1932).)

It was natural that Felix should have come to the conclusion that his new antigen had much to do with the virulence of the typhoid bacillus used, as those strains having this antigen were able to kill mice in smaller doses than those without it (we have always found this too); and accordingly he called this antigen "virulence antigen". However, this term may be found not to have been happily chosen when we attempt to clarify what is meant by "virulence".

The term virulence is usually understood to imply ability to grow in a more highly organized host. But even if we stick to this definition it will soon be found that many different meanings may be applied to this definition.

It may be of interest to examine some of the different types of infections in order to show what we really mean by virulence in any given case; and we may begin with the *Aertrycke* infection in mice. Here there can be little doubt what we mean when we say that the *Aertrycke* bacillus is virulent for mice. It is easy to show that the bacillus given *per os* can penetrate the walls of the whole intestinal tract, and after passing through the mucous membrane, can grow in the peripheral lymph glands, spreading from there and invading the main lymphatics and the blood, finally causing the death of the animal. If we compare this infection with *abortus* bacillus infection in mice, for instance, we find that the development of the latter infection is the same, but it seldom kills the animals, and the bacilli diminish in number after a certain time and ultimately disappear. There is a difference here in the quality of the pathogenic effect of the two bacteria, but the ability to penetrate and spread is a common feature. Paratyphoid B infection in mice is different again, for the

bacilli easily penetrate the mucous membrane of the intestinal tract, but they very seldom cause generalized disease, remaining localized in most cases to the lymph glands of the whole intestinal tract, where they grow well and often persist for quite a long time. A very similar picture is obtained when mice are infected *per os* with typhoid bacilli, but heavier doses must be used than of paratyphoid B bacilli; here, too, a generalized infection of any importance is rarely seen, and it is even rarer still for the animal to die however large the dose of typhoid bacilli administered. In all these infections we may say that by virulence is meant the ability of the infecting organism to penetrate the intestinal wall and multiply actively in the animal.

Typhoid infection in man may be compared with *Aertrycke* infection in mice, and is generally different from what was found by us in typhoid infection in mice. This will be useful to remember when attempting to compare the infection in mice and men.

In another group of bacteria, generally characterized as virulent for mice, the pneumococcus group, we find other features, since these bacteria can seldom infect mice when given *per os*—we know not why. If, on the other hand, they are introduced into the organism by some other route, they produce a generalized disease ending rapidly in the death of the animal; and other well-known bacteria behave similarly: they cannot penetrate the intestinal wall, but they multiply rapidly when infected, even in very small quantities. The same holds good for several different viruses.

Another group of bacteria neither penetrate the walls of the intestinal tract nor can they multiply in the animals even if injected, e.g. the dysentery bacillus, but when given in heavy doses they can kill the animals owing to the toxic products they contain; and when such living bacilli are injected in a dose which will cause acute intoxication in the animal, we find that the animals after death show enormous numbers of living bacteria in situations where we do not find any in animals which survive after a sublethal dose and are killed a short time after the infection. Here we have a picture which has a certain interest in connexion with the problem of typhoid infection in mice; for here is a bacillus with little ability to grow in mice in whose bodies it is destroyed instantly when injected in doses which do not cause marked symptoms of intoxication. If such symptoms do arise the bacteria persist and grow probably on account of depression in some natural immunity reaction. This short account does not, however, exhaust the subject of the different forms adapted by "virulence".

