# STUDIES ON TYPHUS FEVER

## VII. Active Immunization against Mexican Typhus Fever with Dead Virus

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In a preceding paper we have described experiments in which partial or complete immunization against European typhus fever was obtained by preliminary inoculations with formalin-killed tunica material containing *Rickettsia* or Mooser bodies. We also described two methods by which a considerable increase in the yield of *Rickettsiae* could be obtained in infected animals, the first one consisting in a preliminary injury of the animals with benzol, the second, and more regularly successful one being based upon the inoculation of animals in the late stages of experimental scurvy.

These methods were resorted to since we had come to believe from the work of Spencer and Parker (1) and of Connor (2), who studied Rocky Mountain spotted fever, and from our own experiments with Mexican typhus fever, that successful active immunization could be hoped for only if considerable amounts of virus were available. Spencer and Parker obtained adequate virus concentrations in Rocky Mountain spotted fever by using the virus obtained from ticks, a single tick often containing as many as a thousand infectious doses.

An analogous method for typhus fever by the use of lice might be successful, but hardly practicable. There is a single experiment of this kind on record in the work of Anderson and Goldberger (3), in which a large number of infected lice were crushed and killed by heating for 30 minutes at 60°C., such suspensions being injected subcutaneously into a monkey. The monkey was not protected by this against subsequent intravenous injection of 3 cc. of defibrinated blood. In view of the experiments we have reported and are about to report,

it would seem that immunity might be obtained with infected lice, in guinea pigs at any rate, if a method of killing the virus other than heat could be substituted. On the other hand, the concentration of virus for vaccination purposes in lice would be hardly practicable, because lice infected naturally or by the Weigl method must be fed on human subjects for at least 10 days before they are fully virulent, a procedure that requires typhus immune individuals, much labor and a laboratory organization entirely too complex to make this worth considering.

The ideal method of vaccine production of course would be cultivation. The Maitland method is beginning to prove successful in the hands of Nigg and Landsteiner (4), and we, too, have had multiplication of the *Rickettsia* bodies in such cultures, but as far as our own work is concerned, the method has proved so far fraught with so many difficulties and irregularities, and yields amounts of *Rickettsiae* so inadequate that it needs considerable improving before it can be utilized for vaccine production.

We have, therefore, continued with the diet method of producing vaccine for experimental purposes, knowing that thereby we could obtain material sufficiently rich in *Rickettsiae* to permit us a final judgment as to the possibility of producing active immunity with killed virus.

In our preceding papers we have published only upon the results obtained when Mexican *Rickettsia* material was used for vaccination and reinoculation was carried out with European virus. The experiments were carried out in this manner, in the first place, because we were particularly interested in the development of a method of protecting against European typhus, and in the second place, because the reaction of guinea pigs to the living Mexican virus is much more severe than it is to the European variety. In consequence, we thought we would be more likely to be successful in vaccinating against European typhus fever than against the Mexican experimental infection.

In the present communication we wish to report upon an experiment in which we vaccinated against the Mexican variety of the disease.

The vaccine material in this case consisted of peritoneal washings of a guinea pig that was inoculated after the preliminary establishment of experimental scurvy, the animal having lost considerable weight, and showing definite signs of weakness on the day on which he was intraperitoneally inoculated with a suspension of tunica

