FURTHER EXPERIMENTS IN TYPHUS FEVER

IV. Infection with Washed Mexican Rickettsiae and Immunity to European Typhus

By HANS ZINSSER, M.D., and M. RUIZ CASTANEDA, M.D.

(From the Department of Bacteriology and Immunology, Harvard University Medical School, Boston)

Plate 39

(Received for publication, August 18, 1930)

In a preceding (1) communication we have reported upon experiments in which we succeeded, in a certain number of rats treated with benzol, in obtaining large numbers of *Rickettsiae* of the Mooser type in the peritoneum as well as in the tunica and in many organs in which *Rickettsiae* had not been previously observed. In some of these rats we found extra-cellular *Rickettsiae* in sufficient number to permit us to wash them, by centrifugation in Locke's solution, and to utilize them for infection free of plasma or cells, or, at any rate, with these elements reduced to such an extent that they could not be held responsible for subsequent symptoms in the infected animals. These previously reported experiments showed that, with such washed organisms, the typical Mexican disease could be produced.

In the present communication we wish to report upon a repetition of such experiments and the subsequent inoculation with European typhus virus of animals that had sustained a typical Mexican disease after infection with the washed organisms.

On June 20, several rats received subcutaneously 2 cc. of equal parts of benzol and olive oil. 2 days later these rats were intraperitoneally infected with tunica scrapings from a guinea pig that had been kept in the cold room, was in its fifth day of disease, showed definite swelling and an adherent testicle, and smears of which showed many *Rickettsiae*. On June 27, 5 days after infection, the rats were killed. One of them showed relatively few *Rickettsiae*, but the one used in this experiment had plentiful *Rickettsiae* both in the peritoneum and in the tunica.

The peritoneum was moist, with some exudate, but there was no free fluid. Both tunica and peritoneum were scraped, and the material from each washed out in 15 cc. of Locke's solution. The two materials were then separately handled, as follows:

(A) Peritoneal scrapings washed out in 15 cc. of Locke's solution, centrifuged 5 minutes at high speed, sediment of cells discarded, and supernatant fluid centrifuged for 45 minutes at high speed. The sediment from this on microscopic examination showed many *Rickettsiae*, but no cells were found in two drops thoroughly searched. This was again taken up in 10 cc. of Locke's solution, and centrifuged at high speed for 40 minutes longer. The sediment again showed many *Rickettsiae* and no cells. This sediment was taken up in 8 cc. of Locke's solution and 4 cc. respectively were injected intraperitoneally into Guinea pig 1 and subcutaneously into Guinea pig 2. Assuming that there may have been as much as 0.5 cc. of actual blood plasma in the original peritoneal scrapings, dilution in the several washings with the final injection of half the last 8 cc. of Locke's solution added would mean that any plasma left would have been in total amount not more than 1/600th cc. Since in each case supernatant fluid was carefully pipetted off from the sediment, it is likely that even less than that was present. If cells remained, they were present in very small number, and as a matter of fact we found none.

(B) Tunica scrapings were similarly treated, except that the final sediment was taken up in 5 cc. of Locke's solution instead of 8, but since these 5 cc. were divided between two guinea pigs, the result is the same. In this material there were no whole cells, but there was a small amount of cell detritus. This material was injected in amounts of 2.5 cc. each, intraperitoneally into Guinea pig 3 and subcutaneously into Guinea pig 4.

The results of these injections are represented in the accompanying curves. The intraperitoneally injected animals, Guinea pigs 1 and 3, developed some temperature, Guinea pig 1 reaching 105°F. on the fourth and fifth days and 106° on the seventh day. Only Guinea pig 3 was castrated, because we did not wish to risk losing both animals, but the swelling was entirely characteristic and in Guinea pig 3 typical *Rickettsiae* were found.

The subcutaneously inoculated guinea pigs, 2 and 4, developed no temperature and no swelling—a matter which, with subcutaneous injection of Mexican typhus fever, is not unusual; moreover, the washing process had consumed, from the death of the rat to the injection of the guinea pigs, about 2 hours, during which a certain amount of attenuation in the Locke's solution is conceivable.