In most of the investigations on the new typhoid antigen and its significance in regard to the virulence of the bacillus, the experiments were carried out with doses which gave symptoms of acute intoxication in the animals. The first object of this paper will be to endeavour to discover how the infection develops in mice injected with doses which do not give these symptoms, and in this way attempt to find what role the Vi antigen plays in the growth of the "single" typhoid bacillus in the mouse. In this connexion it is necessary to point out also that we consider it dangerous to compare the V form[1] of one strain with the W form of another strain. As there can be no doubt that the V forms of different strains of the typhoid bacillus may differ in regard to average virulence and in regard to toxicity, there will be still more cause to be cautious in comparing the V form of one strain with the W form of another. It is all the more appropriate to compare the V and the W forms of the same strain, as in this way we have a better chance of comparing two forms differing, as far as can be demonstrated to-day, only in one factor, the presence or

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[1] V form = O inagglutinable, contains Vi antigen.
W form = O agglutinable, contains no Vi antigen.

absence of the virulence antigen. We possess at present two different strains which fulfil this claim: (1) the Watson strain, which we have in two more or less constant variants with and without Vi antigen, and (2) the H 901 strain, which was kindly given us by the Lister Institute and which has been constantly found devoid of Vi antigen; this strain one of us (F. K.) succeeded in cultivating from a single mouse in the V form, which has constantly kept this antigen, so that the variants may be compared.

Very early during the experiments we were impressed by the symptoms of acute intoxication which arose within a few hours of the injection of a lethal dose of typhoid bacilli—an effect contrasting very markedly with that of smaller doses. Another point which attracted our attention was that nearly all the animals which die do so within 36 hours of the injections; when they survive, however, they become clinically completely fit again very rapidly. A few sections and cultivations from surviving animals showed that this did not mean that the animals had rid themselves of the bacteria; on the contrary, the organs were often crowded with them, and a constant appreciable bacteriaemia was present for many days; nevertheless, the animals were apparently not ill, nor did they ever die from the infection later.

We were soon able to confirm all the findings of Felix and his collaborators, but in the interpretation of some of them we differ from him.

The first questions we put ourselves were:

(1) What part does the Vi antigen play in the growth of the typhoid bacillus in mice?

(2) How do typhoid bacilli develop in mice vaccinated by different routes?

(3) How do specific sera given prophylactically and therapeutically influence the growth of typhoid bacilli in the mouse?

What part does the Vi antigen play in the growth of the typhoid bacillus in the mouse?

We have already mentioned that we were able to confirm Felix & Pitt's results in regard to the significance of the Vi antigen, in as far as we found also in our experiments that those strains in which this antigen could be demonstrated killed mice in smaller doses than those which had no demonstrable Vi antigen; but we also soon found by means of bacteriological examinations of the animals that the strains which did not contain this antigen grew or at least remained alive in the mice, although they did not kill the animals.

In the following experiments we always compared the variants of the same strain, and in most of the experiments administered the bacteria in such doses that no symptoms of acute intoxication arose. In mice weighing 18 g. and upwards a dose of 1 million typhoid bacilli intraperitoneally will seldom produce any visible disease, even when the most toxic strain is used; but in smaller animals of, say, 15–16 g., this dose may cause marked symptoms of intoxication, generally transitory. In this connexion we compared the

V and the W forms of both the strain Watson and the strain H 901, both of which we have in such constant forms that they can be used experimentally.

The experiments were carried out as follows. A series of mice were injected intravenously or intraperitoneally with 1 million living organisms, and at intervals afterwards animals were killed and cultures made from the appropriate organs. Table I shows the results of such an experiment with the two variants of the Watson strain. The bacteria cultivated from the animals were always examined serologically for constancy in antigenic structure which was found in all these experiments.

Table I

	No. 1 15 min.	No. 2 1.45 hr.	No. 3 2.30 hr.	No. 4 4 hr.	No. 5 5 hr.	No. 6 24 hr.	No. 7 24 hr.	No. 8 4 days	No. 9 5 days	No. 10 7 days
	Nos. 1–10 mice injected intravenously with 1 million typhoid bacilli, "Watson" V form									
Blood	+ +	(+)	0	0	((+))	0	0	0	0	0
Liver	+ + + +	+ + +	+ + +	+ +	+ +	+	+	0	0	0
Spleen	+ + + +	+ + +	+ + +	+ + +	+ + +	+ +	+ +	+	+	+
	Nos. 1–10 mice injected with 1 million typhoid bacilli intravenously, "Watson" W form									
Blood	+	0	((+))	0	0	0	0	0	0	0
Liver	+ + +	+ + +	+ + +	+ +	+ +	+	+	0	0	0
Spleen	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+	+	+	+

((+)) = 1 colony.
(+) = less than 10 colonies.
+ = up to a hundred colonies.
+ + = several hundred colonies.
+ + + = difficult to count.
+ + + + = nearly confluent.
∞ = confluent growth.

