THE FATE OF TYPHOID BACILLI WHEN INJECTED INTRAVENOUSLY INTO NORMAL RABBITS.

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PLATE 54.

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The course of typhoid bacillus infection in the rabbit has been followed by many investigators. Certain facts are established: The typhoid bacilli injected into the blood do not long remain in the circulation; the gall bladder frequently becomes invaded quickly after the injection; and the bacilli survive and multiply there for a long period of time.[1] Ultimately they may disappear from all parts of the body except the gall bladder.

The present study was undertaken to determine more accurately than had hitherto been done the manner in which typhoid bacilli are removed from the blood of normal rabbits. This study was part of a more general study of the mechanism of bacterial immunity in the rabbit,—whether native or acquired. The rabbit may be regarded as possessing a high degree of natural immunity for the typhoid bacillus, and the fate of the bacilli injected was traced from the blood through the various organs in arriving at an explanation of their disappearance.

Source of the Cultures.

The strain of typhoid bacilli used in the mass of the work was obtained from a capsule of Besredka's sensitized vaccine kindly supplied by Captain H. J. Nichols of the Medical Corps, U. S. Army. Three other typical strains from widely different sources were used to check the results obtained with the Nichols strain. All four strains had been under artificial cultivation for some time and should

[1] Nichols, H. J., *Jour. Exper. Med.*, 1914, xx, 573.

be considered as essentially non-virulent, although death of the animal due to intoxication sometimes ensued. The bacilli used for inoculation were cultivated on plain agar and used when twenty-four hours old. For injections they were washed from the medium with 0.85 per cent sodium chloride solution.

Disappearance of Bacilli from the Blood.

From one-thirtieth to one-fiftieth of the bacilli from an agar slant were injected into the ear vein and Petri plates were made from the heart's blood at various intervals, beginning as early as thirty seconds after the injections. It was found that the first specimen taken always contained the largest number of bacilli and that they left the blood with remarkable rapidity. Cultures made ten minutes after the injections contained only a few colonies. Specimens taken fifteen to twenty minutes after injecting the bacteria were often sterile. In some cases a few colonies developed in cultures made several hours after the inoculations or at the time of the death of the animals. Even when the bacilli from an entire agar slant were given and death resulted in two hours the blood was frequently sterile. The following instance will serve as a typical experiment.

Experiment 1.—A rabbit weighing 2,000 gm. was given 1/40 of a 24 hour agar slant of typhoid bacilli in the ear vein. Blood cultures made from the heart at stated intervals gave the results indicated in Table I.

TABLE I.

Time after injection. *min.*	No. of colonies per cc.
1	10,000,000
2	2,500,000
5	100,000
15	40
20	1

In several experiments of this kind the same general results were obtained. In some instances the bacilli left the blood somewhat more slowly than in others, but the variation was a matter of a few minutes only.

The abrupt disappearance of typhoid bacilli from the blood stream was investigated. Why should the bacilli leave the blood

so much more rapidly than other bacteria, namely, streptococci, pneumococci, or dysentery bacilli of the Shiga type, which were studied? If the process was merely a matter of filtration by the capillary systems of the various organs as Wyssokowitsch[2] concluded, this difference should not exist. The notion prevailing has been that the typhoid bacilli are destroyed (dissolved) by the rabbit's blood within a short time. Indeed, it is known that normal rabbit's whole blood or serum *in vitro* kill the bacilli in relatively brief periods of time. Our next inquiry was directed to the organs to determine whether the bacilli taken out of the blood accumulated in them.

Relation of Bacilli to the Tissues.

From one-thirty-fifth to one-fiftieth of the growth from an agar slant was injected into the ear vein of normal rabbits and specimens of blood were taken for culture from the heart one minute after the inoculation to determine the number of bacilli injected, and again at ten minutes to make sure that the bacilli had left the circulating blood. The rabbits were then killed by a stroke on the neck at ten, twelve, or twenty minutes, and the various organs removed and crushed finely in tissue crushers, after which definite quantities of the pulps were thoroughly shaken in sterile salt solution, and plated. The results of this test are given in illustrative Experiment 2. It may be stated here, however, that in no instance were as many bacilli found in any of the organs per unit of measure as had been present in the blood, and moreover that most of the tissues contained very few bacilli at all.

