

ATTEMPTS TO REPRODUCE THE TYPHOID-CARRIER STATE IN THE RABBIT.

(PRELIMINARY COMMUNICATION.)

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With Plate III.

SINCE the work of Blachstein and Welch (1891) who showed that rabbits inoculated intravenously with the typhoid bacillus might continue to harbour that organism in the gall-bladder for a prolonged period, much help in the elucidation of the typhoid-carrier state in man has come from the results of experimental investigation of this condition in the rabbit.

Among those who have contributed most materially to our knowledge of this subject one may cite the names of Forster and Kayser (1905), Doerr (1905) and Koch J. and Chiarolanza (1909), the latter of whom has shown that the infection of the gall-bladder following intravenous inoculation of *B. typhosus* in the rabbit most probably takes place directly through the blood vessels of the gall-bladder wall, although a secondary invasion by infected bile from the liver may certainly take place at a somewhat later period.

Histological examination of the gall-bladder and biliary tracts in such acute cases has revealed the presence of bacillary depôts of embolic origin at the extremities of the rugose papillary formations which give the mucosa the shaggy appearance so characteristic of typhoidal infection in the human gall-bladder.

In rabbits which have been killed at a later period after inoculation and found to be still harbouring the typhoid bacillus, very similar changes have been noted. Thus in one of Chiarolanza's experimental

rabbits which was sacrificed on the 14th day after inoculation, the gall-bladder mucosa showed inflammatory foci near the tips of the shaggy papillae, but bacilli could not always be demonstrated in them by staining methods, although a pure culture of the *B. typhosus* was obtained.

It is obvious that this method of experimentation offers a wide prospect of studying the carrier-state under laboratory conditions and particularly with a view to treatment. Already some preliminary experiments by Conradi (1910) and Hailer and Rimpau (1911) have appeared in which some success has been achieved in freeing the animal from typhoid bacilli by the intrarectal injection of Chloroform, Bromoform, Iodoform and other substances.

Most of these therapeutic attempts have been begun as a rule not later than the first fortnight after infection but a small series of animals was experimented upon by Conradi about the 25th day and in these cases recourse was had to preliminary laparotomy in order to determine whether the gall-bladders were really infected at this date.

The experiments which I propose to record in this short note were commenced in Oct. 1910 and the main object in view was to determine the frequency with which the chronic carrier condition occurs in the rabbit after intravenous inoculation and the length of its duration. It was also hoped that a clue to the infected condition of the gall-bladder might be obtained from the systematic bacteriological examination of the faeces. If such examination proved successful it was intended also to institute various therapeutic measures with a view to influencing the gall-bladder lesions.

Author's Experiments.

Between the dates Oct. 17, 1910, and Oct. 29, 1910, twelve rabbits with an average weight of 2.52 kilos received a single intravenous inoculation of 4 c.c. of a 24-hour broth culture of *B. typhosus*. In six of the animals a strain isolated from an intestinal carrier was employed, and in the other six a strain from a urinary carrier.

Two animals died within 12 hours from the effects of the injection.

The systematic examination of the faeces of the remaining ten rabbits was begun about ten days after inoculation. Before plating on MacConkey's medium the faeces were incubated for 24 hours at 37° C. in 0.5% malachite-green broth.

The agglutinin-content of the rabbits' sera was also estimated at intervals, the homologous organism being employed.

*The Typhoid-carrier State**Results in the individual rabbits.*

Rabbit I. Wt. 2.50 kilos. Inoculated on Oct. 17, 1910.

Agglutinins: 31 Oct., 1 in 2000 + + +; 9 Nov., 1 in 2000 + + +; 15 Nov., 1 in 2000 +; 27 Nov., 1 in 2000 +; 13 Jan. 1911, 1 in 2000 + +; 21 Feb., 1 in 2000 +.

Faeces: Exams. on 1 Nov., 9 Nov., 17 Nov., 23 Nov., 1 Dec., 12 Jan., 4 Feb., 21 Feb., 1 Mar., 3 Mar. All negative.

Rabbit IV. Wt. 1.85 kilos. Inoc. Oct. 18, 1910.

Agglutinins: 31 Oct., 1 in 2000 + + +; 9 Nov., 1 in 2000 + + +; 15 Nov., 1 in 2000 + +; 29 Nov., 1 in 2000 +; 13 Jan., 1 in 2000 + +; 21 Feb., 1 in 2000 +.