As will be seen from the results of the cultures, no difference can be demonstrated in the growth of the two variants; and we may conclude that the virulence antigen cannot play an essential part in the growth of the typhoid bacillus in the mouse.

Table II shows similar results with the two variants of the strain Typh. H 901.

Table II

	2 hr.	21 hr.	48 hr.	48 hr.	3 days	3 days	5 days	6 days	9 days	10 days	10 days	12 days	13 days	13 days
	Mice injected intravenously with 1 million typhoid bacilli, strain H 901, V form													
Blood	(+)	0	0	0	0	0	0	0	0	0	0	0	0	0
Liver	+	+	+	+	+	+	(+)	+	(+)	+	(+)	0	((+))	0
Spleen	+ + +	+ +	+ + +	+ +	+ + +	+ + +	+ + + +	+ + +	+ + +	+ + +	+	+ +	+ + +	+ +
	Mice injected intravenously with 1 million living typhoid bacilli, strain H 901, W form													
Blood	(+)	0	0	((+))	0	0	0	0	0	0	0	0	0	0
Liver	+	+	+	+	+	+	(+)	(+)	(+)	0	0	0	0
Spleen	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + + +	+ + +	+ + +	+ + +	+ +	+ +	+ + +

Table III gives the culture results from animals infected intraperitoneally, and shows that similar results are obtained when this route is used to infect the animals. A few hours after the injection some of the animals infected with the V form were slightly ill, but they soon recovered, and no animals died during the first 3 days. As will be seen, the first cultures were made on the

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Table III

	3 days	3 days	3 days	6 days	6 days	6 days	16 days	16 days	21 days
Mice infected intraperitoneally with 20 million typhoid bacilli, Watson V form									
Blood	0	0	0	0	0	0	0	0	0
Liver	0	0	0	0	0	0	0	0	0
Spleen	+	+	(+)	+	+	+	0	0	0
Mesenterial gl.	+	+++	0	+	+	++	0	0	0
Mice infected intraperitoneally with 20 million typhoid bacilli, Watson W form									
Blood	0	0	0	0	0	0	0	0	0
Liver	+	0	+	0	0	0	0	0	0
Spleen	+	+	++	+	+	+	+	(+)	0
Mesenterial gl.	+++	+++	+++	++	++	++	+	(+)	0

third day. From these two experiments, picked among many similar ones, we see that when typhoid bacilli are given in doses which do not cause acute intoxication, the significance of the virulence antigen in the growth of the typhoid bacilli in the mouse organism is slight. Finally it seemed possible that the V form could penetrate the mucous membrane of the intestinal tract better than the W form. A few experiments showed that this was not so; and we consider it permissible to conclude that the clinical and bacteriological findings we obtained after the injection of heavy doses of typhoid bacilli in mice are due to the combination of Vi antigen and a toxic substance found parallel with it, causing by its toxic effect better growth for the relatively little virulent typhoid bacillus. These views are quite in accordance with those of Petruschky, who made a very elaborate study of the typhoid infection in mice at the suggestion of no less a person than Robert Koch. These results are very similar to the infection mechanism of the dysentery bacillus in mice. A toxic dose will give the bacilli a much better chance of growing than when injected in smaller doses.

The picture may be different in man, in whom the virulence antigen may play a much greater part, since the typhoid infection in man is quite different from that found in mice.

