ON THE RELATIONSHIP BETWEEN *BACILLUS PESTIS* AND *BACILLUS PSEUDOTUBERCULOSIS RODENTIUM* (PFEIFFER).

By ALFRED T. MacCONKEY, M.B., D.P.H.
Bacteriologist-in-charge, Serum Department, Lister Institute of Preventive Medicine.

The *Bacillus pseudotuberculosis rodentium* (Pfeiffer) is the organism which morphologically and culturally most closely resembles *Bacillus pestis.* Galli-Valerio (1903) concluded that these bacilli resemble each other very closely in most respects but that *B. pseudotuberculosis* coagulates milk while the *B. pestis* does not; and while the *B. pseudotuberculosis* is not pathogenic for rats, it is extremely so for guinea-pigs, which succumb to cutaneous, nasal and conjunctival inoculations.

Zlatogoroff (1904) from a comparative study of 2 strains of the *B. pseudotuberculosis* and 22 strains of *B. pestis* (2 from rats and 20 from men) expresses the opinion that morphologically and culturally these organisms are practically identical; though *B. pseudotuberculosis* does not show such extensive pleomorphism as *B. pestis* and is non-virulent for rats and pigeons and only slightly so for white mice. The agglutination titre of the pest serum he tested did not exceed 1 in 500 and "clumping" took place quite as well in the case of *B. pseudotuberculosis* as in that of *B. pestis.* He obtained precipitin reactions in pest serum only with pest filtrates. Pest serum did not protect against infection with the *B. pseudotuberculosis* and *vice versa.* He failed entirely in his attempts to produce cross immunisation.

I have had at my disposal for study 11 strains of *B. pestis* and 7 of *B. pseudotuberculosis rodentium* (Pfeiffer) of various origins. The convention ΨT.R. is used to designate the pseudotubercle bacillus.

When grown on agar or gelatine or in neutral bouillon no *constant* difference could be made out as regards the appearance of the growths or the morphology and staining reactions of the bacilli. The growth of

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B. pestis on agar is usually found to be of a stringy consistency when touched with a needle but this is not a constant feature (cf. Shibayama, 1905). Not infrequently it is short and granular like that of the Bac. ΨT.R. On the other hand none of my strains of ΨT.R. ever gave a viscid growth; and they almost always grew more abundantly than *B. pestis.*

In litmus milk all the 18 cultures both of pest and pseudotuberculosis produced no change for three to four days at 35° C. Then in the case of the ΨT.R. cultures the medium began to turn alkaline and finally became deep blue. The pest cultures were kept at 35° C. for about a week and then at room temperature for five weeks. At the end of this time there was no change apparent in the medium. On subculture an abundant growth of pest was obtained. In bile salt media containing various fermentable substances the strains of each gave the same reactions, and in this connection it is worthy of note that the ages of these strains varied from a few weeks to ten years. Acid but no gas is produced from glucose, mannite, laevulose, galactose and dextrin, while lactose, cane sugar, raffinose, sorbit, dulcit, adonit, inulin, amygdalin and α-methyl glucoside are unaffected (cf. MacConkey, 1905, p. 350). Vourloud (1907) obtained slightly different fermentation reactions, but he used ordinary nutrient agar and a trace of muscle sugar may have been present.

All these races were inoculated on to salt agar (NaCl 3%). At the end of 24 hours' incubation at 30° C. there was nothing to choose between them in the appearance of the growths. The growth of ΨT.R. might have been slightly more abundant but that was all. Microscopically one could pick out a strain of pest which differed somewhat from some one particular strain of ΨT.R.; but taking all the cultures together there was no bacillary form appearing among the films of pest which was not represented among those of ΨT.R. also. After 48 hours' growth at 30° C. the majority of the cultures of ΨT.R. showed a more abundant growth than the majority of the cultures of pest, but still there were some of the cultures of ΨT.R. which had only grown slightly, while one culture of pest (10 years old) had grown more vigorously than any of the strains of ΨT.R. Microscopically the relative appearances were the same as at 24 hours. The general impression gathered from a microscopical examination of all these cultures was that the yeast-like involution forms were not so abundant in the cultures of ΨT.R., though one culture of ΨT.R. was full of such forms, in fact fuller than any of the pest cultures.

We find then culturally only two points of difference between these

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bacilli, namely the frequently viscid character of the growth of pest on agar and the development of an alkaline reaction in tubes of litmus milk inoculated with the Bac. ΨT.R. These results are quite in accordance with those of Zlatogoroff, and as far as one can form an opinion from cultural characters one is constrained to recognise a close resemblance between these bacilli.

