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## THE "TYPHOID-CARRIER" STATE IN RABBITS AS A METHOD OF DETERMINING THE COMPARATIVE IMMUNIZING VALUE OF PREPARATIONS OF THE TYPHOID BACILLUS

### *STUDIES IN TYPHOID IMMUNIZATION. I\**

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#### INTRODUCTORY

Prophylactic immunization against typhoid fever offers sufficient security against subsequent infection to render its general application advocable. Such application is not only becoming wide-spread and mandatory in segregated, controllable bodies like armies, but has proved very advantageous in communities in general. In surveying the already extensive statistical literature, one becomes convinced of the relative protection against typhoid fever that is enjoyed by those who have been immunized with preparations of the typhoid bacillus; one is likewise convinced that the results obtained and the methods of immunization followed leave much to be desired.

Why should the results of immunization against typhoid infection be less perfect than those obtained in small-pox and rabies? Why should artificial immunization with the typhoid bacillus offer so much less perfect protection than recovery from typhoid fever? In respect to the method employed we may well question why 500,000,000 killed typhoid bacilli is the recommended dose, and why the interval at which this dose should be repeated is arbitrarily set at seven or ten days. The fact is that we possess only too little information of an exact sort about methods of prophylactic immunizing with bacteria in general. In the case of antityphoid immunization, empiricism rather than experimentation, has largely determined the method employed. The results attained are so good that they should be better, and it would seem that the time has arrived to return to animal experimentation in order to save time in determining the ultimately best method of protecting man.

The best evidence that the most satisfactory method has yet to be determined lies in the fact that a number of methods are still being

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advocated. Friedberger,<sup>1</sup> in his exhaustive article on antityphoid immunization, mentions no less than twelve well-recognized preparations of the typhoid bacillus which may be employed. Paladino Blandini<sup>2</sup> has actually endeavored to test the comparative immunizing value of seventeen different typhoid preparations. In addition to the actual preparation to be employed, we have to consider the dose used, the method of estimating the dose, the intervals between doses and the like.

The chief reason why animal experimentation has not done more to perfect the method of typhoid vaccination is the reasonable skepticism which obtains at transferring results attained in a vastly different and artificial infection in animals to a very characteristic human disease. There seems little or no resemblance between the more or less common type of peritonitis produced in the guinea-pig by the typhoid bacillus and actual typhoid fever. Until very recently it has been supposed that nothing resembling typhoid fever in man could be produced in animals. In 1911, Metchnikoff and Besredka<sup>3</sup> described a disease in chimpanzees which had been given food contaminated with the excreta from typhoid fever patients, which resembled in practically all details the human syndrome. They further attempted to utilize this experimental disease as a means of comparing the immunizing value of various typhoid preparations. Their results even in the limited aspect of the problem they attacked can hardly be regarded as conclusive in view of the necessarily small number of animals employed and the fact that their tentative results differ from what is known to be true in man. We shall consider their report more fully later, but here it may be noted simply that desirable as tests might be in the experimental disease most closely simulating typhoid fever in man, it would be impossible from the item of expense and supply alone to carry out any extended experiments on these animals.

#### PRODUCTION OF AN ARTIFICIAL TYPHOID-CARRIER STATE IN

##### RABBITS

It has been known since the observations of Blackstein<sup>4</sup> and Welch<sup>5</sup> in 1891, that living typhoid bacilli injected into the circulation of rabbits can subsequently be recovered in pure culture therefrom, for considerable periods of time. Further work has served to explain the nature of this

1. Friedberger: Die Methoden der Schutzimpfung gegen Typhus, Kraus and Levaditi's Handbuch der Immunitätsforschung, 1908, i, 723.

2. Paladino, Blandini: Proflassi specifica del tifo addominale, Ann. d'ig. sper., xv, 1905, 295.

3. Metchnikoff and Besredka: Recherches sur la fièvre typhoïde expérimentelle, Ann. de l'Inst. Pasteur, 1911, xxv, 193.

4. Blackstein: Intravenous Inoculation of Rabbits with Bacillus Coli Communis, and the Bacillus Typhi Abdominalis, Bull. Johns Hopkins Hosp., 1891, ii, 96.