From these experiments it seems justifiable to conclude that, when the bacilli are given in proper doses, the virulence antigen does not play a marked part in the growth of the typhoid bacillus in the mouse, and that when its presence is associated with a greater mortality in a smaller dose, this is due to the initial toxic effect which favours the rapid overwhelming of the organism with bacteria, these two factors causing the death of the animals. Secondly, we may conclude that the typhoid bacillus in our experiments has retained its serological type in the mice.

Our second question was: *How does the typhoid bacillus develop in the mouse vaccinated by different routes?*

Like Felix & Pitt, who demonstrated it clearly, we found that only Vi antigen containing strains, V strains, could be used as vaccines against the rapidly fatal doses of living bacilli containing Vi antigen when tested, for instance, 8 days after the last vaccinating dose has been given. But we have

not obtained a clear demonstration of what happens to the bacilli in organisms vaccinated differently.

Table IV shows the result of such an experiment. Series of animals were vaccinated with V forms and W forms, and infected later with V and W forms respectively. The animals in this experiment were a little smaller than those we used in other experiments, weighing only 15–16 g.; and this has played a certain part, as we can see from the control animals, most of which became very ill after intraperitoneal injection of as small a dose as 1 million of the V forms, many dying within 48 hours. The W form of H 901 causes no acute symptoms of disease, and the generalization which follows is much less marked than when the animals are infected with the V forms. If we compare the results of cultures obtained from the animals vaccinated with V forms and then infected with V forms and those of the control animals, we see a very marked difference in these results and those obtained with the control animals infected with the same V forms: the most striking difference is that the blood is very seldom infected in the vaccinated animals. Another more or less marked feature is the relatively scanty growth obtained from the vaccinated animals in the first 48 hours after infection. In accordance with our earlier experiments with vaccinations we find here also that the vaccination causes only a slow elimination of the infecting microbes, showing that the effect of the vaccination is relative. If we examine the vaccination results obtained with the W forms of H 901 and Watson strain respectively we find a striking difference in the two vaccines: H 901 protects the animals to a degree not essentially different from the effect obtained with the V forms, while the W form of Typh. Watson, especially against the V form of H 901, is little pronounced—three animals died within 48 hours after the infection, showing that these are probably strain differences not wholly covered by the factor of O and Vi antigens. Lastly we must mention that under these experimental conditions the vaccination effect against the W forms is more marked than against the V forms. This is not astonishing since the V forms in the doses used are relatively toxic. If a smaller infecting dose had been used which would not cause any intoxication in the control animals the differences in the controls and vaccinated animals would certainly have been much less marked. In earlier experiments we often observed that within the first 10–14 days after an injection the difference in culture results between specifically vaccinated animals and controls are often so small that they can only be demonstrated with difficulty; but after this time we often do find this difference.

Our third question was: *What part do different anti-typhoid sera given prophylactically and therapeutically play in the growth of the typhoid bacillus in the mouse?*

Since Felix & Pitt we also found that only the Vi serum protected animals against acute lethal doses of Vi-containing typhoid bacilli, V forms, but we did not obtain an answer to the question: how does a certain serum act

