# BACILLI OF THE HOG-CHOLERA GROUP (BACILLUS CHOLERÆ SUIS) IN MAN.

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Hirschfeld (1) has described an epidemic of clinical paratyphoid fever which occurred in Serbia or Greece from which he obtained organisms culturally paratyphoid B or *Bacillus schottmülleri*, but which were not agglutinated by the sera of animals immune to the latter bacillus. These organisms were isolated eighteen times, twice being obtained after death. Sera obtained from patients in this epidemic agglutinated the organisms isolated in some cases in dilutions as high as 1:800. MacAdam (2) obtained similar inagglutinable paratyphoid bacilli in Mesopotamia from the blood stream of patients who clinically showed respiratory rather than enteric symptoms. Mackie and Bowen (3) have described cultures of the same group, as shown by Schütze's (4) work. The latter compared twelve cultures obtained from febrile cases occurring in the Balkan region, the organism isolated by the above writers being included. All the cultures were agglutinated by and absorbed the agglutinin from the serum of an animal immune to one of Hirschfeld's strains. Hirschfeld called his organisms paratyphoid C and Schütze called them Hirschfeld's bacillus.

It seemed possible that by comparing these organisms with the paratyphoids found in animals they might be classed in one of the known groups. Upon request Dr. Hirschfeld kindly sent me two cultures of these organisms and some immune serum. The cultures were labeled Para C and it may be that they were duplicates but I have called them Paratyphoid C I and II and have used both of them in the tests made. No differences have been detected between the two strains.

Agglutination tests were made with the serum received and the results given in Table I were obtained.

The serum agglutinates two strains of the hog-cholera bacillus in as high dilution as it does the organism isolated by Hirschfeld, while *Bacillus schottmülleri*, *Bacillus enteritidis*, and swine-typhus bacilli are agglutinated in only the lowest dilutions.

Anti-hog-cholera bacillus serum was absorbed with the cultures received and the results of agglutination tests with the absorbed as well as the unabsorbed serum are given in Table II.

The paratyphoid bacilli under consideration are agglutinated to the titer limit by anti-hog-cholera bacillus serum and absorb from this serum not only the agglutinins for themselves but also those for the hog-cholera bacilli.

TABLE I.

*Agglutination with Hirschfeld Paratyphoid C Serum.*

| Culture tested. | Limit of agglutination. |
|---|---|
| *B. schottmülleri* 232 | 100 |
| " " 242 | 100 |
| Swine *enteritidis* I | 50 |
| *B. enteritidis* (Mt. Sinai) | 50 |
| Swine-typhus I | 100 |
| " V | 100 |
| Hog-cholera XII | 12,800 |
| " XIV | 12,800 |
| Paratyphoid C I | 12,800 |
| " " II | 12,800 |

TABLE II.

*Tests with Anti-Hog-Cholera Bacillus Serum.*

| Culture tested. | Limit of agglutination using serum. | | |
|---|---|---|---|
| | Unabsorbed. | Absorbed with Paratyphoid C I. | Absorbed with Paratyphoid C II. |
| Paratyphoid C I | 12,800 | 200— | 200— |
| " " II | 12,800 | 200— | 200— |
| Hog-cholera bacillus XII | 12,800 | 200 | 200 |
| " " XVI | 12,800 | 200 | 400 |

Rabbits were immunized, one to each of the two strains, by the injection of unheated bouillon cultures and their sera gave the results in Table III.

Four different hog-cholera bacillus cultures were agglutinated to the titer limit. Three of the hog-cholera bacillus cultures were used for absorption tests, one for one serum and two for the other, and they

took out the agglutinins for the Paratyphoid C bacilli, as well as for the other hog-cholera bacilli.

Serologically these organisms are typical hog-cholera bacilli but culturally they differ in that they form acid and gas in dulcite and arabinose and form hydrogen sulfide, whereas the hog-cholera bacillus, as has been pointed out by Jordan (5) and Krumwiede, Kohn, and Valentine (6), do not act on these carbohydrates or form hydrogen sulfide. Culturally, then, they are the same as *Bacillus schottmülleri*.

Two rabbits given subcutaneous injections of 0.1 cc. of 24 hour bouillon cultures of these organisms showed a slight rise in temperature

TABLE III.

