EXPERIMENTAL TYPHOID INFECTIONS IN THE RABBIT.

By MAJOR S. L. CUMMINS AND MAJOR C. C. CUMMING.

Royal Army Medical Corps.

In the Journal of Medical Research, vol. xxvii, No. II, p. 177 (November, 1912), an interesting and important paper appeared by Dr. J. A. Johnston on the treatment of the “typhoid carrier state” in the rabbit by inoculations with an anti-typhoid vaccine. The animals used in the experiment were infected by inoculation with 0.5 c.c. of an emulsion of one agar slope of Bacillus typhosus, given intravenously.

Certain of these rabbits were left untreated as “controls,” while others were treated by the inoculation of a dose of killed anti-typhoid emulsion administered from thirty-six to forty-eight days after the preliminary infecting inoculation.

The animals were chloroformed at stated intervals and the blood and the organs examined by cultural methods for the presence of B. typhosus. The experiments appeared to indicate some beneficial effect following the vaccine treatment, since out of seven rabbits so treated B. typhosus could only be recovered from two, while out of eleven untreated animals it was recovered from ten.

A further observation of great interest was recorded by Dr. Johnston to the effect that, several days after the preliminary septicæmia resulting from the intravenous inoculation had passed off, the typhoid bacilli reappeared in the blood and could be isolated therefrom, positive results being obtained from the heart blood in animals killed at dates varying from seven to thirty days after inoculation. The importance of this result, as offering a possible line of research in the pathogenesis of typhoid fever, appeared to us to be very great, and we decided to carry out a series of experiments with the object of confirming, if possible, Dr. Johnston's work. At the same time we decided to try the effect of a preliminary anti-typhoid inoculation in a certain number of animals in order to ascertain whether any prophylactic action, as opposed to the therapeutic action investigated by Dr. Johnston, could be demonstrated.

Experiments.

Ten rabbits of approximately equal weight and age were selected, numbered from 1 to 10, and divided into two groups.
Group 1, consisting of rabbits Nos. 1 to 5, was treated as follows:—

On January 20, 1913, each rabbit was inoculated with 50 million killed typhoid bacilli.

On January 30, 1913, each rabbit received a second dose of 100 million bacilli.

Group 2, consisting of rabbits Nos. 6 to 10, was left untreated.

On February 4, 1913, the pooled serum of Group 1 was found to agglutinate B. typhosus up to a dilution of 1 in 300.

Thermostable opsonins, calculated by the dilution method, were as follows:—

<table>
<thead>
<tr>
<th>Serum dilutions</th>
<th>Group 1</th>
<th>Control</th>
</tr>
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<tbody>
<tr>
<td>1/2</td>
<td>169</td>
<td>47</td>
</tr>
<tr>
<td>1</td>
<td>38</td>
<td>1</td>
</tr>
<tr>
<td>1/4</td>
<td>22</td>
<td>1</td>
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<tr>
<td>1/8</td>
<td>24</td>
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<tr>
<td>1/16</td>
<td>2</td>
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</tr>
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</table>

The numbers refer to the number of bacilli counted in 50 phagocytes.

It was recognized that, in order to produce a degree of immunity corresponding to that following anti-typhoid inoculations in man, larger doses would have to be given, but owing to pressure of other work it was decided to continue the experiment without further loss of time.

At noon on February 13, 1913, all the rabbits of both groups were given an intravenous inoculation of 0.5 c.c. of an agar culture of B. typhosus (Eyre) which had been emulsified in 5 c.c. of normal saline.

Blood cultures from an ear vein of each rabbit were made at 3 p.m. on February 13, 14, 15, 17, and 18, and subsequently twice weekly.

The faces of each rabbit were emulsified and plated once a week from February 25 onwards. Two rabbits (one from each group) were chloroformed and examined post mortem, by culture and otherwise, each week from March 3 onwards. The method employed for making cultures from the viscera was as follows: The whole spleen, kidney, or gall-bladder (less such portions as were used for sections), and a whole lobe or more of the liver, were removed in turn with sterile precautions, dipped in spirit and "flamed" to kill any superficial contamination, placed in a sterile glass mortar and pounded up with sterile solution of sodium taurocholate in distilled water (0.5 per cent), the whole contents of the mortar being then transferred to a flask and incubated.