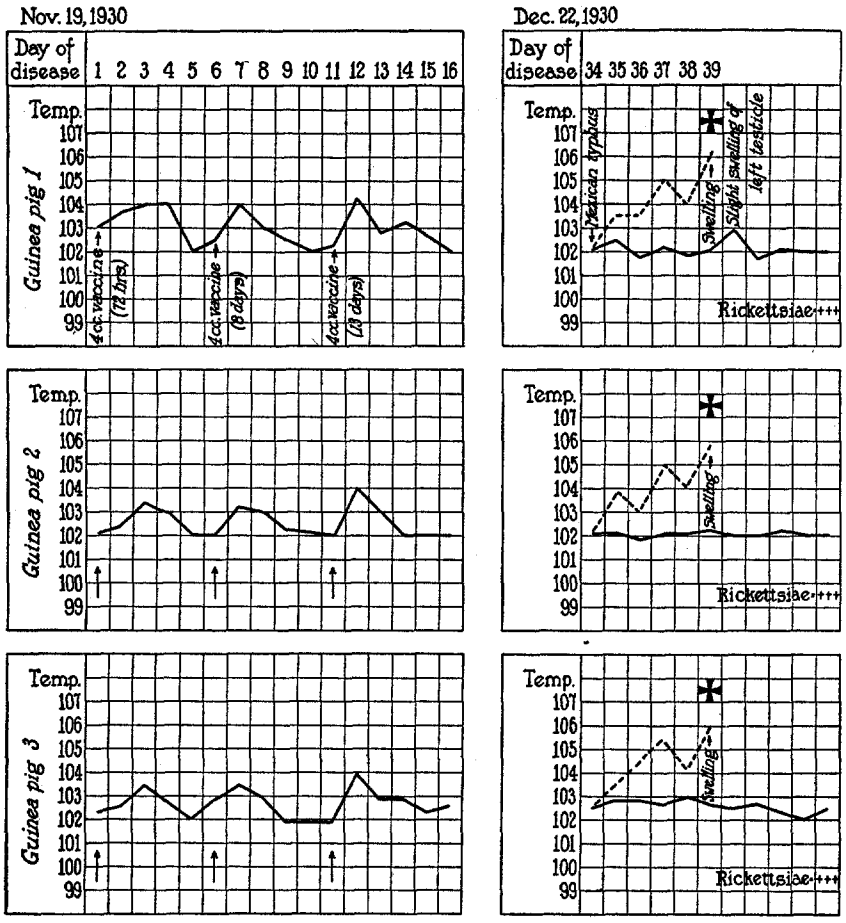


CHART 1. The curves on the left are those of three guinea pigs vaccinated with peritoneal washings of a "diet animal" described in the text. The vaccine was made by treatment with 0.2 per cent formalin and was 3, 8 and 13 days old respectively when injected. On the right are the curves of the same animals inoculated on Dec. 22nd, 23 days after the last vaccination with tunica material from a Mexican typhus animal. The dose was a considerable one and the temperature curves of three controls, inoculated at the same time and with the same material are charted, in broken lines above those of the three immunized animals.

from an active Mexican lesion in which considerable numbers of *Rickettsiae* were present.

This guinea pig was definitely sick on the 5th day after inoculation, reacting in a manner usual to inoculated scorbutic guinea pigs, as described in a preceding paper. In the peritoneal exudate, and in the scrapings from the peritoneum, as well as in the tunica vaginalis, there were large numbers of intra- and extracellular *Rickettsiae*. The material was washed out of the peritoneum with a 0.2 per cent formalin in physiological salt solution. The final suspension, containing cells and many *Rickettsiae*, both intra- and extracellular, represented a concentration of organisms not much inferior to a dilute bacterial culture suspension. The organisms could be found in smears made of the vaccine. It was stored at room temperature for 48 hours before use; after that, in the ice chest. Three male guinea pigs were intraperitoneally treated with this vaccine, 4 cc. of the suspension being injected on Nov. 19th, 25th and 30th. The vaccine, thus, had been in formalin for 3 days, 8 days and 13 days, respectively. This is important as having bearing upon the problem of whether the virus was dead or merely attenuated at the time of injection, a matter of fundamental importance in appraising the value of these experiments. The percentage of formalin is of course of relatively little value in determining its killing power, unless we are dealing with solutions containing equivalent amounts of protein. We cannot, therefore, assume that we worked with killed or attenuated virus—a question of fundamental importance to the significance of the results—merely upon the basis of the concentration of formalin used.

The temperature curves of the three guinea pigs which were intraperitoneally inoculated with the formalinized *Rickettsia* suspension show immediate and sharp rises after each inoculation. We feel confident that these reactions are due to toxicity and not to survival of the virus for the following reasons: The rise of temperature in all cases came immediately, without incubation time, and was followed by rapid return to normal much sooner than this ever occurs in active infection; it was uniform after every inoculation in approximate extent and in time —there being no difference in the general reaction between the first, second and third inoculations, respectively, in all three of the animals; in none of the animals was there, at any time, any sign of scrotal swelling and tunica lesion—a condition which is rarely absent in animals intraperitoneally inoculated with this strain of the disease. We believe for these reasons that, in addition to other things, these experiments show that the *Rickettsia* bodies described by Mooser in this disease possess considerable toxicity. Whether this is in the nature of an excreted poison or of a cellular constituent we cannot state, since we used the entire peritoneal washings in preparing the suspension.

The three animals described were reinoculated, together with their controls, on Dec. 22nd, 33 days after the first and 23 days after the last vaccine injection. The inoculation material consisted of the ground material of the two tunics of a guinea pig in which large numbers of *Rickettsiae* were found. Each animal received about

one-tenth of this suspension, therefore one-fifth of the *Rickettsiae* of one tunica vaginalis. The severity of the dose is indicated by the uniform and violent reactions of the controls, which were entirely typical of the severest form of Mexican typhus infection in regard to temperature, scrotal swelling and *Rickettsia* findings.

Of the vaccinated animals, none showed any temperature reaction. The first guinea pig charted, coincident with the rise to 103°F. on the 7th day after inoculation, had a slight enlargement of the left testicle, which was still reducible, but was—for 1 day—obviously abnormal. This completely subsided within 48 hours, but may have represented a localized temporary lesion.

The series of animals is a very small one, but the entire uniformity of the results and their corroboration of experiments published in our preceding papers on vaccination against the European disease with similar materials persuade us that they are worthy of report.

## CONCLUSIONS

Guinea pigs can be immunized against Mexican typhus virus by peritoneal injections of formalinized *Rickettsia* material, provided sufficient amounts of the organisms are used. Our results in this respect are analogous to those of Spencer and Parker with carbolized virus of Rocky Mountain spotted fever.

The *Rickettsia* suspensions appear to possess considerable toxicity.

We do not wish to be misunderstood as implying that the results in guinea pigs offer anything more than a demonstration of the principle of active immunization with killed *Rickettsiae*. Application to man will have to be worked out, and preliminary to this, we are now attempting to apply the methods to a limited number of monkeys.

## REFERENCES

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