5. Welch: Additional Note Concerning the Intravenous Inoculation of the Bacillus Typhi Abdominalis, Bull. Johns Hopkins Hosp., 1891, ii, p. 121.

"carrier" condition more fully. The injections must be intravenous to yield positive results,<sup>6</sup> and it is evident from the varying success of different observers that not all strains of the typhoid bacillus give equally good results. It is clear from the results of Morgan,<sup>7</sup> Koch,<sup>8</sup> Doerr,<sup>9</sup> and Johnston,<sup>10</sup> that the permanent reservoir of the organism is the gall-bladder, which is invaded within two hours after intravenous inoculation.<sup>6</sup> It may be that, under usual conditions, the circulation is only invaded periodically, which would explain why Johnston failed to get positive cultures from the blood with regularity before the period from the seventh to the tenth day, and also would indicate the reason why Doerr failed to obtain positive blood-cultures after the fourth day. It is certain that failure to produce a carrier should not be judged from a single blood-culture. At all events, cultures from the gall-bladder are positive more constantly and for longer periods than cultures from the blood. Constant lesions of the gall-bladder have been described by Chirolanza in particular and will be further detailed in connection with our own protocols.

The persistence of the typhoid bacillus in the rabbit has been noted for varying lengths of time: in the blood for 58 days (Chirolanza); 109 days (Blackstein); 127 days (Johnston); and in the gall-bladder for even longer periods: 180 days (Uhlenhuth and Messerschmidt<sup>11</sup>); 75 days (Morgan); 120 days (Doerr). The organisms are apparently eliminated through the intestine in which they have been found by Doerr in the mucosa up to 120 days after inoculation. They were likewise repeatedly found in the feces by Morgan and by Johnston.

The actual pathogenicity for rabbits of strains of the typhoid bacillus capable of producing the carrier state in these animals does not appear to have received particular attention from previous observers. Morgan mentions, without further particulars, that two out of twelve of his animals died in twelve hours. Similar observations were made by Chirolanza. Johnston mentions the obtaining of positive cultures from bile and blood in nearly all fatal cases, but does not give the percentage of carrier rabbits that died. Another point of particular interest in view of our employment of the carrier state as a means of demonstrating the

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6. Chirolanza: Experimentelle Untersuchungen über die Beziehung der Typhusbacillen zu der Gallenblase und den Gallenwegen, *Ztschr. f. Hyg. u. Infektionskrankh.*, 1909, lxii, 11.

7. Morgan: Attempts to Produce the Typhoid-Carrier State in the Rabbit, *Jour. Hyg.*, 1911, xi, 202.

8. Koch. Typhusbacillen und Gallenblasen, *Ztschr. f. Hyg.*, 1909, lxii, 1.

9. Doerr: Experimentelle Untersuchungen über das Fortwuchern von Typhusbacillen in der Gallenblase, *Centralbl. f. Bakteriöl.*, 1905, Orig. xxxix, 624.

10. Johnston: Experimental Typhoid-Carrier State in the Rabbit, *Jour. Med. Research*, 1912, xxvii, 177.

11. Uhlenhuth and Messerschmidt: Versuche Kaninchen zu Typhusbacillen-Trägern zu machen und sie therapeutisch zu beeinflussen, *Deutsch. med. Wchnschr.*, 1912, xxxviii, 2393.

absence or presence of an active immunity is the uniform presence of agglutinins in positive rabbits (Morgan, Johnston, Doerr).

The typhoid-carrier state in rabbits has been used as a means of testing certain therapeutic measures. Conradi<sup>12</sup> has shown that daily doses of chloroform in oil or milk given by the rectum suffice to cure the carrier rabbits. Hailer and Rimpau<sup>13</sup> have obtained similar results with methyl iodid and iodoform. Johnston has been able to cure the bacteremia by two doses of 20,000,000 killed typhoid bacilli.

To meet our experimental needs, it was necessary that we should be able to produce a typhoid bacillemia with regularity, since the efficacy of a given method of vaccination could be judged only by the failure of properly protected animals to become carriers, whereas the normal controls should give positive results uniformly. The irregularity in the results of other observers was not very encouraging, and our own first results were even less so.

