color obtained with carotene develops its maximum intensity at once and fades within 2 minutes, leaving a dirty yellow brown color. The green color obtained with cholesterol develops its maximum intensity at the end of 15 minutes.

Carotene does not interfere with the quantitative estimation of cholesterol in the blood. The extraction with chloroform of a mixture of blood and plaster of Paris removes the cholesterol, but does not release the carotene present.

7327 P

Immunization of Guinea Pigs with Formalized Cultures of European Strain of Typhus Rickettsia.

I. J. KLIGLER AND M. ASCHNER.

From the Department of Hygiene and Bacteriology, Hebrew University, Jerusalem.

We have reported[1] the successful cultivation of the European strain of Typhus *rickettsia* in flask cultures containing guinea pig tunica. These cultures have now passed successfully through 7 subcultures during a period of 4 months.

In view of successful immunization of guinea pigs with formalized rickettsia suspensions from infected lice[2] and Zinsser's[3] experience with formalized rickettsia suspensions of the Mexican type from infected X-rayed rats, it appeared desirable to test the possibility of using flask tissue cultures for this purpose. Our efforts to immunize guinea pigs with formalized infected brain tissue proved unsatisfactory. Dead virus produced at best only a slight degree of immunity, when large amounts (10 gm.) of formalized infected tissue were injected. Solid immunity could only be obtained with tissue formalized for 1-2 hours, that is tissue still containing live (attenuated?) virus. The difficulty seems to lie in the quantity of organisms, or in other words the amount of antigen, injected. The cultures appeared to offer a satisfactory solution to the problem. On the one hand, the organisms are present in fairly large quantities and, on the other, the cultures are more easily handled than lice.

1 Kligler, I. J., and Aschner, M., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 349.

2 Kligler, I. J., Olitzki, L., and Aschner, M., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 456.

3 Zinsser, H., and Castaneda, M. R., *J. Exp. Med.*, 1933, **57**, 381.

Guinea pigs have been successfully immunized with formalized suspensions of fresh virulent cultures as well as with old cultures no longer infective for guinea pigs.

Experiment 1. The material from 2 flasks of an 11-day-old virulent culture, second generation, was mixed, centrifuged, the supernatant fluid decanted, the sediment thoroughly triturated in a glass mortar and resuspended in the clear supernatant fluid. A formalin solution was added to the suspension to give a 0.1% concentration and the material left overnight at ice box temperature. Each of 2 guinea pigs was given 3 injections intraperitoneally of 1.5 cc. of the diluted formalized material, at intervals of 3 days. Each animal received the material of one culture. None of the animals developed any sign of fever or even a slight rise in temperature. The temperature during the immunization did not exceed 39°C., that is, it remained within the normal range of temperature of our guinea pigs.

Ten days after the last injection the guinea pigs received 80 infective doses and 16 days later 800 infective doses of brain virus. Neither manifested temperature or any other reaction. Appropriate controls came down with typical infections.

Experiment 2. In this experiment a series of old cultures, 30 to 55 days old, 4th to 6th generation, was used. They apparently no longer contained viable organisms since 1 cc. of a heavy suspension of the triturated tissue failed to infect a guinea pig. These cultures were treated as above and 4 guinea pigs immunized by 3 injections of 2 cc. each, at intervals of 3 days. Tested 6 and 18 days after the last injection all 4 guinea pigs proved immune to 80 and 800 infective doses of brain virus.

It is apparent, therefore, that formalized flask tissue cultures of the European strain of Typhus *rickettsia* provide as effective a vaccine for guinea pigs as do formalized *rickettsia* suspensions from infected lice. These cultures offer a relatively simple procedure for the production of vaccine *in vitro,* since old cultures produce as effective immunity as fresh cultures.