We desire to lay stress on this method as it is much more likely to afford a positive result than the method usually adopted of cutting
Experimental Typhoid Infections in the Rabbit

into the viscus, and removing a loopful of such tissue and blood as is obtainable in the incision. The temperatures of the individual rabbits were taken once daily up to February 17, and twice daily from that onwards in the hope of eliciting the occurrence of pyrexia in association with any secondary septicæmia that might develop. As a control the temperature of three normal rabbits was also observed at the same time. The main points observed in the case of each rabbit will now be recorded in order.

Rabbit 1.—Beyond the initial rise for forty-eight hours after inoculation and the fall on the third day, the temperature showed no marked disturbance. The animal remained in good health up to March 4 (the nineteenth day after the inoculation), when it was chloroformed. On no occasion was B. typhosus isolated from the faeces during life.

<table>
<thead>
<tr>
<th>DATE</th>
<th>FEB.</th>
<th>MARCH</th>
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<tbody>
<tr>
<td></td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>DAY of DIS.</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

![Chart comparing the temperature of Rabbit VI with the average temperature of the whole series of ten animals.]

Post-mortem.—The heart’s blood, spleen, bile, gall-bladder, and the contents of the small intestine were all negative as regards the isolation of B. typhosus. This bacillus was, however, isolated in pure culture from the pounded-up substance of the liver. Blood culture was positive on February 13, 14, and 15, after which it was always negative.

Rabbit 6.—This rabbit was distinctly affected by the inoculation, the temperature rising to 107°F. on the day following inoculation, and reaching 104°F. or upwards in 13 out of 31 observations. (On no occasion did the average temperature of the three control rabbits reach 104°F.) Blood culture was positive on February 13, 14, and 15, negative on 17, 18, and 20, positive again on February 24 (the eleventh
day), negative on February 27 and March 3. The strain isolated on February 24 was only agglutinated with great difficulty and incompletely after three hours by an anti-typhoid serum in a dilution of 1 in 500. The normal titre of this serum was up to 1 in 10,000. Culturally the bacillus was typical B. typhosus. It is of interest to note that the strain used for the intravenous inoculation was easily agglutinated by this serum, and it seems that this strain must have acquired, within the animal, relatively serum-fast properties.

The serum of Rabbit 6 tested on March 3 agglutinated B. typhosus (Eyre) up to a dilution of 1 in 1,000. B. typhosus was never isolated from the faeces of this animal. The rabbit was chloroformed on March 4, the nineteenth day after inoculation.

Post-mortem.—The heart's blood, bile, gall-bladder, and the contents of the small intestine were all culturally negative for B. typhosus. This organism was, however, isolated from the spleen and the liver in pure culture. The strains isolated from these organs were agglutinated with comparative ease by the antityphoid serum before mentioned. It is to be noted that this rabbit was the only one of the series to show a continued modification of the temperature, the only one in which a secondary septicemia occurred, and the only one in which the spleen gave a positive culture.

Rabbit 2.—B. typhosus was isolated from the blood on the day of inoculation only. Subsequently to the three days' rise following the inoculation, which was noted in most of the animals, this rabbit twice showed a temperature of 104°F or over (on February 22 and 25). On no occasion was B. typhosus isolated from the faeces. When the rabbit was chloroformed on March 12 cultures from the heart's blood, spleen, liver, gall-bladder, bile, contents of small intestine, urine, and kidney were all negative as regards B. typhosus.

Rabbit 7.—In all essentials this rabbit gave results similar to Rabbit 2. It was chloroformed on the same date, March 12, 1913, and as in the case of Rabbit 2, B. typhosus could not be isolated.

For the reason that they showed excretal evidence of infection, it was thought desirable to chloroform Rabbits 4 and 9 out of their turn, in order to ascertain as far as possible on what pathological condition the excretion of bacilli depended.

Rabbit 4.—B. typhosus was isolated from the blood on the day of inoculation and again on February 15 (forty-eight hours after inoculation). In all subsequent cultures the blood was found negative.
After the preliminary pyrexia, the temperature reached 104°F. on two occasions only (March 25 and April 1). *B. typhosus* was isolated from the faces on March 11 (twenty-six days after inoculation). The animal was chloroformed and examined on March 19. *B. typhosus* was present in cultures from the liver and from the contents of the small intestine. Cultures from the heart's blood, spleen, gall-bladder, and bile were all negative.