It seems to us that no wholly satisfactory method of obtaining an exact dosage of living bacteria has been devised. Perhaps the least objectionable method consists in employing a given portion of a suspension of a standard agar surface, particularly when the dose is of necessity large, as in the present case. In order to obtain comparable results in different experiments, we have used thick-walled culture-tubes of uniform size (inside diameter 18 mm.), containing 10 c.c. each of a 2 per cent. peptone agar, or in the majority of our experiments agar containing 10 per cent. defibrinated rabbit blood. These tubes are slanted at a fixed angle of about 6 degrees, so that the entire solidified surface is, within a small margin of error, uniform. Twenty-four-hour growths of such cultures suspended in salt solution have been used in amounts representing fractions of an original culture. By plating out these blood-cultures, we estimate that each one contains 1,400,000 million organisms. The blood-cultures are more abundant than corresponding agar-cultures.

The first five strains of *Bacillus typhosus* tested, which included three from the collection of the California State Board of Health Laboratory, Army and Navy Strain 5 obtained from the Cutter Laboratory, and one strain (65), obtained from Professor Zinsser, in an intravenous dose of the entire standard agar-culture previously referred to, failed to produce carriers, to kill, or seriously to affect rabbits which weighed from 1,500 to 2,000 gm. Two other cultures gave positive results, Culture 6 killing a small rabbit in twenty hours, and Strain 3, which had been isolated two weeks previously from a human blood-culture, produced a carrier that gave a positive culture ten days later. The reisolations from these two

12. Conradi: Ueber sterilisierende Wirkung des Chloroform im Tierkorper, Ztschr. f. Immunitätsforsch., 1910, vii, 158.

13. Hailer and Rimpau: Versuche über Abtötung von Typhusbacillen in Organismus, Arbeit. a.d.k. Gsndtsamte, 1911, xxvi, 409.

animals were made on rabbit-blood agar. All of our subsequent experimental work has been carried on with cultures grown on this medium. To this procedure we attribute, at least in part, our present success in producing a bacteriemia regularly. Since our first success in producing a carrier with a whole standard agar-culture of Strain 3, we have failed with subsequent generations of the culture grown on agar to produce carriers, whereas the blood-agar cultures produce carriers regularly. Inasmuch as the growth on the blood-medium is more abundant, a little less than twice the amount of the agar growth by weight, we have used a whole agar-culture in comparing its infectiousness with half a blood-agar culture. Culture 6, which in a dose of a whole agar-culture killed the first rabbit in twenty hours, in the first blood generation from this animal (Culture 6a) failed to produce a bacteriemia in a dose of one-half of a culture, and the strain was abandoned for Culture 3a and subsequent generations, which gave positive results in this dosage.

A survey of our protocols in which groups of vaccinated rabbits were tested with normal controls, shows that one-half of a standard blood-culture of our Strain 3 of *B. typhosus* produces with almost perfect regularity characteristic pathogenic effects which do not occur in properly protected animals. Smaller doses, as one-quarter of a culture, produce less consistent results. Thus of seventeen control animals used in our latest experiments we find: 1. Three died in from two to twenty-four hours with symptoms of acute intoxication. 2. Thirteen gave one or more positive cultures of *B. typhosus* from the blood<sup>14</sup> at periods from the fifth to the twenty-eighth day, and of these animals five died between the fifth and twentieth day. Of the other eight, only one certainly recovered, as shown by negative cultures from bile and blood; through error, four animals were not observed over a sufficient period of time to know whether they would have died or recovered. 3. Only one animal gave apparently negative results. Cultures from the blood were negative on the sixth, eighth and twentieth day, and when the animal was killed by bleeding on the thirty-fifth day, cultures from the gall-bladder and blood were also negative. The animal, however, showed extreme loss of weight (2,440 to 1,710 gm. on the thirtieth day), and the blood-serum on the thirty-fifth day agglutinated the typhoid bacillus in a dilution of 1 to 2,000.

