

STUDIES ON TYPHUS FEVER

VI. REDUCTION OF RESISTANCE BY DIET DEFICIENCY

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The average guinea pig or rat possesses a resistance against typhus infection which limits the distribution of the virus and leads to almost invariable recovery of the animals. Many of our efforts during the last 2 years have focused upon the production of increased susceptibility, in order that we might convert the milder, experimental disease into a fatal one or at least obtain an increased yield of *Rickettsia* bodies for immunological studies.

As reported in a preceding paper (1), preparatory benzol injections into rats yielded us a sufficient number of successful experiments to furnish peritoneal exudates rich in *Rickettsiae* and suitable for the etiological demonstration which we reported. Although we have at the same time studied cultural methods by the tissue-plasma technique and by the Tyrode-serum method of the Maitlands, and have obtained some multiplication, we continued to concentrate upon the susceptibility-enhancement experiments because—wherever successful—the peritoneal exudates of animals inoculated in the stages of depression yielded not only many intracellular, but plentiful extracellular *Rickettsiae* which were, therefore, easily freed of plasma and cells by simple washing in the centrifuge. The benzol method was successful in only about one out of three times, since individual differences between rats seemed to necessitate adjustment of time factors and the number and amounts of the benzol injections which could not be approximated without a very large series of experiments. Although we employed the benzol method, nevertheless, in the previously re-

ported studies—in which we believe we proved the etiological significance of the *Rickettsiae*—our desire to obtain more regular results, particularly for vaccine production, led us to cast about for other methods of reducing resistance.

About 2 years ago, with Batchelder, one of the writers (2) endeavored to reduce the resistance of guinea pigs by feeding them on vitamin-free diets. The idea underlying this was the well-known fact that typhus epidemics have throughout history been most virulent and fatal in times of famine. While there are of course other factors besides nutritional deficiency which participate in rendering typhus particularly severe in times of hardship, war and crop failures, nevertheless, as analyzed by Hirsch (3) and subsequently by Kurschmann (4), the association with famine and consequent malnutrition appears to be one of the very important influences that contribute to the severity of epidemics. Moreover, there has been much experimental evidence in recent years to indicate that nutritional disturbances of various kinds exercise a beneficent influence upon susceptibility to various infections.

At the time when we first entertained the idea of applying this reasoning to typhus, we carried out a number of experiments which showed that animals fed on vitamin-deficient diets often responded to infection with severe and manifest illness, during which there was no rise of temperature, the temperature not infrequently dropping to subnormal within 2 or 3 days after inoculation, the animals sometimes dying on the 4th to the 8th day without having exhibited any of the characteristics of an experimental typhus. Owing to the fact that we were still using the Giemsa method of searching for *Rickettsiae*, we were not satisfied with the results of these experiments in regard to any evidence of increased development of *Rickettsiae*.

We resumed experiments of this nature in the course of the last year, making more careful *Rickettsia* studies by means of the formalin-buffer-methylene-blue-saffranin stain perfected by one of us and elsewhere published (5).

Experiments on Guinea Pigs

In accordance with the experience of Abels (6) and of several other investigators (7) in regard to the resistance-depressing effects of

scurvy, we fed guinea pigs on a diet consisting of rolled oats, skimmed milk, autoclaved 1 hour at 15 pounds, and water. After anywhere from 14 to 20 days on such a diet, the guinea pigs showed signs of weakness, and sometimes joint swelling, and when one or another of the animals of each lot exhibited distinct symptoms of deficiency, we inoculated intraperitoneally with tunica material from Mexican typhus animals.

Altogether, we have in this way investigated thirteen guinea pigs, weighing about 250 gm. each.

Of this series, two animals were of little significance. They died without the development of temperature, both on the 6th day after inoculation, of secondary infection with bacteria. It is of some interest that in one of them on the 6th day, in addition to the bacteria there were numerous *Rickettsiae* in the peritoneal exudate.

Another animal of the series is of relatively little interest because it developed an irregular typhus fever, not in principle differing from the ordinary experimental disease. In this guinea pig the diet had obviously not been carried far enough.

All the rest of the guinea pigs, however, yielded results of the greatest interest to us in our efforts to produce vaccines for the practical application of the principles recorded in our Study V (8). In three, out of the ten remaining animals, the temperature curve showed a tendency to rise immediately, reaching between 104°F. and 105°F. on the day after inoculation, touching 105°F. in two of these on the 3rd day, and 106°F. in one of them on the 4th day. Examined on the 6th, 7th and 8th days, respectively, when the temperatures had returned to normal in all of them, there were plentiful *Rickettsiae* in the tunica. In the remaining seven guinea pigs, the temperature either did not rise at all, or went up very slightly on the 3rd or 4th day; in all but one of them it remained at or below 103°F.; in only one it rose to 104°F. for 1 day.

