STUDIES ON THE INTEREPIDEMIC SURVIVAL OF LOUSE BORNE EPIDEMIC TYPHUS FEVER[1]

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In an excellent recent study, Murray and Snyder (Am. J. Hyg., **53**, 22, 1951) isolated seven strains of *Rickettsia prowazeki* from patients ill with Brill's disease (recrudescent typhus), thus confirming and extending the work of previous workers. They furthermore made the significant observation that patients with Brill's disease can infect lice feeding on them. Final proof of Zinsser's hypothesis that man can serve as an interepidemic reservoir of *R. prowazeki* requires isolation of this agent from healthy individuals, known to have had typhus in the past, under conditions which would rule out any possible environmental source of infection. This note describes two such isolations which appear to conform to these requirements.

Both patients had come to this country from Russia more than twenty years ago. Antibody tests previously had shown them to have specific complement fixing (CF) and toxin neutralizing antibodies to *R. prowazeki*. When one patient underwent an abdominal operation, inguinal lymph nodes were taken and cut into 1 mm pieces.[2] For every ten such pieces 3.0 ml of a tissue culture medium containing 4 parts of Baker's solution (Science, **83**, 605, 1936) and 1 part of human serum were used. Incubation was carried out at 30.5 C for 10 days, changing the medium when necessary. A 1 per cent tissue suspension was prepared, and 0.2 ml inoculated intraperitoneally into twenty cotton rats. Six were sacrificed at 12 days, a 3 per cent brain suspension prepared, and 0.5 ml inoculated into the yolk sacs of 6 day old chick embryos. Of the remaining 14 animals seven challenged intracardially with 2 to 4 LD_{50} of the classical epidemic typhus Breinl strain lived, while the other seven challenged with 2 to 4 LD_{50} of the murine typhus Wilmington strain died. White mice immunized with the Breinl but not those immunized with the Wilmington strain were completely protected against 2 to 4 LD_{50} of the fourth egg passage of the isolated strain. Fifteen guinea pigs inoculated with the first cotton rat brain passage showed at least a 10-fold higher CF titer against *R. prowazeki* than *R. mooseri* using the specific antigens (Murray and Snyder, Am. J. Hyg., **53**, 22, 1951). Infected yolk sac smears of the first egg passage stained with Macchiavello's stain showed typical rickettsiae. The second human isolation gave similar results. Part of the lymph nodes injected directly into cotton rats without first being put in tissue culture yielded no *R. prowazeki* from either patient.

A study of the medical records and epidemiological studies showed that neither person very likely could have picked up the disease in his environment, and had no symptoms suggesting epidemic typhus infection for at least six years up to the time of his operation. Furthermore, periodic bleedings over the previous 14 months preceding the operations and 4 months after the operations showed no increase in their 1:10 CF titer to *R. prowazeki*.

The final problem that remains to be solved in the role of man as a reservoir of *R. prowazeki* is the mechanism that causes the recrudescences of the illness. Although we have found latent epidemic typhus infections in cynomolgus monkeys to be activated by injections of cortisone acetate, extensive attempts to cause recrudescences in humans by continued daily injections of 50.0 mg of cortisone or 20 units of ACTH for a period of three weeks have been negative (Price, Emerson, Johnson, Hubbard, Preston, and Nagel, *data to be published*). Of interest is that 3

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[2] A great many precautions and controls were used to rule out, insofar as possible, laboratory contamination. All data indicate the above represent isolations from the patients. Fourteen attempts from similar patients were negative.

of the patients receiving ACTH showed a complete loss of detectable serum antibodies to *R. prowazeki* as measured by CF tests, agglutination tests, and toxin neutralization tests for a period of 4 to 6 weeks. These patients showed no clinical symptoms suggesting epidemic typhus and their antibody levels returned to a very low level not indicative of typhus infection. Extensive attempts to isolate *R. prowazeki* from the blood of these patients during the above period by injection into cotton rats were completely negative.

A STEREOSCOPIC METHOD FOR COUNTING BACTERIAL COLONIES

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Plate counts are usually made with the aid of a magnifying glass and controlled illumination. Some workers have used stereoscopic microscopes for the purpose, generally marking a petri dish into a number of areas with wax pencil lines to facilitate counting. It was noted that when a Wolffhuegel plate was placed on the stage of a stereoscopic microscope equipped with a 1× objective and 9× oculars, one cm^2 of the ruled area practically filled the field of view. Colonies could be observed very readily at this magnification, and at the same time the rulings on the glass plate were in fairly sharp focus. The combination of limiting the field of view to not much more than a single square and the stereoscopic effect greatly facilitated both differentiation of colonies from foreign matter and counting of colonies in and on the agar in a petri dish placed on the ruled glass plate. Transmitted light was employed, but it is desirable to use reflected light in addition to transmitted light when fairly opaque agars are used. This method of observing colonies is particularly valuable when large numbers of colonies are present, and estimates of the bacterial population in densely populated plates can be made quite readily with counts of colonies in the smaller ruled squares on the Wolffhuegel plate. The method also appears to be less tiring to the eyes than is the standard method.

It was necessary, owing to limitations of the stage area, to cut the Wolffhuegel plate along one side of the ruled area in order that all parts of a petri dish placed on the plate could be observed on moving the ruled plate around. Movement of the plate can be controlled with one hand, sliding the plate with the petri dish on it down one line of squares as viewed in the microscope and back along the next one. This process is continued until all colonies have been counted or until the ones in a sufficient number of squares to give a satisfactory average have been enumerated. If a mechanical stage could be provided, it would greatly facilitate moving the ruled plate from square to square covered by the petri dish. The method works well with either poured or spread plates; reliable estimates of the population can be made after five or six hours' incubation. At the same time that counts are being made one can also note differences in colonial or other characteristics of the colonies very readily, and, with proper illumination, the method can be employed for making bacteriophage plaque counts.