On examining sections of the gall-bladder, large agglomerations of gram-negative bacilli were found close to the walls, while the epithelium was degenerated and the more superficial cells of the gall-bladder wall, those immediately under the epithelial lining, were found to show intense pyknosis of their nuclei. It is difficult to explain why the gall-bladder wall and the bile gave a negative result in culture unless some error occurred in our technique or the bacilli were dead. The isolation of living *B. typhosus* from the contents of the duodenum, these having presumably reached their situation by the bile, points to the former as the more probable explanation. Sections of the liver showed marked fatty degeneration of the liver cells.

*Rabbit 9.*—*B. typhosus* was isolated from the blood on the day of inoculation, on February 14 and 17 (twenty-four and ninety-six hours after inoculation). On all subsequent cultures the blood was found negative. The temperature after the usual preliminary rise never attained 104°F. on any subsequent date. *B. typhosus* was isolated from the faces on February 25 (twelve days after inoculation). The faces were negative on March 4, 11, and 19.

The rabbit was chloroformed on March 19, and the usual post-mortem examination made.

*B. typhosus* was isolated from the liver, the gall-bladder, and the bile in pure culture. The heart's blood, spleen, and the contents of the small intestine were negative.

Sections of the gall-bladder showed appearances exactly similar to those already described in Rabbit 4. Liver sections showed intense fatty degeneration of the liver cells. In neither Rabbit 4 nor Rabbit 9 were we able to find bacilli in the sections of the liver, although both livers gave a positive result on culture.

*Rabbits 3 and 8* were chloroformed and examined on March 25, and *Rabbits 5 and 10* on April 3. All of these rabbits gave positive blood cultures on the day of inoculation, and twenty-four hours later. In none of them was *B. typhosus* isolated from the blood on any subsequent date, though attempts at culture were continued in all of them up to the date of post-mortem. In no case
Clump of typhoid Bacilli in the gall-bladder of rabbit 9 (Leishman's stain).

Portion of the gall-bladder wall of rabbit 9 showing pyknotic changes in the nuclei (Weigert's and Van Gieson's stain).
S. L. Cummins and C. C. Cumming

was *B. typhosus* isolated from the faeces of these animals, nor did any of them give a positive culture from tissues or organs on post-mortem examination.

On May 1, 1913, two more rabbits were inoculated intravenously with *B. typhosus*, "G. Ford," a strain isolated a year previously from an acute case, and kept in an agar "stab," without subculture since the date of its verification by cultural and serological tests. Of these two, Rabbit A was chloroformed on May 29, and the usual post-mortem carried out, followed by cultures from the tissues and organs. *B. typhosus* was isolated, together with a large non-motile gram-negative bacillus, from the liver. The spleen, kidney, gall-bladder, bile, and heart's blood were all negative, and no bacilli could be found in sections of the gall-bladder.

Rabbit B was similarly treated a week later, June 5, with entirely negative results.

The results of the above experiments may be summarized as follows:

**Comparison of Group I and Group II.**

As regards any prophylactic effect of the preliminary inoculation with anti-typhoid vaccine in Group I (Rabbits 1 to 5), we may say at once that the inoculated group of rabbits showed no definite protection as compared with the non-inoculated group, nor is this result surprising when it is recalled that we administered directly into the blood-stream a large dose of living typhoid bacilli, thus opposing to the limited equipment of protective substances conferred on the inoculated animals a task out of all proportion to that which has to be encountered when an inoculated human being ingests into the intestine a relatively small number of bacilli in contaminated food or water. In comparing prophylactic with therapeutic inoculation, it must always be remembered that the former is intended to initiate a relative immunity sufficient to inhibit the development within the body of a comparatively small number of germs, while the latter is intended to supplement the efforts at immunization already initiated by body cells attempting to cope with an established attack. We believe that work on similar lines to these experiments may throw much light on the effects of protective inoculation, provided that due weight is given to such quantitative factors as the degree of antibody production and the number of living bacilli subsequently inoculated.
It is open to question whether rabbits are the best animals to use for such investigations. Possibly monkeys might furnish results more comparable with human beings. Large numbers of observations are essential both for the preliminary elucidation of conditions affecting the relative dosage of the prophylactic vaccine and the infecting inoculation, and for the subsequent experiments, so that the question of cost takes a prominent place in deciding upon what animal to use. The experiments of Metchnikoff and Besredka point to chimpanzees as the ideal animals for this purpose, but the expense is prohibitive.