Faeces: Exams. on 1 Nov., 9 Nov., 17 Nov., 23 Nov., 1 Dec., 12 Jan., 4 Feb., 21 Feb. All negative.

Rabbit V. Wt. 3.50 kilos. Inoc. 21 Oct. 1910.

Agglutinins: 31 Oct., 1 in 2000 + + +; 9 Nov., 1 in 2000 + + +; 22 Nov., 1 in 2000 + +; 29 Nov., 1 in 2000 +; 13 Jan., 1 in 2000 + + +; 21 Feb., 1 in 500 +.

Faeces: Exams. on 1 Nov., 14 Nov., 17 Nov., 23 Nov., 1 Dec., 12 Jan., 4 Feb., 21 Feb. All negative.

Rabbit VI. Wt. 2.68 kilos. Inoc. Oct. 22, 1910.

Agglutinins: 31 Oct., 1 in 2000 + + +; 9 Nov., 1 in 2000 + + +; 22 Nov., 1 in 2000 + + +; 29 Nov., 1 in 2000 + + +; 13 Jan., 1 in 2000 + + +; 21 Feb., 1 in 2000 + +.

Faeces: Exams. on 1 Nov., 14 Nov., 17 Nov., 23 Nov., 1 Dec., 12 Jan., 4 Feb., 21 Feb. All negative.

Rabbit VII. Wt. 2.0 kilos. Inoc. Oct. 24, 1910.

Agglutinins: 1 Nov., 1 in 2000 + + +; 10 Nov., 1 in 2000 + + +; 22 Nov. 1 in 2000 + + +; 30 Nov., 1 in 2000 + + +.

Faeces: **Positive** on 4 Nov. 1910.

Further examinations on 14 Nov., 19 Nov., 28 Nov., 1 Dec. were all negative.

Animal died on 6 Jan. 1911 (see below).

Rabbit VIII. Wt. 2.65 kilos. Inoc. Oct. 25, 1910.

Agglutinins: 1 Nov., 1 in 2000 + + +; 10 Nov., 1 in 2000 + + +; 22 Nov., 1 in 2000 + + +; 30 Nov., 1 in 2000 + + +; 24 Jan., 1 in 500 + +, 1 in 2000 O; 23 Feb., 1 in 500 + +.

Faeces: Exams. on 4 Nov., 14 Nov., 19 Nov., 28 Nov., 1 Dec., 18 Jan., 6 Feb., 22 Feb. All negative.

Rabbit IX. Wt. 2.35 kilos. Inoc. Oct. 26, 1910.

Agglutinins: 1 Nov., 1 in 2000 + + +; 10 Nov., do.; 28 Nov., do.; 6 Dec., do.; 24 Jan., 1 in 2000 +; 23 Feb., 1 in 2000 +.

Faeces: Exams. on 4 Nov., 15 Nov., 19 Nov., 28 Nov., 5 Dec., 18 Jan., 6 Feb., 22 Feb. All negative.

Animal showed paralysis of hind legs and diarrhoea and was killed (see below).

Rabbit X. Wt. 2·85 kilos. Inoc. Oct. 27, 1910.

Agglutinins: 7 Nov., 1 in 2000 + + ; 10 Nov. 1 in 2000 + + + ; 28 Nov. 1 in 2000 + + ; 6 Dec., do.; 24 Jan., 1 in 2000 +.

Faeces: **Positive** on 4 Nov. 1910.

Further examinations on 15 Nov., 19 Nov., 28 Nov., 5 Dec., 18 Jan., 6 Feb. were all negative.

Animal died on 13 Feb. 1911 (see below).

Rabbit XI. Wt. 1·97 kilos. Inoc. Oct. 28, 1910.

Agglutinins: 1 Nov., 1 in 2000 + + + ; 15 Nov., do.; 28 Nov., do.; 6 Dec., do.; 24 Jan., 1 in 2000 + ; 23 Feb., 1 in 500 + + , 1 in 2000 O.

Faeces: Exams. on 9 Nov., 15 Nov., 23 Nov., 28 Nov., 5 Dec., 6 Feb., 22 Feb. All negative.

Rabbit XII. Wt. 2·17 kilos. Inoc. Oct. 29, 1910.