So far, then, the experiments showed a complete confirmation of our previous work, in that guinea pigs intraperitoneally injected with washed *Rickettsiae* developed a disease in every way identical with that conveyed by blood, whole tunica scrapings or other infectious material from Mexican typhus fever animals. In regard to the time

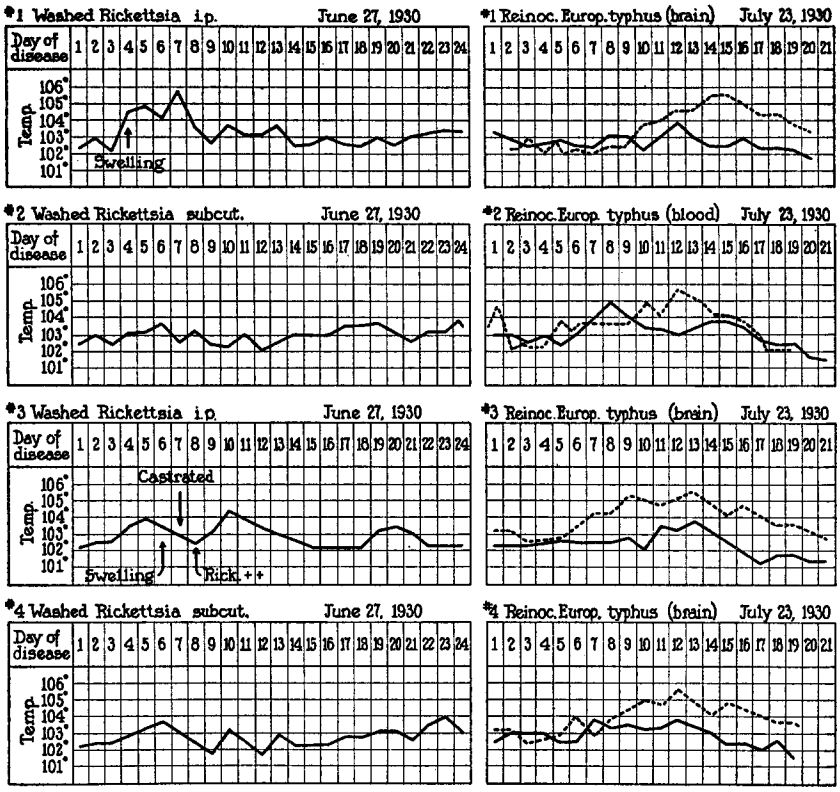

CHART 1. The above curves represent the results of the inoculations described in the text. The four on the left are curves of the temperatures of four guinea pigs inoculated with washed *Rickettsiae* of the Mexican variety, Guinea pigs 1 and 3 intraperitoneally, Guinea pigs 2 and 4 subcutaneously injected. The curves on the right are continuations of the same guinea pigs respectively after inoculation on July 23 with virulent material from European typhus animals. In Guinea pigs 1, 3, and 4 the injection was made with brain emulsion, in Guinea pig 2 with citrated blood. In each case, in the curves on the right, the European control animal is shown in broken lines above the continuous line which represents the temperature of the experimental animal. It will be understood of course that every one of the controls is applicable to every one of the experimental animals except in the case of Guinea pig 2, where experimental animal and control were inoculated with blood. Discussion of the interpretation to be applied to this chart is incorporated in the text.

at which temperature and swelling appeared, the disease resembled that produced by whole tunica scrapings, since with blood and organ materials the symptoms are usually deferred for several days. That the subcutaneously inoculated animals did not react is not surprising, since this happens not infrequently when ordinary virus material is injected.

Reinoculation was deferred until July 23 because, in the 4 or 5 days preceding this, there was an extremely hot spell, during which about twenty-five normal guinea pigs, on which temperatures were taken, reached from 103.8° to 105°F., and it was not regarded safe to continue the experiment until the hot weather had abated. It is the heat which we believe accounts for a temporary rise of temperature apparent in the curves of all but one of these guinea pigs on the 21st of July, the date on which the temperatures of the normal controls were taken, the laboratory temperature at that time ranging between 88° and 92°F.

