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Cultivation of Vaccinia in Agar-Slant Tissue Cultures.

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The method most widely used for *in vitro* cultivation of vaccine virus is the modified Maitland technic with chick embryonic tissue suspended in Tyrode's solution. In this medium, multiplication of the virus occurs in the cells but not in the fluid in which they are suspended. It seems conceivable, therefore, that any medium which is capable of preserving metabolic activities of the cell may also provide conditions necessary for the growth of vaccine virus. Zinsser, *et al.*,[1, 2] devised a solid medium, consisting of Tyrode's solution, horse or beef serum and agar, which the authors successfully employed for the cultivation of different Rickettsiae. Since these organisms are intracellular parasites and require for their growth the presence of viable cells, it is likely that the medium permitting their development may also be satisfactory for the cultivation of vaccine virus. The medium has already been successfully used in this laboratory by FitzPatrick for the cultivation of the Eastern strain of equine encephalitis. In the present paper, the successful cultivation of vaccine virus is described.

The virus used was a strain of vaccinia propagated for some time in chorio-allantoic membranes of developing hen's eggs. The material for initiation of cultures was prepared by removing an infected membrane and mincing it in a large test-tube with scissors. A small, arbitrary amount of this material was then inoculated into finely minced tissues of chick or mouse embryo and again thoroughly mixed. The inoculated tissue was deposited upon agar slants prepared as described by Zinsser and his coworkers. From 60 to 80 mg of the tissue by wet weight was placed upon each agar slant contained in test-tubes measuring 6x1 inches. Usually, the amount of tissue obtained by mincing one chick embryo of 8 to 9 days old or that of a mouse weighing 0.5-0.6 g was sufficient for inoculation of 6 or 8 agar slants. The tubes were tightly stoppered with rubber stoppers and kept at 37°C for 5 days. Subsequent serial transfers were made by inoculating freshly prepared chick or mouse embryonic tissues with the contents of one agar-slant culture. In this manner,

1 Zinsser, H., Wei, H., and FitzPatrick, F., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 285.

2 Zinsser, H., FitzPatrick, F., and Wei, H., *J. Exp. Med.*, 1939, **69**, 179.

the virus has been propagated for 16 generations in chick embryonic tissue and for 12 generations in mouse embryo. Determinations of potency were carried out every 3rd generation of both series. Since the initial cultures were inoculated with an arbitrary amount of an infected chorio-allantoic membrane, the determination of the titer of these cultures before incubation was also performed. In all instances, the material for titration was weighed under sterile precautions, ground, diluted to different concentrations, and 0.2 cc of each concentration injected into shaved rabbits' skins. It was found that the infected tissue used for the first cultures and titrated before incubation gave positive skin reactions in dilution 1 :250. The culture grown in chick embryonic tissue reached in the 3rd generation the titer of 1 :50,000. The 6th generation showed a titer of 1 :100,000. This titer remained unchanged for the 9th and 12th generations. The 15th generation gave a titer of 1 :1,000,000. The cultures propagated in mouse embryo increased to 1 :100,000 in the 6th generation, but the titration of the 9th generation gave only 1 :10,000. The 12th generation showed again the titer of 1 :50,000. These fluctuations of titer of cultures propagated in mouse embyonic tissue were thought to be due to the inferior qualities of the mouse embryo as compared with those of chick. To prove this point, the 9th generation culture grown in chick embryo, showing a titer of 1 :100,000, was transferred into mouse embryo tissue. Subsequent titrations of this new culture indicated immediate and considerable drops in potency of the original culture to 1 :10,000 and 1 :1,000 in the 1st and 3rd generations respectively. When, however, the latter culture of a low titer was transferred back into chick embryo, rapid rise of the titer to the original level of 1 :100,000 was observed. Likewise, the 9th generation of the culture grown in mouse embryo tissue having a titer of 1 :10,000 was transferred into a chick embryo tissue culture and an immediate rise of the titer to 1 :10,000 was recorded.

It is difficult to explain why the cultures initiated with the infected chorio-allantoic membrane and propagated in mouse embryo tissues exhibited during the first few generations progressively increasing titers, whereas those started with the chick embryo tissue culture showed rapid diminution of potency.

The multiplication of the virus grown upon solid medium used here occurred exclusively in the tissue cells. This followed from the result of titrations in which tissue, washings of the agar surface after the removal of the tissue, condensation water, and different layers of the agar slant were used. It was found that whereas the tissue showed the titer of 1 :100,000, washing prepared by using 5 cc of normal saline to an agar slant and condensation water gave weak

skin reactions when employed in the undiluted state. No reaction was elicited when ground agar removed from different depths of the slant was tested.

To determine the optimal time for incubation of cultures, a series of 7 tubes was prepared by inoculating chick embryo with an infected chorio-allantoic membrane. One tube was immediately placed in the icebox, and the remaining 6 tubes were incubated for 3, 4, 5, 7, 9, and 11 days, respectively. Titration of all of these cultures, performed upon the same rabbit, showed the titer of 1:1,000 for the first tube, and of 1:10,000, 1:10,000, 1:10,000, 1:1,000, 1:0 and 1:0 for the remaining 6 cultures. The experiment showed that the multiplication of the virus took place during the first 5 days. Deterioration of potency observed on further incubation was due, in all probability, to the effect of incubator temperature. This is suggested by the fact that cultures grown for 5 days and then kept in the icebox showed no decrease in their titer during the first 3 weeks of storage.

Summary. The cultivation of the vaccine virus in agar-slant tissue cultures was shown to be possible both with chick and mouse embryo tissues. The former was found to be distinctly superior so far as the growing qualities of the tissue are concerned.

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Toxic Manifestations After Oral Administration of Sodium Sulfapyridine.

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In 2 previous papers[1, 2] we have reported the occurrence of uroliths consisting mainly of 2-(acetylsulfanilyl amino) pyridine, in rats, rabbits and monkeys following oral administration of sulfapyridine. Similar results were also published by Gross, Cooper and Lewis.[3] Marshall, *et al.*,[4] have shown that sulfapyridine in the form of its soluble sodium salt is more rapidly and completely absorbed than sulfapyridine, and have recommended its intravenous injection in

1 Antopol, W., and Robinson, H., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 428.

2 Molitor, H., and Robinson, H., *Arch. Internat. de Pharm. et Ther.*, in press.

3 Gross, P., Cooper, F., and Lewis, M., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 448.

4 Marshall, E. K., Bratton, A., and Litchfield, J., *Science*, 1938, 597.