Table IV

Time after infection		24 hr.	48 hr.	48 hr.	3 days	3 days	7 days	7 days	10 days	10 days	10 days
Vaccinated with Typh. 2 V form, infected with Typh. 2 V form	Blood	0	0	0	0	0	0	0	0	0	
	Liver	++	0	+	+	+	0	0	0	0	
	Spleen	+++	+	+	+++	+++	0	+	0	0	
Vaccinated with Typh. 2 V form, infected with Typh. H 901 V form	Blood	0	0	0	0	0	0	0	0	0	
	Liver	(+)	0	0	(+)	0	0	0	0	0	
	Spleen	(+)	++	(+)	+++	+++	0	++	++	+	
Vaccinated with Typh. 2 V form, infected with Typh. H 901 W form	Blood	0	0	0	0	0	0	0			
	Liver	0	+	0	0	0	0	0			
	Spleen	+++	+++	0	+++	0	0	0			
Vaccinated with Typh. H 901 W form, infected with Typh. H 901 V form	Blood	0	+	0	0	++	0	0	0	0	
	Liver	((+))	++	0	(+)	++	0	0	0	0	
	Spleen	+++	+++	++	++	+++	+	+	+	+	
Vaccinated with Typh. H 901 W form, infected with Typh. 2 V form	Blood	0	0	0	(+)	(+)	0	0	0	0	
	Liver	+	0	0	+	+++	0	0	0	0	
	Spleen	+++	++	+++	++++	++++	++++	++	+	+++	
Vaccinated with Typh. H 901 W form, infected with Typh. H 901 W form	Blood	0	0	0	0	0	0	0	0	0	0
	Liver	0	+	0	(+)	0	0	0	0	0	0
	Spleen	0	+++	+++	+++	+++	++	++	+++	+++	+
Vaccinated with Typh. Watson V form, infected with Typh. 2 V form	Blood	0	0	0	0	0	0	0	0	0	
	Liver	(+)	++	+	+	+	++	++	++	++	
	Spleen	++	+++	+++	+++	+++	+++	+++	+++	+++	
Vaccinated with Typh. Watson V form, infected with Typh. H 901 V form	Blood	0	0	0	0	0	0	0	0	0	
	Liver	+	+	0	+	+	+	+	+	+	
	Spleen	+++	++	0	+++	+++	+++	+++	++	++	
Vaccinated with Typh. Watson V form, infected with Typh. H 901 W form	Blood	0	0	0	0	0	0	0	0	0	0
	Liver	0	0	0	(+)	(+)	0	0	0	0	0
	Spleen	+++	0	0	+++	++	+	++	++	++	+

Table IV (continued)

Vaccinated with Typh. Watson W form, infected with Typh. 2 V form

Time after infection	24 hr.	48 hr.	48 hr.	48 hr.	3 days	3 days	7 days	7 days
Blood	+	++	(+)	0	0	0	0	0
Liver	++++	++++	+++	++	++	(+)	++	+++
Spleen	++++	++++	++++	+++	+++	++	++++	+++++

Vaccinated with Typh. Watson W form, infected with Typh. H 901 V form

Time after infection	24 hr.	48 hr.	48 hr.	48 hr.	3 days	3 days	7 days
Blood		†	†	†	0	0	0
Liver	∞	∞	∞	∞	+	+	0
Spleen	∞	∞	∞	∞	++	+++	++

Vaccinated with Typh. Watson W form, infected with Typh. H 901 W form

Time after infection	24 hr.	48 hr.	48 hr.	48 hr.	3 days	3 days	7 days	10 days
Blood	0	0	0	0	0	0	0	0
Liver	0	0	0	0	0	0	0	0
Spleen	0	++	0	+++	+++	++	++	0

Control animals infected with Typh. 2 V form

Time after infection	24 hr.	48 hr.	48 hr.	48 hr.	48 hr.	48 hr.	3 days	3 days
Blood	∞	†	†	†	†	(+)	(+)	+
Liver	∞	∞	∞	∞	∞	+	+++	++++
Spleen	∞	∞	∞	∞	∞	∞	++++	+++++

Control animals infected with Typh. H 901 V form

Time after infection	24 hr.	48 hr.	48 hr.	48 hr.	48 hr.	3 days	3 days	7 days
Blood	++	†	†	†	0	0	+	0
Liver	∞	∞	∞	∞	++	++	+++	0
Spleen	∞	∞	∞	∞	+++	+++	++++	+++

Control animals infected with Typh. H 901 W form

Time after infection	24 hr.	48 hr.	48 hr.	48 hr.	3 days	3 days	10 days	10 days
Blood	0	(+)	0	0	0	0	0	0
Liver	(+)	+	0	0	0	0	0	0
Spleen	++	+++	+++	+++	+++	++	++	++