On July 23 the four guinea pigs described were reinoculated intraperitoneally with virus from a European typhus animal on the fifth day of its fever, when the temperature was slightly above 105°F. Guinea pigs 1, 3 and 4 were injected with 2 cc. each of brain suspension. Guinea pig 2 received 4 cc. of citrated heart's blood. Four controls were injected at the same time in a similar manner, three with brain, one with blood.

The results of these inoculations are shown in the accompanying chart. In every case the temperature of a control is drawn in broken lines, the temperature of the previously inoculated animal in a continuous line. They are charted in this way for convenience and in order to save space, since of course all the controls are applicable to each experimental animal. It is apparent from the chart that every single one of the control animals ran the typical temperature curve of European typhus. In every case the temperature reached 104°F. or above between the eighth and the tenth days, remaining above this point for from 6 to 7 days. The experimental animals, on the other hand, showed in no case a typical European typhus curve. The two guinea pigs that had been originally inoculated intraperitoneally with washed *Rickettsiae* and Guinea pig 4, inoculated subcutaneously with washed *Rickettsiae*, could under no circumstances be regarded as having had a European typhus temperature. Guinea pig 2, which had shown no reaction from subcutaneous inoculation with the washed *Rickettsiae*, did develop a sudden rise of temperature on the eighth day, touching 105°F. The temperature came down immediately, however.

We are quite confident in interpreting this rise of temperature in this guinea pig, and the more moderate temperature rise of Guinea pig 1, as due to the hot weather, which returned at about this time. Nevertheless, however rigidly one criticizes the chart, it is quite obvious that Guinea pigs 1, 3 and 4 showed a definite immunity, and Guinea pig 2 either immunity or increased resistance to an amount of European virus which in every one of four controls gave rise to typical European typhus fever.

Incidental to these observations is the interesting fact that the European control guinea pig charted with experimental Guinea pig 3 showed on the fifth day a slight enlargement of the scrotum, without adhesions, and that on castration and examination of the tunica we found in this European typhus animal a few cells filled with *Rickettsiae* indistinguishable from those of the Mooser type found in the usual Mexican typhus animal. This is in corroboration of previously recorded observations by Pinkerton and by ourselves (3), and confirms us in the belief that, in order to find *Rickettsiae* in the tunica vaginalis of European animals, it is necessary to examine early, certainly before the seventh day, since later examinations are almost invariably negative. Moreover, Dr. Pinkerton tells us that it has been his experience in searching for such organisms in the European strain that tunica lesions and *Rickettsiae* are more apt to occur during the hot summer months.

SUMMARY

The experiments recorded in the preceding paragraphs justify, we believe, the following statements:

They confirm our previous observations that *Rickettsiae* of the Mooser type, obtained in considerable numbers in the peritoneal cavities of some benzolized rats and free from cells and plasma to an extent excluding the participation of these substances in any experimental inoculation effects, can produce the typical Mexican disease in guinea pigs.

Animals which have been subjected to infection with such washed *Rickettsia* material prove themselves subsequently either completely immune or highly resistant to inoculation with considerable amounts of European typhus material which produces typical temperature curves in control animals.

The fact that animals which have passed through the disease caused by the washed *Rickettsiae* are subsequently resistant to European tyhus infection justifies a number of interesting conclusions. In the first place, knowing what we do about cross immunization between Mexican and European typhus infection, these experiments show that the disease conveyed by the washed *Rickettsiae* represents the entire picture of Mexican typhus fever in these animals and that it is not necessary, in consequence, to postulate the co-existence of some virus other than the *Rickettsia* in infectious blood, organs or tunica scrapings. In the second place, it seems clear from these experiments that if washed Mexican *Rickettsiae* will immunize to European typhus fever, the etiological agent of European typhus is likely to be a *Rickettsia* organism identical with or closely related to that causing the Mexican disease and, incidentally, the disease observed by Maxcy (4) in the southern United States. The last conclusion is corroborated by our confirmation of Pinkerton's results in finding organisms indistinguishable from the Mooser type of *Rickettsiae* in early tunica lesions of some European animals.

