6105

A Method of Obtaining Large Amounts of Rickettsia Provaceki by X-ray Radiation of Rats.

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Eventual interepidemic control of typhus fever will depend upon the effective sanitary utilization of the epidemiological facts now available. Even with our almost complete knowledge of transmission and animal reservoir, however, the circumstances under which typhus epidemics occur usually exclude any hope of adequate control. Students of typhus, therefore, have long felt that in this disease particularly a method of specific prophylaxis was needed.

The *Rickettsia provacceki* may now be accepted as the established etiological agent, and all rational methods of vaccination must be based on (1) active immunization with Rickettsia, killed, attenuated or in subinfectious doses; (2) immunization with living Rickettsia, sensitized or neutralized with convalescent or immune serum; and (3) passive prophylaxis with such serum alone.

The ideal method at which first attempts should be aimed is active immunization with killed Rickettsia material. The first fact to be ascertained is whether or not such a thing is possible.

Active immunization of guinea pigs with carbolized Rickettsia was first accomplished by da Rocha-Lima,[1] confirmed by Weigl,[2] Breinl,[3] and Rosenberger.[4] Doerr and Schnabel[5] obtained negative results, possibly, as Otto[6] suggests, because of deterioration of their vaccines. This is rendered likely by Kemp,[7] who found that our own early vaccine material lost potency within about 4 weeks. The above writers, as well as Otto, believe that the results obtained with louse vaccines, in contrast to the negative attempts made with virulent tissues—brain, blood, etc.,—are attributable to the high concentration of Rickettsia in the louse vaccines.

[1] Da Rocha-Lima, *Mediz. Klinik.*, 1917, 1147; also *Münch. Med. Woch.*, 1918 **2**, 1454.

[2] Weigl, *Mediz. Klinik.*, 1924, 1046.

[3] Breinl, F., *Z. f. Immunitätsforsch.*, 1924, **41**, 97.

[4] Doerr u Schnabel, *Wien. Klin. Woch.*, 1919, 523, 891.

[5] Otto, R., and Munter, H., *Fleckfieber, in Kolle u Wassermann Handbuch*, 3rd Edition, 1930, **8**, 1200.

[6] Rosenberger, cited from Otto and Munter, *loc. cit.*

[7] Kemp, H. A., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 353.

In our first vaccinations with formalinized tunica material of Mexican typhus with Batchelder[8] we were encouraged chiefly by the results of Spencer and Parker[9] and of Conner[10] with Rocky Mountain Spotted Fever, which favored the belief that active immunization with dead Rickettsia was feasible provided a sufficient concentration could be attained.

We proceeded, therefore, with the production of vaccine materials from tunica tissues of guinea pigs infected with the Mexican strain, with encouraging results. We used 0.2% formalin as the sterilizing agent, because formalin seemed, in our studies with bacteria, to exert the least denaturizing effect upon antigen, and because Dunkin and Laidlaw[11] had found it suitable in experiments with a filterable virus. Subsequently, we carried out concentration of Rickettsia bodies in the peritoneal cavities of infected rats in which resistance had been reduced by scorbutic diets and by injections of benzol in olive oil.[12] We showed that increased resistance and not infrequently complete immunity can be obtained in guinea pigs by vaccination with large doses of such formalinized suspensions.

Kemp[6] has confirmed the basic fact that formalinized tunica virus can actively immunize guinea pigs, and has pointed out the relative instability of the vaccine.

We ventured to make no predictions in our earlier papers of the possible applicability of this method to the prophylaxis of man. Casco[13] has tested on man our later vaccine, the formalinized exudate of benzolized and infected rats. His results are not discouraging, perhaps encouraging, since only 3 out of 11 vaccinated individuals, and 2 out of 3 controls developed typhus on inoculation with virus. But the numbers are too small and the histories of native Mexicans too uncertain to permit ourselves too much happiness on these figures. What is more encouraging than the actual reinoculation results is the development of Weil-Felix reactions in a large proportion of these and of other vaccinated people observed by Casco.

The present aspects of the problem may be summarized as follows: 1. Active immunization with killed typhus virus is possible.

[8] Zinsser, H., and Batchelder, A. P., *J. Exp. Med.*, 1930, **51**, 847.

[9] Spencer and Parker, *U. S. Public Health Rep.*, 1926, **41**, 35.

[10] Conner, *J. Immunol.*, 1924, **9**, 4.

[11] Dunkin, G. W., and Laidlaw, P. P., *J. Comp. Path.*, 1926, **39**, 201, 213.

[12] Zinsser, H., and Castaneda, M. R., *J. Exp. Med.*, 1930, **52**, 649.

[13] Sanchez-Casco, R., Thesis, 1932, Faculdad de Medicina, National University of Mexico.

2. The immunizing properties of a vaccine seem to depend directly upon the concentration of Rickettsia contained in it.

From this point of view we have attempted to evolve a reliable method for obtaining still higher concentrations of Rickettsia from the Mexican strain. The benzol method was the one employed for Casco at the Mexican Hygienic Institute. While this procedure, especially when combined with the subjection of the inoculated rats to a temperature of about 10 C., usually gave excellent results, we found on consistent study throughout a year that it was subject to variations and that it occasionally failed entirely during the hot months. There is undoubtedly a seasonal fluctuation of typhus susceptibility both in rats and in guinea pigs which complicates experimental procedures.

For these reasons, we attempted many modifications of the procedures for Rickettsia concentrations. We finally succeeded in developing a method which has been carried out on more than 50 rats, and has yielded, with a regularity of approximately 90%, a Rickettsia suspension always equal to the best materials obtained by the benzol procedure, and occasionally superior, even simulating the concentration of bacteria in cultures.

