GUINEA-PIGS AS CHRONIC CARRIERS OF AN ORGANISM BELONGING TO THE FOOD-POISONING GROUP.

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A good deal of research has been devoted during the last few years to the subject of "carriers" in connection with various diseases of man, but up to the present time less attention has been directed to the existence of the same condition in animals.

In November last an epizootic broke out amongst a stock of about 500 guinea-pigs at the Lister Institute and all but 21 of them died. A bacillus of the food-poisoning group was closely associated with the epizootic[1]; its relationship to the transmission of the disease is discussed in another paper, but from its frequent occurrence in animals during the epizootic, in the intestines as well as in the organs, an examination of the faeces of the survivors of the epizootic appeared to be desirable.

Two of the animals were killed for other purposes, while of the remaining nineteen, ten had been tested for evidence of acquired immunity by the subcutaneous inoculation of a broth culture of the bacillus above mentioned. The subcutaneous inoculation of the bacillus is often followed by its appearance in the intestine so that any carriers found amongst these ten animals could not be called natural carriers, and the results of the examination of their faeces are therefore not included in Table I, which shows the results of the examination, at different dates between January and May, of the faeces of nine of the guinea-pigs which survived the epizootic.

The pellets of faeces were incubated in dulcite broth at 37° C. for 48 hours, and from the tubes that showed gas a loop of broth was spread on MacConkey lactose plates to which saccharose had been added[2].

[1] The identification of the bacillus will be discussed later.

[2] The use of plates containing both lactose and saccharose was found to facilitate the isolation of the bacillus, since many organisms present in faeces which do not ferment lactose but produce acid in saccharose are excluded by this method.

TABLE I.

Results of examination of faeces of nine of the survivors of the epizootic.

Date of examination	Results of examination : occurrence of bacillus in the faeces			
1. 1. 1910 to 8. 4. 1910	Guinea-pig "A"—8 examinations, all positive.			
	Guinea-pig "B"	Guinea-pig "C"	Guinea-pig "D"	Guinea-pig "E"
5. 4. 1910	...	...	−	−
14. ,,	+	−	...	...
19. ,,	+	+	...	...
22. ,,	−	...	...	...
23. ,,	+	−	...	...
24. ,,	−	−	...	...
25. ,,	+	−	...	...
27. ,,	−	−	...	...
28. ,,	−	+	...	...
2. 5. 1910	+	...	...	...
3. ,,	+	−	+	+
4. ,,	−	−	−	−
9. ,,	+	−	−	−
30. ,,	+	...	−	−

The four other survivors examined on five occasions with "D" and "E" gave negative results throughout.

The dulcite bile salt broth strongly favours the growth of bacilli of the food-poisoning group in a mixture of these organisms with lactose fermenters, but it was rather interesting to find that specimens from the carriers "A" and "B" frequently gave a pure culture of the bacillus on the plates; plates similarly inoculated from carriers "C," "D" and "E" generally showed many lactose fermenters and very few non-fermenters. In two of the early examinations of faeces from "A" the pellets were emulsified in salt solution and a loop of emulsion at once spread on a lactose-saccharose MacConkey plate with the result that an almost pure culture of the bacillus was obtained. In human typhoid carriers an almost pure culture of the typhoid bacillus has been frequently reported and the observation above suggests that the normal balance of intestinal flora is here similarly overthrown, resulting in the diminution or disappearance of lactose fermenting organisms.

From this table we see that in the case of animals "D" and "E" the bacillus was recovered once in five times (*i.e.* 20 %), "C" gave the same percentage over ten examinations, while "B" gave eight positive results in thirteen examinations (= 61 %) and "A" was examined eight times and gave positive results on every occasion (= 100 %).

Thus out of nine survivors of the epizootic whose faeces were tested five proved to be carriers of the bacillus.

Agglutination of the bacillus by the serum of the carrier guinea-pigs.

It next became of interest to determine whether the serum of these animals agglutinated the bacillus, and it was found that the serum of all four examined agglutinated the bacillus in a dilution of 1 in 50 or 1 in 100, whereas of six stock guinea-pigs tested, one only gave agglutination in a dilution of 1 in 20. The details appear in Table II.

TABLE II.

Agglutination limits with serum of "carriers" and of normal animals tested against the bacillus.

Guinea-pig tested	Result of agglutination test with serum from each animal in various dilutions			
Dilution:	1 in 20	50	100	500
B	+	+	+	−
C	+	+	−	−
D	+	+	−	−
E	+	+	+	−
Stock animal 1	−	−	−	−
2	−	−	−	−
3	−	−	−	−
4	−	−	−	−
5	−	−	−	−
6	−	−	−	−
7	+	−	−	−

Immunity of survivors of the epizootic.

Interesting results were obtained from the inoculation of varying doses of broth cultures of the bacillus into ten of the survivors from the epizootic. A strain of the bacillus found in the organs of a guinea-pig dying during the epizootic was grown in broth and after 20 hours varying doses were injected subcutaneously into each of the ten animals. The lethal dose for stock animals of the particular culture used was ·00001 c.c. of a 24 hours' broth culture. Of the ten animals only three died; the bacillus was not recovered post-mortem in one case (No. 6, see Table III) and it is probable that it died from some other cause. From animal No. 8 the bacillus was recovered at autopsy. These results justify the conclusion that eight of the ten animals showed a definite degree of immunity to varying multiples of the minimal lethal dose of the bacillus.

The immunity of the carriers to subcutaneous inoculation of the bacillus has not yet been tested but this will be done after further observations have been made on the duration of the excretion of the bacillus.

TABLE III.