We believe it likely that Tabardillo and the disease of Maxcy are caused by organisms similar to but not identical with those causing European typhus, differing particularly in capacity for selective localization in the body of guinea pigs, the Mexican variety localizing with great regularity and causing violent local reactions in the tunica vaginalis, the European variety localizing without much reaction only during the very early periods of the disease, rarely causing noticeable gross changes and distributing from there to the organs more slowly than is the case in the Mexican guinea pig disease.

Although our present paper does not deal with the particular problem, we would like to add that all our experience with animal inoculation, tissue culture, etc., is against the conception of *Rickettsiae* as true bacteria, since, so far, they have been rigidly dependent for multiplication upon survival of susceptible animals cells, whether insect or mammalian.

CONCLUSIONS

Precise interpretation of our experiments seems to impose the following conclusions:

Guinea pigs inoculated with washed *Rickettsiae* from Mexican typhus fever develop a disease identical with that resulting from inoculations with whole tunica scrapings, blood or other virulent material, and become thereby immunized to European typhus fever.

The etiological agent of Mexican typhus fever is the *Rickettsia* body of the type described by Mooser (5) in the tunica vaginalis of infected guinea pigs; and it is likely that the etiological agent of European typhus fever is an organism similar to this, but not identical with it in some of its minor biological characteristics.

REFERENCES

1. Zinsser, H., and Castaneda, M. R., *Jour. Exp. Med.*, 1930, **52**, 649.
2. Pinkerton, H., *Jour. Inf. Dis.*, 1929, **44**, 337.
3. Zinsser, H., and Castaneda, M. R., *J. Exp. Med.*, 1930, **52**, 661.
4. Maxcy, K. F., *U. S. Public Health Service Rep. 1087*, June, 1926.
5. Mooser, H., *Jour. Inf. Dis.*, 1929, **44**, 186.

EXPLANATION OF PLATE 39

FIG. 1. One of two tunica cells seen in smears of a European typhus guinea pig castrated on the fifth day after inoculation, when slight swelling was noted. Only two such cells were found in an extensive search, but these were absolutely characteristic.

FIG. 2. Washed *Rickettsiae*. × 900.

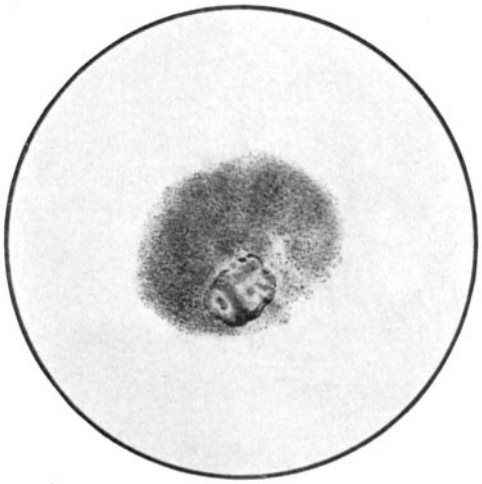

FIG. 1

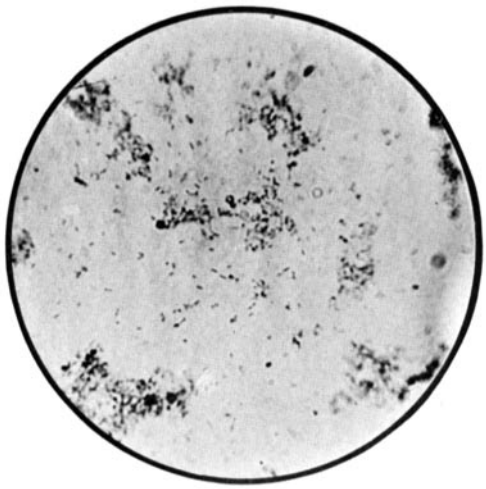

FIG. 2

(Zinsser and Castaneda: Typhus fever. IV)