Two of these guinea pigs died, one on the day after inoculation, after a drop from a temperature of 102.4°F. to 101°F. Even in this animal a moderate number of *Rickettsiae* were found in the tunica. The other animal that died spontaneously on the 7th day showed a few small pneumonic lesions in the lungs, but the

peritoneal exudate was extraordinarily rich in *Rickettsiae*.* In all the other animals, without exception, *Rickettsiae* were found both in peri-

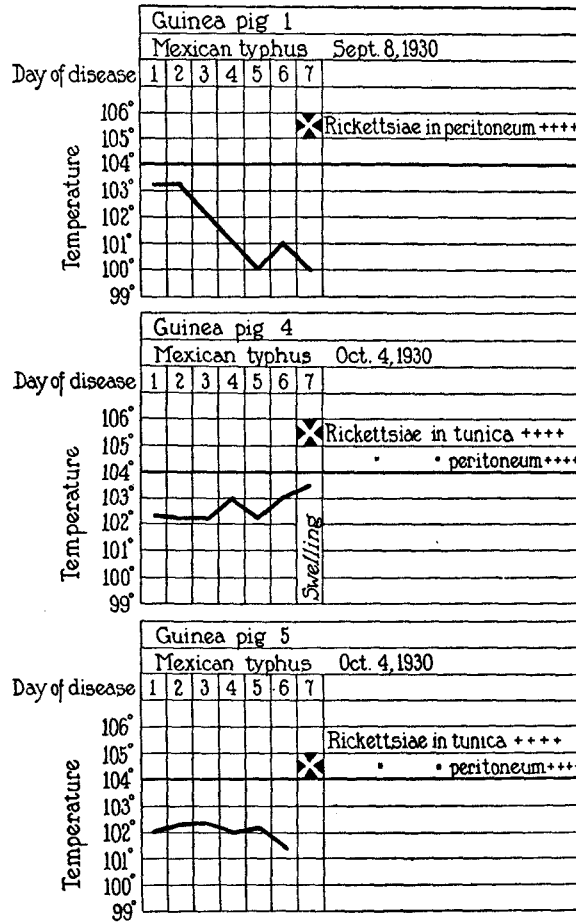


CHART 1. Temperature curves of three guinea pigs inoculated with Mexican virus from tunica material. In all of them, in spite of the absence of a characteristic typhus curve, there was serious illness and extraordinary numbers of *Rickettsiae* were found both in tunica and peritoneum.

* We do not include a photograph of the exudates containing the large numbers of *Rickettsiae* in these animals since they differed in no essential respects from similar exudates from the successful benzol animals, pictures of which were published in a preceding paper.

toneum and in tunica in profuse, even cultural numbers, and in many of them—to our surprise—large numbers of the *Rickettsiae* were extracellular. Typical curves of such guinea pigs, in which large numbers of *Rickettsiae* were found, are shown in Chart 1.

Experiments on Rats

Rats were fed on slightly baked biscuits containing vitamin-free casein, vitamin-free lard and vitamin-free starch, salts and water, 5 to 7 gm. of this being fed daily. As a rule, the rats showed the effects of this diet within 14 days. At the time when the first effects of the diet appeared, the animals were intraperitoneally inoculated with tunica material from infected guinea pigs.

Of six animals so investigated, two were apparently too weak to stand the inoculation, and died without result.

The other four animals, however, showed a remarkable departure from the ordinary course of rat typhus. All of them became noticeably ill between the 4th and the 6th day after inoculation. Two were killed on the 5th and the 6th day after inoculation, when they were noticeably sick, both of them showing considerable numbers of *Rickettsiae* in the tunica and peritoneal exudate, and one of them having an abnormal amount of peritoneal and pleural exudate, in both of which enormous numbers of *Rickettsiae* were found. In this animal, also, scrapings made from the visceral pleura showed very large numbers of the organisms within the endothelial cells, and the *Rickettsiae* were also present in smears from the blood, the endocardium, the spleen, liver and pia mater.

A third rat very sick on the 4th day after inoculation, was killed and showed numerous intra- and extracellular organisms in the peritoneum, and large numbers were found in scrapings of pleural cells—indeed, more than were found in the peritoneum.

A fourth animal died spontaneously 6 days after inoculation. Again *Rickettsiae* were plentiful in peritoneal exudate and in the pleura. In all of these animals, with the exception of those especially mentioned, which were apparently too far gone with the diet before the inoculation was undertaken, infection was far more severe, and the yield of *Rickettsiae* extraordinarily increased over that usually observed after the inoculation of normal rats.

SUMMARY OF DIET EXPERIMENTS

The above experiments demonstrate that guinea pigs and rats subjected to vitamin-deficient diets to a point at which deficiency symptoms appear, and then inoculated with typhus virus, exhibit clinical pictures which indicate a far more severe infection than that observed in normal animals after inoculation. There is also a wider distribution of *Rickettsiae* and a concentration of organisms which, in pleural and peritoneal exudates, amounts to almost cultural proportions.

Important from our point of view is the fact that these experiments furnished a step toward the accomplishment of our purpose, which was to obtain amounts and concentrations of *Rickettsiae* suitable for immunological studies until such a time when tissue culture may have developed to a practically useful stage.

The experiments are of immediate importance in that they furnish us a method for improving our technique of active immunization reported upon in the preceding paper, No. V (8).

From the epidemiological point of view these experiments at least suggest an explanation of one of the important factors which enter into the historical association of high typhus mortality with war and famine.

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