Series of mice are vaccinated with formol treated vaccines made from different variants of typhoid bacilli: Typh. 2 V form, Watson V form, Watson W form and H 901 W form. Doses 200 and 400 million given subcutaneously at 5 days interval. Fourteen days after the last injection the animals are infected intraperitoneally with 1 million living typhoid bacilli typh. 2 V form, H 901 V form and H 901 W form respectively.

against the infecting bacilli when given prophylactically? It is difficult to demonstrate the difference in the number of bacteria in experiments when large doses, e.g. 100 million, are used intraperitoneally, because the serum, though able to protect the animals against the acute toxic effect of the injection, cannot eliminate the bacteria within the first few days of infection to such an extent that differences would be easy to estimate. We therefore decided to examine the progress of infections arising after smaller and less toxic doses. In one of the following experiments the mice were rather small (under 16 g.), and the dose of 1 million was large enough to cause the animals to be clinically ill for some time, although it did not kill them. The difference in the number of bacilli in the two experiments will thus be due to the difference in size of the mice, though it cannot be excluded entirely that the strain used may not itself have differed a little in usual virulence.

We see from Table V that the Lister serum containing Vi anti-bodies can eliminate the typhoid bacilli very markedly, but we also note that an O serum (Africa) is nearly as potent. In the next experiment—Table VI—the mice were smaller, as already mentioned, so that a dose containing 1 million causes a much severer infection than in the above experiment. Here we find the same excellent effect of the Lister serum given prophylactically, while the effect therapeutically cannot be demonstrated. In the same table we give the results of an experiment with concentrated diphtheria horse serum; for we found that rabbit O sera caused a less marked elimination of the typhoid bacilli than horse sera. There can be little doubt that the non-specific serum had a certain effect.

We consider that we may conclude from these and many other similar experiments that both typhoid Vi and O sera given prophylactically have a marked bactericidal effect on typhoid bacilli, and that non-specific sera may have some, though a smaller, effect. Therapeutically Vi serum causes no demonstrable elimination of the bacilli.

During the work on the above questions we came to the conclusion that even the V form of typhoid bacillus is only slightly virulent for mice, and that the death of the animals is always due to an acute intoxication. When doses were used which only killed some of the animals we found by cultures from the surviving animals that they were astonishingly heavily infected for several days, the blood showing massive growth of typhoid bacilli although the animals clinically behaved like healthy animals. Survival did not consequently coincide with a rapid destruction of the injected bacteria.

SUMMARY

We have been able to confirm all the experimental results of Felix and his collaborators in regard to the Vi antigen and its significance in mouse infections. In the interpretation of some of the findings we are, however, not quite in agreement with Felix.

Table V

Prophylactically 0·2 Lister Vi serum intraperitoneally, 24 hr. later Typh. 2 V form. 1 million intraperitoneally

	3 hr.	24 hr.	48 hr.	4 days	5 days	6 days	9 days
Blood	0	0	0	0	0	0	0
Liver	(+)	((+))	0	0	0	0	0
Spleen	++	((+))	((+))	0	0	0	0

Prophylactically 0·2 c.c. *Africa O serum intraperitoneally, 24 hr. later Typh. 2 V form intraperitoneally

	3 hr.	24 hr.	48 hr.	4 days	5 days	6 days	9 days
Blood	0	0	0	0	0	0	0
Liver	(+)	((+))	0	0	0	0	0
Spleen	++	++	0	+	0	(+)	(+)

Control animals Typh. 2 V form, 1 million intraperitoneally

	3 hr.	24 hr.	48 hr.	4 days	5 days	6 days	9 days	9 days
Blood	0	0	0	0	0	0	0	0
Liver	+	+	+	0	+	(+)	0	((+))
Spleen	+++	+++	++	+++	+++	++	+++	+++

Mice weighing 18 g. * From the South African Institute, Johannesburg.