*Saturation of Paratyphoid C Sera with Hog-Cholera Bacilli.*

| Culture tested. | Limit of agglutination with serum of Rabbit A, immune to Paratyphoid C I. | | Limit of agglutination with serum of Rabbit B, immune to Paratyphoid C II. | | |
|---|---|---|---|---|---|
| | Unabsorbed. | Absorbed with Hog-cholera bacillus XVI. | Unabsorbed. | Absorbed with Hog-cholera bacillus X. | Absorbed with Hog-cholera bacillus XII. |
| Hog-cholera X, Pig 30 | 12,800 | 200 | 12,800 | 400 | 200— |
| " XI | 12,800 | 200— | 12,800 | 400 | 200— |
| " XII | 12,800 | 200— | 12,800 | 800 | 800 |
| " XVI | 12,800 | 200— | 12,800 | 400 | 400 |
| Paratyphoid C I | 12,800 | 200— | 12,800 | 400 | 800 |
| " " II | 12,800 | 200— | 12,800 | 400 | 800 |

and local lesions. Neither was very sick and recovery was prompt. A typical hog-cholera bacillus should kill rabbits in from 6 to 10 days, so that these cultures resemble the animal typhus group rather than the hog-cholera bacilli in their virulence for rabbits.

20 days after the subcutaneous injection these rabbits were given an intravenous injection of 0.01 cc. of 24 hour bouillon cultures of the same organisms. This produced no effect and 25 days later they were given a subcutaneous injection of 0.00001 cc. of a 24 hour bouillon culture of a virulent hog-cholera bacillus. This organism in a dilution 100 times the one used here will kill normal rabbits in from 6 to 10 days, but in these injected rabbits it produced no rise in temperature, loss in weight, or local lesion. The rabbits were

killed 6 weeks after the final injection and aside from a slight enlargement of the spleen appeared normal. Cultures from the spleen showed hog-cholera bacilli from one rabbit but not from the other.

The interpretation of this experiment is not clear. As has been pointed out in the preceding paper (7), various animal typhus cultures will immunize rabbits to this virulent hog-cholera bacillus, but no tests have been made with the amount used here. The fact that these rabbits withstood such a large injection without showing any evident disturbance seems to indicate that they had a specific immunity.

A pig weighing 30 pounds was fed 100 cc. of one of the cultures received from Dr. Hirschfeld mixed with its food. For the next 4 days its temperature was increased and it ate very little. Its feces were diarrheal in character but the organism fed was not obtained on Endo plates made from the feces the 5th day after the feeding. On this day its temperature approached normal, it was eating well and appeared to be much livelier than on the days following the feeding. On the 5th day after the feeding virulent hog-cholera virus was injected intramuscularly, as it has been found in similar experiments that hog-cholera bacilli introduced into the digestive tract by feeding will act as secondary invaders when the pig is infected with virus. The pig died 8 days after the injection of the virus and was autopsied soon after death. It showed lesions characteristic of hog-cholera with the addition of a grayish, membranous necrosis of the mucosa of the large intestine. Plate cultures were made from the spleen, two mesenteric lymph nodes, the exudate in the colon, the mucous membrane of the colon after removing the exudate, the kidney, and the liver. In all, thirty-five subcultures from these plates were studied. They resembled the organism fed, with the following exceptions. Of six cultures from the mucosa of the colon, one failed to form hydrogen sulfide or ferment dulcite, but acted on arabinose. The other cultures were the same as the culture fed. Of two cultures from the kidney, one was the same as the organism fed, while the other differed in that it failed to act on dulcite. It did, however, produce hydrogen sulfide and on the second test fermented dulcite promptly. Only two cultures from the liver were examined and neither acted on dulcite, while one did and the other did not ferment

arabinose. Both formed hydrogen sulfide and on the second test both fermented dulcite and arabinose. The culture from the colon is, then, the only one that has shown any permanent change, and this culture 2 months after its isolation still failed to form hydrogen sulfide or ferment dulcite. It has, then, approached the hog-cholera bacillus in its cultural characters. The organisms from the liver and kidney also approached the hog-cholera bacillus in cultural character, but they soon regained the properties lost. The question as to whether the cultures recovered are the ones fed cannot positively be decided. Seven other pigs of the same litter have been infected with hog-cholera virus and bacteriological examination has failed to show the hog-cholera bacillus, though from some of them swine-typhus bacilli have been isolated. All the cultures from this pig resembled the hog-cholera bacillus serologically so the probabilities are that they were the descendants of organisms fed.