The carrier state in rabbits is not accompanied by any distinctive or continuous rise in temperature.

Chirolanza has given a full description of the lesions he found associated with the typhoid-carrier state in rabbits, and a few observations

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14. The routine cultures are taken by allowing 10 drops of blood to drop from the carefully cleaned ear vein into bile bouillon. If contaminations are evident, which is rarely the case, the culture is plated out. (Culturally probable typhoid bacilli are tested with an agglutinating serum.)

have also been made by Morgan. The lesions affect the gall-bladder primarily and to a less extent the liver and bile ducts. According to Chirolanza, animals dying in the first few hours after inoculation show cloudy swelling, subcapsular hemorrhages and zonal necroses of the liver. The gall-bladder is not at first affected, but after a day or two the mucosa becomes necrotic. The later changes in this organ are more characteristic and consist in proliferation of the mucosa of the gall-bladder, more rarely a loss of mucosa and thickening of the fibromuscular coat; thickening of the bile-duct and gradual lymphoid infiltration and cirrhosis of the liver.

Although we do not pretend to offer any systematic study of the morbid anatomy of this condition, certain constant lesions are noted in our protocols.

In rabbits in which death is rapid, that is, within twenty-four hours, the gall-bladder shows no changes; the liver shows chronic passive congestion and usually central necrosis of the lobule. We have not studied the intermediate period in any detail, but beginning with the second week certain characteristic changes are to be noted. At necropsy the gall-bladder is uniformly distended and frequently is four or five times the usual size. The bile is usually cloudy or contains visible flocculi and at times a mass of inspissated bile salts is present that fills and distends the gall-bladder. In our experience complete destruction of the mucosa and reduction of the wall to a thickened fibromuscular coat is more frequent than the proliferation described by Chirolanza. We have been unable to convince ourselves of any constant changes in the liver beyond the early zonal necrosis, that is characteristic of bacterial infections in general.

It is of importance in justifying our use of the typhoid-carrier rabbit and in showing that conditions in these animals are analogous to the conditions in typhoid infection in man, to note that the lesions of the gall-bladder in typhoid cases in man are quite similar to those in the infected rabbits. J. Koch has compared these conditions very carefully, using his own and Chirolanza's material. He has further shown that the infection of the bile in man and in the rabbit is through the circulation and not from the intestine by way of the bile duct. The analogy of the lesions in man and in the rabbit is also emphasized by Uhlenhuth and Messerschmidt.<sup>11</sup>

We have further found marked injection of the cecum in two instances, and actual hemorrhage in one instance in carriers, similar to those produced regularly by the toxin of the typhoid bacillus.<sup>15</sup>

We find, then, that the intravenous injection of a uniform amount of a rabbit-blood agar-culture of our strain of *B. typhosus* produces an almost invariable and characteristic syndrome in normal rabbits (94 per

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15. Arima: Ueber die Typhustoxine und ihre pathogene Wirkung, Centralbl. f. Bakteriol., Orig., 1912, lxxiii, 424.

cent. positive). They either die with acute symptoms of intoxication within the first few hours and without characteristic lesions (18 per cent.) or become more or less chronic carriers (76 per cent.), yielding positive cultures from the blood for two or three weeks, within which time many of them die. Cultures from the gall-bladder of these animals are even more uniformly positive than the blood-cultures, and the bladder itself shows characteristic lesions in the distention and necrosis of the mucosa. The bile becomes inspissated in the majority of cases. Inadequately immunized animals give the same result.

We do not wish at this time to consider in detail the methods of comparative prophylactic immunization with the preparations of the typhoid bacillus that we have employed, but reserve it for a separate report, when our already considerable data have been further amplified. It may suffice to say that we have been comparing dead and living cultures of the organisms, sensitized (Besredka) and unsensitized cultures, and, finally, various fractions of dried and ground bacilli. It will suffice at this point to state that animals which have been fully immunized with, let us say, whole dried and ground preparations of *B. typhosus*, show no ill effects on injection of the standard amount of living blood-culture. The properly protected animals, following the test intravenous inoculation, show no considerable loss of weight, give uniformly negative cultures from twenty-four hours on, and when killed at considerable periods (from twenty-one to thirty-five days) subsequently, present uniformly negative findings at necropsy. In other words, this intravenous method of inoculation has given a nearly perfect method of comparing the relative immunizing property of various preparations of the typhoid bacillus. The small margin of error (6 per cent. in our series) incident to the failure of the exceptional normal animal to give positive results may be corrected by using a sufficient number of animals in testing each immunizing preparation.