Table VI

Prophylactically 0·2 c.c. Lister Vi serum intraperitoneally, 24 hr. later infected with Typh. 2 V form 1 million intraperitoneally

	24 hr.	24 hr.	24 hr.	48 hr.	48 hr.	48 hr.	3 days	4 days	4 days	6 days	8 days
Blood	0	0	0	0	0	0	((+))	0	0	0	0
Liver	(+)	0	0	0	0	0	0	0	+++	0	0
Spleen	++	0	0	(+)	0	0	+++	+++	+++++	0	0

Typh. 2 V form 1 million intraperitoneally, 24 hr. later 0·2 Lister Vi serum therapeutically intraperitoneally

	24 hr.	24 hr.	24 hr.	48 hr.	48 hr.	48 hr.	3 days	3 days	3 days	5 days	7 days
Blood	0	++	0	++	++	0	+	+	+	+	0
Liver	+	++++	++	+++++	++++	+++	+++++	++++	++++	++	++
Spleen	++	+++++	++++	+++++	+++++	+++++	+++++	+++++	+++++	+++	+++

Three animals die within the first 48 hr. of infection.

Control animals infected with 1 million Typh. 2 V form intraperitoneally

	24 hr.	24 hr.	24 hr.	48 hr.	48 hr.	48 hr.	3 days	3 days	4 days	6 days	8 days
Blood	∞	∞	∞	+++	+++++	+++++	0	+	++	++	0
Liver	++++	++++	++++	+++++	+++++	+++++	+	++	+++	+++	+
Spleen	+++	+++++	+++++	+++++	+++++	+++++	+++	+++	+++++	+++	+++

Prophylactically 0·2 c.c. diphtheria serum intraperitoneally, 24 hr. later Typh. 2 V form 1 million intraperitoneally

	24 hr.	24 hr.	24 hr.	48 hr.	48 hr.	48 hr.	3 days	3 days	5 days	7 days
Blood	0	++	0	0	0	0	0	0	0	0
Liver	+++	++++	++	++	0	0	++	++	++	+
Spleen	+	+++++	++	+	(+)	+	+++	+++	+++	+++

Mice weighing 15 g.

When large doses of living V forms of typhoid bacilli are injected into the mouse they cause acute intoxication followed by death within 2 days—if the animals die. Surviving animals are clinically healthy after this time, often earlier, but they are nevertheless found to be massively infected in the organs and blood for several days.

When smaller, non-intoxicating, doses are used it is not possible to demonstrate any difference between the V form and the W form of the same strain in regard to the severity of the infection they cause; so that we may conclude that the Vi antigen plays no part in the virulence of the typhoid bacillus in the mouse. (Remember in this connexion that the dysentery bacillus is also very toxic for mice injected in large doses, while its virulence, i.e. the ability to grow in the mouse is = 0; but when rapidly fatal doses are used a massive growth of dysentery bacilli can also be demonstrated here, the cause being that the natural immunity has become partly neutralized by the intoxication.)

Like Felix we find that only the V form can be used both for active vaccination and the production of sera which protect against lethal doses of V forms in mouse experiments. We consider that this is due to the antitoxic effect. Our experiments show that the anti-bacterial effect in vaccination experiments differs little whether the V or the W form is used as vaccine, so long as the vaccinal effect is tested with non-toxic doses of bacilli. As shown in earlier experiments, the bactericidal effect in actively vaccinated animals is often a relatively slow process.

Only the Vi antibody containing sera can protect prophylactically with certainty against the toxic effect of living typhoid bacilli. On the other hand, the bactericidal effect prophylactically of O and O + Vi sera is very similar when tested against non-toxic doses of "virulent" typhoid bacilli.

The therapeutic value of potent Vi sera under the experimental conditions given was nil.

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