One similar test has been made with a swine-typhus culture and in this one case the feeding failed to cause a rise in temperature. Cultures made at autopsy, the animal having been infected with hog-cholera virus, failed to show swine-typhus bacilli.

When hog-cholera bacilli are fed, the pig reacts as did the animal fed the Paratyphoid C culture. There is an increased temperature beginning the day after the feeding and lasting for from 3 to 4 days. If at the end of this time hog-cholera virus is injected into the pig, hog-cholera bacilli will be found in the organs at autopsy.

## DISCUSSION.

While these organisms isolated by Hirschfeld (1) are not typical hog-cholera bacilli in that they ferment dulcite and arabinose, produce hydrogen sulfide, and are not virulent for rabbits, their serum reactions are so characteristic that they should be placed in the hog-cholera bacillus group. These serum reactions are very fundamental, much more so than are the fermentations of the rarer carbohydrates.

There are several possible explanations which might account for these differences. One is that in the region from which these organisms were obtained atypical strains of hog-cholera bacilli exist in swine. Another is that the organisms in swine may be typical but

after passing to man they have become modified. A third possibility is that these organisms did not come from swine. They do not, however, correspond to any of the animal paratyphoids that have been described. What appear to be culturally typical hog-cholera bacilli do exist in nearby regions, as is shown by the observations of Trawinski (8). He isolated forty-two cultures from swine imported into Germany from Poland. All the cultures failed to ferment dulcite and arabinose, while three cultures of so called *Bacillus suipestifer* obtained from Kral's collection acted on these carbohydrates. One so called *suipestifer* strain obtained from Budapest acted the same as the cultures he isolated and was agglutinated to the titer limit by serum of an animal immune to one of his strains. The cultures from Kral were not agglutinated to the titer limit by this serum. He does not record the virulence of the cultures for rabbits or the production of hydrogen sulfide, but notes that all of his forty-two cultures formed acid but no gas in sorbite, whereas the control cultures of *Bacillus suipestifer* formed gas.

It is an interesting fact that the hog-cholera bacillus, which at one time was so commonly present in swine infected with hog-cholera that it was regarded as the cause of the disease, has not been found more frequently in man. In the older literature of food poisonings some of the organisms isolated were virulent when injected subcutaneously into rabbits and in some cases necroses were found in the livers of these animals. These facts indicate that the hog-cholera bacillus may have been the organism that was being studied, but the evidence is not conclusive.

Reed and Carroll (9) made a comparative study of *Bacillus icteroides* (Sanarelli) and the hog-cholera bacillus and concluded that they were the same. In cultural characters, virulence, and the disease produced in animals the two cultures were identical. They made only a few agglutination tests and the results are not very clear-cut but they indicate a relation between the two organisms. We are fortunate in having in our collection a culture of *Bacillus icteroides* that was received directly from Sanarelli and it is agglutinated to the titer limit in anti-hog-cholera bacillus serum and absorbs the agglutinins from this serum. In addition it fails to ferment dulcite and arabinose and does not produce hydrogen sulfide, thus resembling the hog-

cholera bacillus, while it differs from the latter organism in that it is not virulent for rabbits.

There is another culture in the collection labeled paratyphoid B Longcope, which culturally and serologically is a hog-cholera bacillus but which is not virulent for rabbits. The chances are that this culture came from a case of paratyphoid reported by Longcope (10) in 1902, but we cannot be sure of this fact. The probabilities are that it is at least of human origin.

As far as I know these are the only cultures from man that correspond closely to the hog-cholera bacillus so that an outbreak in the Balkan region with which organisms of the hog-cholera bacillus group are associated is of great interest. There must be many opportunities for hog-cholera bacilli to infect man, but they either rarely find conditions such that they can grow in the human body or, what is less likely, they do grow and quickly lose their distinguishing characters.

## CONCLUSIONS.

1. The organisms isolated by Hirschfeld from febrile cases resembling paratyphoid fever and named Paratyphoid C can be placed in the hog-cholera bacillus group by their agglutination absorption properties though they are not typical culturally.

2. When fed to a pig a febrile disease resulted from which the animal recovered. After injection of hog-cholera virus the organisms fed were found generally distributed and some of them had lost cultural characters so that they are brought into the class of typical hog-cholera bacilli except for their low virulence for rabbits.

3. While hog-cholera bacilli have many opportunities to infect man they either are not able to grow in the human body or, what is less likely, they do grow and lose the characters that distinguish them.

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