Turning to the cultural and post-mortem results of the experiment under consideration, the apparent absence of any prophylactic result from the preliminary inoculation makes it possible to treat the whole series as one group. Of ten rabbits examined, a secondary typhoid septicemia was only demonstrated in one, this being on the eleventh day after inoculation. This rabbit also differed from the others in showing irregular temperature for several weeks after inoculation, and in giving a positive splenic culture on post-mortem nineteen days after inoculation.

This failure to confirm Johnston's observation in more than one case out of ten may be due to our having worked with a less virulent strain of \textit{B. typhosus}. The strain used, \textit{B. typhosus} "Eyre," had been isolated by one of us a year and a half before from the blood of an acute case, but had been frequently subcultured in the interval, so that its virulence was undoubtedly very low. It is interesting to note that out of seven rabbits post-mortemed up to the thirty-fourth day after inoculation, \textit{B. typhosus} was isolated from the liver in five, while there was only evidence of infection of the bile and gall-bladder in two.

These results differ from those recorded by other workers who have invariably obtained a large number of positive cultures from the bile and the gall-bladder, while positive cultures from the liver have been much less frequent. Johnston mentions negative findings in the liver in four cases, and makes no record of positive cultures from that organ, though the bile was infected in a majority of the animals examined.

Doerr ("Experimentelle Untersuchung über das Fortwuchern von Typhusbazillen in der Gallenblase," \textit{Centralblatt von Bakt.}, Bd. xxxix, p. 625, 1905) records four positive liver cultures from nine rabbits, all of which gave positive cultures from the bile. In none of his cases was liver culture mentioned as positive after the fourteenth day.
Hailer and Rimpau ("Versuche über Abtötung von Typhusbazillen im Organismus," Arbeit aus dem Kaiserlichen Gesundheitsampte, Bd. xxxvi, p. 409) obtained many positive cultures from the liver but, apparently, none after the fourteenth day.

Somewhat similar results were obtained by Chiarolanza (Zeit. Hygiene, Bd. lxii, p. 2), but he, too, only found the liver positive at short intervals after inoculation. We think the discrepancies may be explained as follows:

(1) The small number of positive results from the bile and gall-bladder are owing to the fact that we made no post-mortem before the nineteenth day after inoculation. Had we killed the animals within the last fortnight after inoculation, our results would probably have been comparable with those of others in this respect.

(2) Our success in isolating B. typhosus from the livers of five out of seven rabbits killed between the nineteenth and thirty-fourth day after inoculation may be attributed to our having worked with very large quantities of liver substance, whereas most of the recorded liver-cultures have consisted in the addition of a loopful of liver substance to some culture medium. We think it probable that B. typhosus could be recovered from the liver in nearly every case within the first two or three weeks after intravenous inoculation, if a sufficient volume of the liver substance were used. In another series of four rabbits post-mortemmed by us within twenty-four hours of intraperitoneal inoculation, the liver substance was found to give positive cultures in all.

It is probable that the isolation of B. typhosus from the bile in the first fortnight after inoculation usually depends on the presence of germs coming from the infected foci in the liver and is really an indication of infection of that organ. Only in cases where the gall-bladder itself becomes infected is it possible to isolate B. typhosus from the bile at long intervals after inoculation.

CONCLUSIONS.

(1) Prophylactic inoculations, in the doses given, had no appreciable effect, two rabbits out of three in each group showing the presence of living typhoid bacilli in the tissues up to nineteen days and over after inoculation, and one rabbit out of each group becoming an excreter of typhoid bacilli, the excretion being associated with bacterial deposits and histological changes in the gall-bladder.
Experimental Typhoid Infections in the Rabbit

(2) Positive cultures of *B. typhosus* were obtained from the livers of five out of seven rabbits post-mortem between the nineteenth and the thirty-fourth day, and in five out of twelve rabbits post-mortem between the nineteenth and forty-seventh day, after intravenous inoculation of one-tenth of an agar culture of *B. typhosus*.

(2) These results deserve consideration in connexion with the use of living anti-typhoid vaccines administered intravenously, as suggested by Nicolle, Conor, and Conseil (*Comptes-Rend. Acad. Sciences*, July 15, 1913, p. 147), though, of course, the difference in dose must be borne in mind.
Experimental Typhoid Infections in the Rabbit

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