Experiments to ascertain the degree of acquired immunity of survivors from the epizootic against a strain of the bacillus of which ·00001 *c.c. of a* 24 *hours' broth culture was fatal to a stock animal when injected subcutaneously.*

Guinea-pig	Dose of 24 hours' broth culture injected subcutaneously	Result
1	1 c.c.	Died in 18 days. (Control stock animals given this dose died in 14 hours.)
2	1	Survived.
3	·5	,,
4	·1	,,
5	·01	,,
6	·001	Died. The bacillus was not recovered postmortem.
7	·001	Survived.
8	·001	Died. The bacillus was recovered.
9	·00001	Survived.
10	·00001	,,

Pathogenicity of the bacillus excreted by the carriers.

A number of the strains of the bacillus recovered from the organs of animals dying during the epizootic were fatal when given subcutaneously to normal guinea-pigs in very small doses. The pathogenicity of the strain of the bacillus excreted in the faeces of one of the carriers was tested and found to be on the whole somewhat less than that of the original epizootic strain. A dose of ·001 of a 24 hours' broth culture of the original bacillus recovered during the epizootic was invariably fatal to stock animals, death ensuing generally after five days, whereas of the three stock animals tested with this dose of the carrier's bacillus, one lived and the other two died after periods of 13 and 20 days.

TABLE IV.

Experiments to determine the pathogenicity of the bacillus excreted by one of the carriers, when inoculated subcutaneously into guinea-pigs.

Dose of 24 hours' broth culture	Result	Result of cultures + = the bacillus recovered
·5 c.c.	Died, 5 days	Heart-blood +
·001	Died, 20 days	,,
·001	Died, 13 days	,,
·001	Survived	—
·0001	,,	—

A dose of ·001 c.c. of a similar culture of the original bacillus recovered from the epizootic killed in 5 days.

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Identification of the bacillus associated with the epizootic and occurring in the carriers' faeces.

The cultural reactions, fermentation of carbohydrates, etc., were those of the food-poisoning group; the bacillus was not agglutinated by the serum of a rabbit immunised against the *B. enteritidis* (Gaertner) and therefore evidently belonged to the paratyphoid B. group. It was then tested against the sera of two rabbits immunised against the *B. aertryck* and the *B. paratyphosus* B. respectively (see Table V).

TABLE V.

Identification of the bacillus by agglutination and absorption tests. "A" is the bacillus recovered from the faeces of carrier "B."

Agglutination results with various dilutions of the serum of a rabbit immunised against *B. aertryck*.

Dilution of serum: 1 in 40	2000	5000	10,000	Control
B. paratyphosus B.	+	+	–	–
B. aertryck	+	+	–	–
B. "A"	+	+	–	–

Agglutination results of similar experiment with the same serum after absorption with the *B. paratyphosus* B.

Dilution of serum: 1 in 40	2000	5000	Control
B. paratyphosus B.	–	–	–
B. aertryck	+	+	–
B. "A"	+	+	–

Agglutination results with various dilutions of the serum of a rabbit immunised against the *B. paratyphosus* B.

Dilution of serum: 1 in 40	500	1000	2000	5000	Control
B. paratyphosus B.	+	+	+	–	–
B. aertryck	+	+	+	–	–
B. "A"	+	+	+	–	–

Agglutination results of similar experiment with the same serum after absorption with the *B. aertryck*.

Dilution of serum: 1 in 40	500	1000	2000	Control
B. paratyphosus B.	+	+	–	–
B. aertryck	–	–	...	–
B. "A"	–	–	...	–

The agglutination results do not permit the bacillus to be definitely assigned to either the aertryck or the paratyphoid B. group, for the serum of the rabbit immunised against the *B. aertryck* agglutinated that bacillus and the *B. paratyphosus* B. equally, and conversely the serum of the rabbit immunised against the *B. paratyphosus* B. agglutinated that bacillus and the *B. aertryck* equally.

Accordingly from these sera the heterologous agglutinins were in each case absorbed with an emulsion of the corresponding heterologous

bacillus to such a degree that each serum in a dilution of 1 in 40 no longer agglutinated the heterologous bacillus whilst retaining enough of the homologous agglutinins to agglutinate the homologous bacillus in a dilution of 1 in 500 or over. A clear difference between the *B. paratyphosus* B. and the *B. aertryck* then appeared in their agglutination reactions to these "absorbed" sera, as had already been demonstrated by Bainbridge (*Journ. of Pathology and Bacteriology*, Vol. XIII., 1909, p. 443).

The bacillus recovered during the epizootic and the bacillus recovered from the carriers' faeces gave the same results as the *B. aertryck*, so that the bacillus is indistinguishable by any test at present available from the *B. aertryck* or the *B. suipestifer*[1].

Contact experiments.

The carriers "B" and "D" were placed in separate cages and into each cage were put two normal stock guinea-pigs. One of the contacts placed with "B" died but the bacillus was not recovered from the organs or the intestinal contents post-mortem. The other three contact animals lived and apparently remained healthy for the period of two months they were under observation; an examination of the faeces was made on two occasions but the bacillus was not recovered.

Summary.

A stock of 500 guinea-pigs at the Lister Institute was attacked by an epizootic and only 21 survived. These survivors showed definite immunity to a bacillus of the food-poisoning group (indistinguishable from *B. aertryck* and the *B. suipestifer*) recovered frequently from animals dying during the epizootic. Five of them have been proved to be carriers excreting the bacillus intermittently five months later and the serum of all of them agglutinates the bacillus. Spread of infection apparently did not occur amongst contacts placed with these carriers in the few experiments carried out.

I have to thank the staff of the Lister Institute for much assistance during this research and Dr F. A. Bainbridge for much practical help in the determination of the bacillus by the methods described in his paper on members of this group.

[1] Bainbridge (*vide supra*) has also shown that the *B. suipestifer* and the *B. aertryck* are indistinguishable by agglutination and absorption tests.