approximately 0.07 cm² these film resistances were found to be 94 and 52 ohm cm², respectively. The changes of impedance with frequency between these two limits were due to the capacitative impedances of the films, which had constant phase angles of 68° and 65° and capacities of 1.07 and 1.23 microfarad/cm² at 1,000 cycles/second, respectively.

The resistance of these films is in marked contrast to the extremely low resistances found in untanned protein films.[1] This reopens the possibility that complex lipo-protein films may have an appreciable resistance and be able to produce diffusion potentials in biological systems. The constant phase angle and a capacity of about one microfarad/cm² is a striking characteristic of all living cell membranes so far measured[5] and we believe these to be the first artificial films produced between two aqueous phases which had these properties.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

MASS PRODUCTION OF VACCINE AGAINST TYPHUS FEVER OF THE EUROPEAN TYPE

IT is unnecessary to emphasize the importance at the present time of the development of prophylactic vaccination against typhus fever. Until 1925, it was supposed that immunization against the Rickettsiae diseases was possible only as a result of actual infection. Since then, however, various attempts to employ living, supposedly attenuated virus for prophylactic purposes have been made[1,2]—all of them, in our opinion, dangerous both for the individual and for the community. Spencer and Parker (1925)[3] were the first to demonstrate that active immunization against spotted fever could be achieved with killed, phenolized Rickettsiae obtained from infected ticks. Following this (1930), Weigl[4] reported analogous results with phenolized European typhus Rickettsiae harvested from the intestinal contents of lice artificially infected. Subsequently (1930), one of the writers with Batchelder[5] and then with Castaneda[6,7] and Macchiavello[8] demonstrated that active immunization against the murine and the European varieties of typhus fever could be produced in animals with formalinized suspensions of the respective micro-organisms. The principles were thus established, but difficulties still remained in the way of obtaining the relatively large amounts of Rickettsiae necessary for practical immunization. Numerous reports have been made by investigators who claim to have been able to cultivate Rickettsiae on media without tissue. The present writers, however, because of the negative results reported by other workers and their own failure, after long experience and persistent effort in similar attempts, feel that, up to the present, such tissueless cultivation of virulent Rickettsiae has not been achieved.

We may summarize the present state of affairs in regard to typhus vaccine production as follows:

As far as the murine variety of typhus fever is concerned, tissue culture methods of various kinds are easily carried out. By none of these procedures can such large quantities of Rickettsiae be produced as by the technique of intraperitoneal inoculation of x-rayed rats, a method sufficiently described in preceding communications. As first developed by one of us with Castaneda,[9] this method has been utilized in its original form and with modifications on a considerable scale by Castaneda in Mexico and Veintemillas in Bolivia.

Unfortunately, the rat methods have, in the course of years of effort, proved inapplicable to the classical European virus. In consequence, other procedures have been suggested.

The Weigl louse technique is effective, but entirely unsuitable for large-scale application.

Tissue culture methods based on the modified Maitland technique as first developed for European Rickettsiae by Nigg and Landsteiner[10] were used for immunization, first, by Kligler and Aschner[11] and

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were enlarged for greater quantity production by one of the writers with Macchiavello.[12] While immunologically valid and quantitatively more useful than the Weigl method, the Maitland technique still remains inadequate for large-scale work.

Recently, two further techniques have been described. The first of these is our own agar tissue procedure which, now adapted to use with Kolle flasks, furnishes considerable amounts of vaccine;[13,14] the second is the Cox method of inoculation of fertile hen's eggs.[15] In 1934[16] Dr. Zia demonstrated in our laboratory that European and murine Rickettsiae would grow on the chorio-allantoic membranes of hen's eggs, but the yield was too small for practical purposes. Cox inoculated directly into the yolk sac. Microscopically, he finds few if any Rickettsiae of various types in the chorio-allantois and in tissues of the embryo itself. In the yolk sacs, however, Rickettsiae are numerous with the spotted fever and murine typhus infections, though none were found in the European variety of typhus.

We have repeated the Cox method, using the European strain, and have passed the virus to date through 15 egg passages. The embryos die regularly after 4 days. After several passages, we begin to find Rickettsae in the yolk membranes, although they are rarely very numerous. We have never found Rickettsiae in the embryos themselves. Comparisons between the egg method and our own agar technique indicated that, as far as yield of active material was concerned, the two procedures were of the same order, although the yolk membranes of the eggs infected with the European virus titrated somewhat higher in guinea pigs than did the material from the agar tubes—in the proportion of about 10^{-7} to 10^{-6}. There are, however, some advantages in the agar method, such as greater ease of cellular elements from the vaccine and more accurate morphological control.

The method which we now employ to secure large numbers of Rickettsiae consists in a combination of the agar method—using considerably enlarged surfaces for cultivation—and the egg technique as a source of inoculum. Specifically, the minced embryonic tissue or macerated yolk sac taken from eggs on the fourth day following infection is used to inoculate large quantities of normal minced chick tissue from 10-day embryos. The tissue thus infected is distributed in large amounts on the agar surfaces of modified Kolle flasks. The neck of the ordinary flask is replaced by one which will receive a No. 6 rubber stopper. After 6 or 7 days' incubation at 37° C., cultures very rich in Rickettsiae are obtained. We emphasize the fact that considerable quantities of tissue may be employed. Transplants can then be made by using the culture Rickettsiae or new cultures inaugurated with material from infected eggs.

By this method, with a personnel of one bacteriologist and two technicians, one liter of vaccine sufficient for 300 complete immunizations can be produced in a week. Increase of production is only a matter of enlarging equipment and personnel.

Since embryonic mouse tissue has also been used successfully in the agar method, it could be substituted for the chick material. For the present, however, the latter gives excellent yields and has proved satisfactory.

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