Experiment 2.—A rabbit was given 200,000,000 typhoid bacilli, as determined by test plating, into the ear vein, and the following data were determined: blood from the heart 1 minute after the injection gave 3,000,000 colonies per cc.; 6 minutes after, 80; and 16 and 20 minutes after, no colonies per cc. The tissues removed at 21 minutes gave colonies per cc. of crushed tissue pulp as follows: spleen, 2,000,000; liver, 1,600,000; lung, 100,000; mesenteric lymph node, 1,000; skeletal muscle, brain, and other tissues, comparatively very few. The weights of the organs were determined and the total number of bacteria recovered, with the finding that it was far below the actual number injected. In none of the organs was the number of colonies per unit of measure as great as the number originally found in the blood.

[2] Wyssokowitsch, W., *Ztschr. f. Hyg. u. Infectionskrankh.*, 1886, i, 3.

From this and similarly conducted experiments it was concluded that the bacilli did not accumulate in the tissues, and it was therefore considered desirable to follow the course of events in an organ for several hours after the bacilli had been injected into the blood. The liver was selected for this study since it is possible to remove portions of the organ at various intervals over a period of time. A typical result is given in Experiment 3.

Experiment 3.—A rabbit was given 1/35 of an agar slant of a culture of typhoid bacilli into the ear vein and estimates were made as follows: Blood 1 minute after the injection gave 15,000,000, and 10 minutes after, 10 colonies per cc.; liver 3 minutes after the injection gave 12,000,000, 14 minutes 6,000,000, 1 hour 700,000, 2 hours 80,000, and 3 hours 1,000 colonies per cc. of crushed pulp.

The data supplied by Experiment 3 support, on first examination, the prevailing notion that certain bacteria and, in this instance, typhoid bacilli fall a ready victim to the destroying and presumably the dissolving effect of the blood, whether in the general circulation or in the capillaries of the organs. This view is based in large part on the classical studies of Wyssokowitsch, and is upheld by the well known fact that fresh serum and the whole blood of rabbits are highly destructive *in vitro* to the typhoid bacilli. Without, however, adopting the usual explanation of the disappearance of the typhoid bacilli from the blood and organs, as illustrated by the experiments on the liver, it was deemed desirable to repeat certain of the experiments on the bactericidal action of normal rabbits' blood.

Action of the Serum and Whole Blood of the Rabbit in Vitro on Typhoid Bacilli.

Agglutinins.—The serum of normal rabbits was tested for agglutinating value. The bacilli were grown upon agar slants and washed off with 5 cc. of normal saline solution. One drop of the suspension of bacilli was added to one cc. of the serum dilution. The agglutinating value of normal serum varied from zero in full serum to positive in 1 to 100 dilution. About one-fifth of the sera examined were devoid of demonstrable agglutinins. The agglutinins are thermolabile and are destroyed at 56° C.

Bactericidal Power.—The fresh serum obtained from the heart's

blood after coagulation was diluted with saline solution and from 2,000 to 4,000 typhoid bacilli (estimated by test plating) were added to one cc. of the dilution. The mixtures in test-tubes were incubated at 37° C. for two hours and the entire contents of the tubes were plated. In order to eliminate the clumping of the bacilli and adhesion to the sides of the tubes, melted agar was poured into them after the plating and they were incubated and examined.

The results can be stated as follows: The sera of different rabbits vary considerably in destructive effect on typhoid bacilli. Full serum and serum diluted 1 to 10 usually destroyed all the bacilli. Serum diluted 1 to 50 usually caused diminution but never complete destruction of the bacilli. The defibrinated whole blood and hirudinized blood act in a manner similar to the serum.

There can, therefore, be no doubt that fresh rabbit serum and the whole rabbit blood destroy considerable numbers of typhoid bacilli *in vitro*. Whether the same means operate in the destruction which takes place *in vivo* remains to be determined.

Fate of Typhoid Bacilli in the Blood and Organs of Inoculated Rabbits.

The experiments performed with the pneumococcus[3] suggested that a similar study be made of typhoid bacilli injected into the circulation of rabbits, especially in view of the fact that while rabbits readily succumb to typhoid intoxication they are highly resistant to typhoid infection. Experiment 4 will serve as an illustration of this class of tests.