It remains to discuss the extent to which we believe we are justified in regarding the carrier condition in rabbits as analogous to a typhoid infection in human beings. The work of recent years on typhoid fever in man has led to considerable readjustment of our ideas as to its pathogenesis. To quote Kolle and Hetsch,<sup>16</sup> we no longer regard typhoid fever as an intestinal disease, but primarily as a bacteriemia. This statement is based first on the fact that blood-cultures taken in the first week of the disease in an appropriate manner yield positive results in 100 per cent. of cases (Brion and Kayser).<sup>17</sup> The bile also gives positive cultures in

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16. Kolle and Hetsch: Die experimentelle Bakteriologie und die Infektions-Krankheiten, Berlin, 1911, i, 250; 3d edit., Urban und Schwarzenberg.

17. Brion and Kayser: Neuere Klinisch-bakteriologische Erfahrungen bei Typhus und Paratyphus, Deutsch. Arch. f. Klin. Med., 1906, lxxxv, 552.

practically all cases and at all stages of the disease (Forster and Kayser<sup>18</sup>). The occurrence of cases of typhoid fever without intestinal lesions is another reason for regarding these typical lesions as secondary rather than primary.<sup>19</sup> There is evidence, indeed, that the lesions in the ileum may be eliminative and secondary to the bacteriemia rather than a primary focus of the disease. The proliferation of lymphoid tissue in the intestine and elsewhere characteristic of the disease has been logically attributed to the typhoid toxins (Mallory) rather than to simple multiplication of the bacillus.

The mere fact, then, that the injection of living typhoid bacilli in rabbits does not lead to the characteristic swelling and necrosis of the agminated follicles of the intestine, as is usual in typhoid fever, is no reason for regarding the rabbit syndrome that we have described as essentially dissimilar from the condition in man. In both rabbit and man the typhoid bacillus remains for considerable periods in the circulating blood, and in both it promptly invades the bile by means of the circulation and not by the bile duct (J. Koch, Doerr, Chirolanza). It remains in both human and rabbit carriers in the gall-bladder even after it has disappeared from the circulation and in both cases it is subsequently eliminated through the intestinal mucosa. The characteristic result of gall-stone formation which may follow typhoid infection in man has also been produced in rabbits by Richardson<sup>19</sup> by giving calcium. It has been noted that the intestinal lesions may more properly be attributed to eliminated toxins of the typhoid bacillus, and it is interesting to note that similar lesions may be produced in rabbits by the use of typhoid toxins<sup>15</sup> instead of living cultures. We have obtained similar results with killed cultures of *B. typhosus* in sufficient doses. Arima produces by means of intravenous injection of his "endotoxin" of *B. typhosus*, bloody diarrhea, hyperemia, swelling and hemorrhage of the intestinal lymph-nodes.

We regard these facts as indicating the very close analogy of infections produced by the typhoid bacillus in man and in the rabbit. Apart from its intrinsic value as a clean-cut experimental method, the rabbit "carrier" condition would seem to offer, next to experimentation on anthropoid apes, the best means for the comparative testing of various immunizing preparations of the typhoid bacillus, designed to be used on man.

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18. Forster and Kayser: Ueber das Vorkommen von Typhusbacillen in der Galle von Typhuskranken und "Bacillenträgern," München. med. Wehnschr., 1905, lii, 1473.

19. Possett: Atypische Typhusinfektion. Typhus ohne Darmerkrankung. Lubarsch and Ostertag: Ergebn. d. allg. Pathol., 1912, xvi, 184.

19. Richardson: On the Rôle of Bacteria in Gall-Stones, Jour. Boston Soc. Med. Sc., 1899, iii, 79.