The method depends upon the use of X-rays. Actual radiation was carried out for us at the Huntington Memorial Hospital by Dr. Richard Dresser, without whose cooperation the work would have been impossible. We abstain from reporting preliminary studies and comparative blood counts. As far as the changes in circulating white cells are concerned, our results are consistent with those of others.

The ideal aimed at for our purposes was radiation with short wave lengths sufficiently severe to affect the resistance of the animal, but permitting it to live long enough for adequate multiplication of the Rickettsia. We used rats, guinea pigs and a few rabbits. The exposure which has given us the best results with rats is, under Dr. Dresser's control, as follows: 170 K. V. constant potential; 80 cm. distance; 0.5 mm. copper filter plus 4 mm. celluloid; 8 milliamperes; the effective wave length 0.160 Angstrom units; intensity 10 "r" units per minute. The most suitable time for the exposure of rats has been found to be one hour—*i. e.*, 600 "r" units.

The rats are intraperitoneally inoculated immediately after radiation with a suspension of a Mexican typhus tunica. The material for inoculation need not be richer in Rickettsia than the average obtained in routine transfer. Control rats treated by X-ray in the same way begin to get sick on the 4th or 5th day, but may live 6, 7,

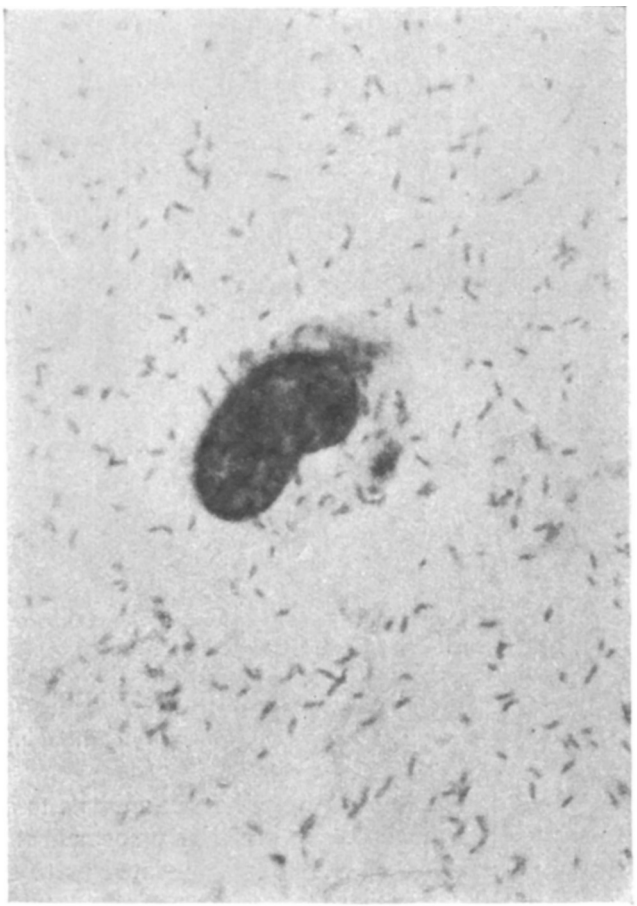

FIG. 1.
Rickettsia in Peritoneal Exudate of X-rayed Rat. Average yield.

or 8 days. The inoculated rats are sicker than the controls by the 3rd day, when some of them may die. This is too early for an adequate yield of Rickettsia. On the 4th day many of the rats appear ill, and by the 5th day they have begun to die. According to the condition of the rats, they are killed on the 4th or 5th day. The skin is dissected from the abdomen and the peritoneum washed and gently scraped with 0.2% formalin salt solution, as in the case of the benzol rats.

The large number of extracellular Rickettsia are not, in our opinion, organisms that have developed outside of the cell. If one examines rats treated in the above manner a day earlier than the optimum, one finds few extracellular organisms, but enormous quan-

tities enclosed within cells. The bursting of these cells results in the large numbers of extracellular organisms.

This method of vaccine production should be undertaken only by immunes, since it is next to impossible to avoid eventual infection.

We do not believe that it will be possible to obtain the results described unless the Mexican strain is used. But since the Mexican vaccine gives a definite though imperfect immunity to the European virus, a sufficient cross immunization can be expected to justify the testing of this method against the European disease.

We have not been able to obtain results in guinea pigs analogous to those obtained in rats. Experiments with rabbits are being made, but the proper exposure to X-ray has not yet been ascertained. Rabbits may die within 24 hours of an exposure to X-ray which rats survive for a week.

6106

Influence of the Concentration of Iodoacetic Acid Upon Excitability and Chemical Changes in Muscle.

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The report of Lundsgaard that muscle from animals treated with iodoacetic acid gives large numbers of relatively normal contractions without perceptible lactate accumulation stimulated us to study the phenomenon in order to rule out several possible sources of error. It seemed possible that iodoacetic acid might accelerate the removal of lactate rather than prevent its formation, and that the poison served to diminish the irritability and the number of contractions rather than influence the lactic acid formation. In studying this problem an important relation between concentration of iodoacetic acid and the rate of lactic acid formation has appeared.

Iodoacetic acid was dissolved in Ringer's fluid, and injected, unneutralized, into the ventral lymph sac, in doses ranging from 1 part in 5,000, on the basis of body weight of the frog, to 1 part in 30,000. Similar concentrations were used in experiments in which iodoacetic acid was added to normal intact muscle or to muscle brei.

Numerous experiments upon the effect of changing the concentration of iodoacetic acid were made. At 1:5,000 lactic acid production is completely inhibited. At 1 to 30,000 there is a consider-