Experiment 4.—An agar slant of typhoid bacilli was suspended in about 5 cc. of salt solution and injected into the ear vein of a normal rabbit. Specimens of the heart's blood were taken and films prepared at 30 seconds, 1, 2, 3, 5, and 7 minutes. The films were stained by Manson's method. Clumps of bacilli were found even in the first film (Fig. 1). The second film showed a larger number of clumps, while the number diminished in the next specimen, and none were found in the last, or 7 minute, specimen. At the end of 7 minutes the rabbit was killed by a stroke on the neck and the organs were immediately removed and finely crushed in tissue crushers. Films were prepared from the pulp and stained by Manson's method. Microscopical examination of the slides showed that clumps of bacilli had accumulated in the capillaries, sinusoids, and blood spaces

[3] Bull, C. G., *Jour. Exper. Med.*, 1915, xxii, 457.

of the various organs, especially the liver, lungs, and spleen, and a large proportion of the clumps had already been phagocyted by the polymorphonuclear leucocytes (Figs. 2, 3, and 4) which had accumulated in the organs following the injection of the bacteria. Free and unclumped bacilli were also found.

This experiment was repeated in its essential aspects several times with wholly concordant results. The experiment was varied in such a manner as to permit the inoculated rabbits to survive for different periods of time, after which the blood and organs were examined. It was found that the largest number of leucocytes enclosing clumps of bacilli were present in the organs of animals killed from thirty to ninety minutes after inoculation. The organs of animals permitted to survive three or four hours still contain many leucocytes, but very few leucocytes containing distinct bacteria are met with. Leucocytes containing granules of disintegrated bacilli or bacilli which have lost power of staining may be found in small number. On the other hand, the three hour specimens still show a certain number of unagglutinated bacilli, outside cells with staining properties unimpaired (Fig. 5).

Still another variation of the experiment was to remove portions of the liver from ten minutes to two hours after injecting the bacilli. The findings just described were corroborated by this procedure. Specimens taken from ten to ninety minutes contained many phagocyting cells; while later specimens showed fewer phagocyting cells and finally cells which enclosed only disintegrating bacteria.

DISCUSSION.

If we review the findings described in this paper we shall arrive at somewhat conflicting results as to the manner in which typhoid bacilli are disposed of, respectively, by the body and the blood of the rabbit.

Directing attention first to the phenomena observed outside the body it may be affirmed, in keeping with usual knowledge, that the fresh blood serum as well as the fresh whole blood of the rabbit is capable of destroying, apparently by a process of solution, considerable numbers of typhoid bacilli. There is no reason, moreover, to doubt that the process of destruction in this instance is the

common one of bacteriolysis in which amboceptor and complement play the decisive part.

But the essential question at issue is not the extracorporeal but the intracorporeal method of destruction of typhoid bacilli. It is upon that point that light is especially needed. To apply directly the results of test-tube experiments to the explanation of what takes place in the body itself has not proven wholly illuminating. We already know that typhoid bacilli may appear and survive in the blood of human typhoid fever patients at a time when the shed blood is highly bacteriolytic for the bacilli.

The observations which this paper records indicate a wide disparity between the processes involved in the destructions of the bacilli in test-tubes and in the living body. In the latter, the bacilli introduced into the blood are quickly agglutinated, after which they are removed by the organs. In the interstices of the organs they come into close relation with polymorphonuclear leucocytes (themselves assembled in the organs as result of the bacterial injection) which englobe and destroy them. There is no evidence at hand which connects ordinary bacteriolysis with *intra vitam* destruction of typhoid bacilli. The unagglutinated and unphagocyted bacilli in the organs resist longest and stain best; and complement has yet to be detected in the circulating plasma.

This view of the process of *intra vitam* destruction of typhoid bacilli may serve to explain the fact that typhoid bacilli sometimes circulate in the blood of typhoid patients. It is known that bacilli cultivated from the circulating blood are often inagglutinable. We have found no indications that phagocytosis of the bacteria studied by us takes place in the blood or on a grand scale in the unagglutinated state. Hence, as the bacilli cannot be agglutinated and removed by the organs and also cannot be phagocyted in the blood stream, they continue to circulate, under some conditions, until they are removed and destroyed by the phagocytes.

The indications therefore are that in the body the destruction of typhoid bacilli by means of bacteriolysis does not take place. The question arises, however, whether in the tests of the survival of the bacilli in the pulp the conditions produced do not render the operation

of the bacteriolytic processes impossible. To test this point, crushed liver, spleen, and kidney were added to fresh normal rabbit sera just previous to introducing the typhoid bacilli. The test-tubes were incubated for two hours, after which plates were made. It developed that the action of the spleen and kidney pulps was negligible, while the liver pulp caused complete inhibition of bactericidal effect. Further tests indicated that it is the biliary constituent of the liver that is responsible for this action, since bile in quantities themselves non-hemolytic inhibits the activity of a hemolytic system apparently through its anticomplementary effect.

SUMMARY.

Typhoid bacilli are agglutinated promptly in the circulating blood of normal rabbits and quickly removed from the blood stream.