Agglutinins: 7 Nov., 1 in 2000 + ; 15 Nov., 1 in 2000 + + + ; 28 Nov., do.; 6 Dec., do.; 25 Jan., do.; 23 Feb., 1 in 2000 +.

Faeces: Exams. on 9 Nov., 15 Nov., 23 Nov., 28 Nov., 5 Dec., 13 Feb., 22 Feb. All negative.

In two instances only, therefore, was the typhoid bacillus recovered from the faeces, at the end of eight days after inoculation in the case of Rabbit X and at the end of 11 days in the case of Rabbit VII. Rabbit VII was found dead on 6 Jan. 1911, *i.e.* about 2½ months after inoculation. The *B. typhosus* was recovered in pure culture from the bile and gall-bladder wall but not from the heart blood or colon. Plate III shows a cross section of the gall-bladder in this case. Apart from the gall-bladder lesion and the finding of *B. typhosus* no obvious cause of death could be found. Although the heart blood gave a negative result, the possibility is not excluded that death may have been due to an autoinfection. One has also to consider the possibility of some anaphylactic disturbance as a contributory cause of death owing perhaps to the sudden liberation of typhoid antigen from the gall-bladder area. Dr Ledingham informs me that on several occasions he has isolated *B. typhosus* from the gall-bladder of guinea-pigs which had received sublethal doses of the living organism 4–5 weeks previously.

Rabbit X was found dead on 13 Feb., 1911. There were no obvious lesions at autopsy and the *B. typhosus* was not recovered. (Examination of Bile, Gall-bladder wall, Duodenum, Ileum, Colon, Spleen, Liver and Heart Blood.)

Rabbit IX was killed after showing paralysis of the hind legs and diarrhoea. *B. typhosus* was not recovered from any of the organs.

Attempts to exhibit B. typhosus in the faeces by the use of purgatives.

Owing to the persistently negative results obtained by examination of the faeces in the majority of the rabbits, it was thought that the administration of purgatives might help matters. The rabbits however proved to be extraordinarily resistant to nearly all the well-known purgatives.

The following drugs were given by the mouth, one after the other without effect: Mag. Sulph. $\frac{1}{2}$ oz., Phenolphthalein gr. XV, Pulv. Jalap. Co. $\frac{1}{2}$ oz., Paraffinum liquidum $1\frac{1}{2}$ oz., and finally Croton oil in doses of 13 minims.

Arecolin $\frac{1}{10}$ grain was given hypodermically with no better result, also $\frac{1}{10}$ grain of Eserine, the only effect of which was to produce violent spasms in the hind legs, lasting for about two hours but which led to no purgation. At last it was found that Calomel in doses of 4 grains produced slight purgation on the following day but not invariably. A dose of Calomel was therefore given to each rabbit on the day before the faeces was collected but in spite of the purgation it was impossible to recover the typhoid bacillus.

Laparotomy and Puncture of Gall-bladder.

Between Feb. 28, 1911, and Mar. 9, 1911, laparotomy was performed on the remaining seven rabbits but in four only was the gall-bladder found infected at the operation. In all four cases the bile, removed by puncture, proved sterile. One cannot conclude, however, that the animals are uninfected as it has been shown that the bile may be free from typhoid bacilli while the bladder wall may still yield a positive result on culture.

All the animals made an excellent recovery from the operation and it is proposed to reinoculate them in order to determine whether, according to Fornet's view (see Ledingham, 1910), the carrier-state is more likely to follow a second infection.

The blood of these 7 rabbits was still found to agglutinate the typhoid bacillus, four months after injection in a dilution of 1 in 500.

SUMMARY.

Preliminary record of attempts to produce the typhoid-carrier state in the rabbit and to obtain evidence of the presence of this condition by bacteriological examination of the faeces.



Illustrating the paper by Mr H. de R. Morgan.
(Description: see p. 207.)

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DESCRIPTION OF PLATE III.

Section of mucosa of gall-bladder (Rabbit VII), from which *B. typhosus* was recovered at death 2½ months after inoculation. Rugose condition well marked. Inflammatory foci with numerous chromatic debris (A, B, C) present in some of the papillae, and in one of them (A) the focus surrounds the central vessel of the papilla. Organisms were not definitely recognised in these foci by staining methods. These partially necrotic foci obviously represent the advanced stage of the embolic bacillary depôts observed in early cases.