The results of precipitin tests emphasize this relationship. On the addition of pest filtrate to pest serum a cloudiness appeared almost at once and an abundant precipitate was deposited at the end of three hours at 37° C. When ΨT.R. filtrate was added to pest serum these changes did not take place so rapidly. After three hours at 37° C. there was a distinct precipitate suspended in the serum but no deposit at the bottom of the tube. After three hours at 37° C. the tubes were placed in the cold room overnight. The precipitate caused by ΨT.R. serum was not completely deposited at the end of 24 hours. On the other hand the pest filtrate precipitate was completely deposited and the supernatant fluid was quite clear.

The filtrates used were those employed to immunise guinea-pigs in the experiments on cross-immunisation detailed later.

Cross-immunisation.

Guinea-pigs are so very susceptible to infection by the Bac. ΨT.R. that it is not to be wondered at if, in trying to immunise them with this bacillus, the mortality is considerable.

It is necessary to proceed very slowly with the process of immunisation and a month is a good interval to place between the injections. If the inoculations are not properly spaced the mortality is too high. Even when one proceeds with the greatest care one cannot count upon keeping all the animals alive until the test inoculation.

This state of affairs obtains also in the case of rats, though rats are said to be immune to ΨT.R. It is true that they do not succumb to an acute disease but they do not all remain in as good health as before the inoculations. They waste and seem to become more susceptible to the attacks of other organisms. For instance in one experiment 12 white rats received killed cultures of the Bac. ΨT.R. mixed with their food, about 9 c.c. of a 24-hour broth culture each day. Ten of these 12 rats died and from five of these ten I isolated a bacillus belonging to the group of the *B. enteritidis* (Gaertner). During this time there was not a single death among our stock rats.

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The experiments detailed below show that animals which have withstood the process of immunisation with the Bac. ΨT.R. are in most cases insusceptible to subsequent infection with pest.

Experiment I.

Four guinea-pigs were inoculated subcutaneously first with killed and then with living cultures of the Bac. ΨT.R. Two of these animals died after the second dose. The remaining two received a third injection and then two months later were inoculated subcutaneously with pest, one control being infected at the same time. The pest inoculations were performed by Captain S. R. Douglas, I.M.S. The control animal died of pest on the fifth day while the other animals showed no signs of illness. Two months later they were killed and examined. One showed no signs of disease whatever. In the other there was a caseous inguinal gland from which no growth could be obtained. A film made from the caseous material showed appearances which might have been bacilli.

Experiment II.

In this experiment two series of animals were used.

Series I. Twelve guinea-pigs were inoculated subcutaneously with a quantity of killed emulsion equal to $\frac{1}{40}$ of an agar culture of Bac. ΨT.R. Subsequently it was found that five does were pregnant. These were not used any more. Thirty-three days after the first injection a second inoculation of killed emulsion was given. This dose equalled $\frac{1}{10}$ of an agar culture. Thirty-seven days later each pig received killed emulsion equal to $\frac{1}{5}$ of an agar culture. Thirty-eight days later 1 c.c. of a living horse serum bouillon culture was given. Three pigs died after this dose. Forty-six days later the remaining four animals were inoculated with pest and found to be immune.

Series II. Twelve guinea-pigs were inoculated subcutaneously with killed emulsion of Bac. ΨT.R., the first six receiving a dose equal to $\frac{1}{20}$ and the remainder a dose equal to $\frac{1}{10}$ of a 2-day agar culture. One of the second six died in three weeks. Thirty-eight days after the first injection each animal received subcutaneously 1 c.c. of a 5-day horse serum bouillon living culture of the Bac. ΨT.R. Forty-six days later they received a test inoculation of pest at the same time as the animals in Series I and 10 controls.

The animals in Series II, like those in Series I, were found to be immune.

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Of the controls seven died within a week and the *B. pestis* was isolated from every one. The remaining three were alive and well seven weeks later.

The result may be summarised thus:

Controls (10) mortality 70 %.
Immunised (15) mortality 0 %.

Experiment III. *To test the duration of the immunity.*

Ten guinea-pigs were inoculated subcutaneously with an emulsion of Bac. ΨT.R. which had been killed by heat. Each animal received an amount of emulsion equal to $\frac{1}{20}$ of an agar tube.

Another series of 10 animals was treated in precisely the same manner except that an emulsion of another strain of Bac. ΨT.R. was used.

Four of the first series and one of the second series died during the following 11 days.