The clumped bacilli accumulate in the organs and are taken up by assembled polymorphonuclear leucocytes in the liver, spleen, and possibly other organs.

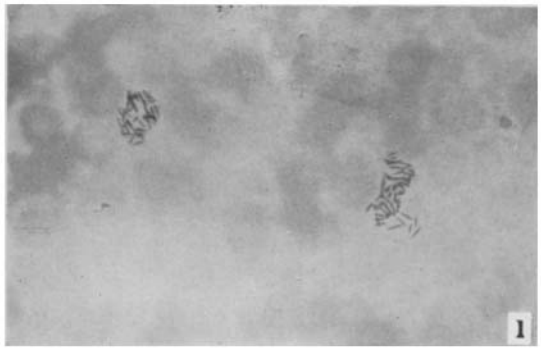
The phagocyted clumps of bacilli are digested and destroyed by the phagocytes.

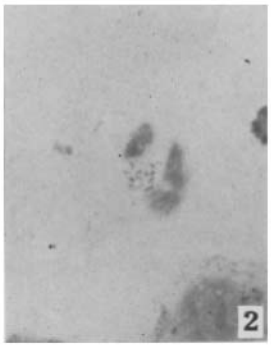
Hence, destruction of typhoid bacilli *intra vitam* is brought about by an entirely different process than is the destruction by serum and whole blood *in vitro*. While the latter is caused by bacteriolysis, the former results from agglutination and intraphagocytic digestion.

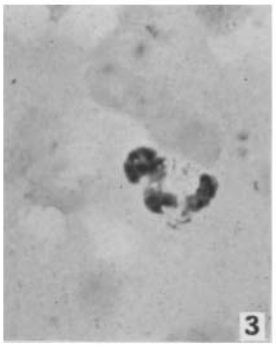
Lysis by fresh blood serum is not appreciably affected by spleen or kidney pulp, but it is inhibited by liver pulp. The action of the liver is referable to its biliary constituents, which exert anticomplementary action.

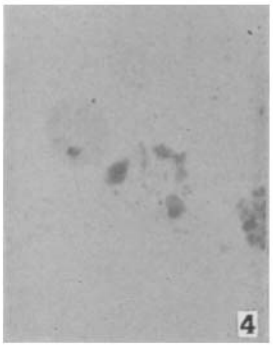
Probably in certain examples of typhoid fever in man the typhoid bacilli in the circulating blood being inagglutinable cannot be removed by the organs and hence are not phagocyted and destroyed.

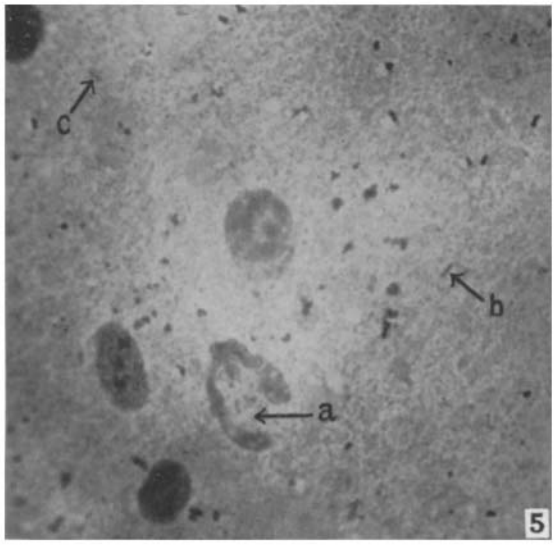
The observed disparity between the ready destruction of typhoid bacilli by serum and shed blood and the resistance sometimes offered by the bacilli in the infected body is explained by the essential differences in the destructive processes in operation within and without the body.











(Bull: Fate of Typhoid Bacilli Injected into Normal Rabbits.)

EXPLANATION OF PLATE 54.

FIG. 1. Heart's blood from a rabbit containing 2 clumps of typhoid bacilli. The specimen was taken 30 seconds after the bacteria were injected into the ear vein. Stained by Manson's method.

FIGS. 2, 3, and 4. Polymorphonuclear leucocytes from lung, liver, and spleen, respectively, containing clumps of typhoid bacilli. The tissues were removed from the animal 30 minutes after the bacteria were injected. Manson's stain.

FIG. 5. A smear of crushed liver tissue. The specimen was removed from a rabbit 2½ hours after an intravenous injection of typhoid bacilli. (a), leucocyte containing disintegrated bacilli; (b) and (c), free bacilli staining deeply. Manson's stain.