Thirty-two days after the first injection a second dose was given subcutaneously, the quantity being equal to $\frac{1}{10}$ of an agar tube. A pig of the second series died after this inoculation.

After an interval of twenty-two days the animals received a third injection—the dose being 1 c.c. of a 24-hour living broth culture.

The animals were then kept for 204 days, i.e. nearly seven months, and were then given a test inoculation of pest; seven controls being inoculated at the same time.

The result was that six of the seven controls died of pest within eight days, three of the second series of immunised animals died on the seventh, eleventh, and twelfth days respectively, while one control and 11 immunised pigs remained alive and healthy.

Summarised result:

Controls (7) mortality 85·7 %.
Immunised (14) mortality 11·5 %.

It would appear then that in the majority of cases the immunity lasts at least six months.

Experiment IV. *To ascertain whether protection can be conferred by means of filtrates from cultures of ΨT.R. bacilli.*

A Roux bottle of nutrient agar was inoculated with ΨT.R. and incubated for three days at 37° C., when the growth was washed off the agar and emulsified in 200 c.c. of NaCl (0·85 %) solution. The emulsion

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was heated in a flask for an hour at 60° C., a few drops of chloroform added, the flask plugged with a rubber cork and autolysis allowed to proceed for eight days at 37° C. The contents of the flask were then filtered through a Berkefeld filter, thus giving "ΨT.R. filtrate."

An exactly similar procedure was carried out with a strain of pest, and "pest-filtrate" obtained.

Ten guinea-pigs were inoculated subcutaneously with 1 c.c. of pest filtrate and another 10 pigs with 1 c.c. of ΨT.R. filtrate. One pest pig and two ΨT.R. pigs died five days later.

After an interval of 14 days all the animals were re-inoculated, the dose being 2 c.c. of filtrate. Two pigs of each series died during the subsequent three weeks.

One month after the second injection the animals received each 5 c.c. of filtrate.

Thirty-seven days later two pest pigs, two ΨT.R. pigs and two controls received a test inoculation of pest.

The result was that both controls died of pest within eight days, one pest pig died on the tenth day, and the remaining pest pig and both the ΨT.R. pigs remained alive and well.

The remaining animals were kept to be tested later.

At the end of four months it was found that the virulence of the pest bacillus used for testing had greatly diminished and that a test could not be carried out. So each pig received 1 c.c. of filtrate subcutaneously to keep up the immunity.

Four months after this injection these animals and six controls received a test inoculation of pest.

As a result four out of five pest pigs, one out of three ΨT.R. pigs and five out of six controls died of pest.

The results of both parts of this experiment may be taken together and summarised thus:

Controls (8) mortality 87·5 %.
Pest immunised (7) mortality 71·5 %.
ΨT.R. immunised (5) mortality 20 %.

These results show that immunity to pest can be conferred by means of filtrates of cultures of the ΨT.R. bacillus and that this immunity lasts several months.

So far the experiments had been confined to guinea-pigs, animals which are acknowledged to be very susceptible both to pest and to ΨT.R. The question then naturally arose as to whether rats could be similarly

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protected by inoculations of the bacillus ΨT.R., an organism to which they are more or less immune.

Experiment V.

Two series of 10 rats each were inoculated subcutaneously with 0·5 c.c. of a living 24-hour broth culture of the Bac. ΨT.R., a different strain being used for each series. Seven weeks later they received 1 c.c. of a 24-hour living broth culture subcutaneously.

Seven animals died during immunisation.

One hundred and sixty (160) days after the second immunising injection Dr C. J. Martin kindly tested these rats with pest. Nine of the one series, four of the other series and five controls were inoculated at the same time. The result was that all the controls were dead of pest on the fourth day. Of the immunised animals three died on the fourth day, one on the fifth day and one on the seventh day. The remaining eight remained alive and healthy.

Thus of the

Controls (5) mortality 100 %,
Protected (13) mortality 38·5 %,

when tested five months after the last immunising injection.

Summary.

(1) Morphologically and culturally the *Bacillus pseudotuberculosis rodentium* (Pfeiffer) bears a strong resemblance to *B. pestis.*

(2) The filtrate from an autolysed agar culture of *B. pseudotuberculosis rodentium* (Pfeiffer) and a similar filtrate from a *B. pestis* culture both gave a precipitin reaction with pest serum.

(3) It has been found possible to immunise both guinea-pigs and rats against plague by means of inoculations of cultures of *B. pseudotuberculosis rodentium* (Pfeiffer), and this immunity lasted in many cases at least six months.

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