the ability to cause clotting appears at this same time. The control 0.05 cc. of either heated or fresh unactivated extract never causes clotting.

6945

Influence of Oxygen on Survival of Tissue Suspensions.

JOSEPH T. KING AND LYDIA LUX. (Introduced by F. H. Scott.)

From the Department of Physiology, University of Minnesota.

Living cells are necessary for the growth of filterable virus. In certain instances cultures have been incubated with free access to oxygen. Li and Rivers[1] point out that a gradual simplification of the medium has taken place recently.[2, 3, 4] They also emphasize the advantages of chick embryo tissue in saline over the more complex media employed earlier. Flexner and Noguchi[5] and Long, Olitsky and Rhodes[6] on the virus of poliomyelitis are typical of work done on cultures under oil. Dochez, Mills and Kneeland[7] and Powell and Clowes[8] used chick embryo tissue under vaseline seal.

In this type of culture apparently intact cells are seen after incubation. Muckenfuss and Rivers[9] and Muckenfuss[10] found that the addition of dead cells to the virus improved survival. Eagles and McClean[11] emphasized the unsatisfactory state of our knowledge regarding the rôle of tissue which had access to oxygen.

We attempted to define more accurately the condition of the cells supposed to be living in such preparations. Saline suspensions of chick embryo were studied since they are coming into general use.

Ten-day chick embryos were shaken with freshly broken pyrex

---

[1] Li, C. P., and Rivers, *J. Exp. Med.*, 1930, **52**, 465.

[2] Carrel, A., and Rivers, T. M., *Compt. rend. Soc. Biol.*, 1927, **96**, 848.

[3] Maitland, H. B., and Maitland, M. C., *Lancet*, 1928, **2**, 596.

[4] Muckenfuss, R. S., and Rivers, T. M., *J. Exp. Med.*, 1930, **51**, 149.

[5] Flexner, S., and Noguchi, H., *J. Exp. Med.*, 1913, **18**, 461.

[6] Long, P. H., Olitsky, P. K., and Rhodes, C. P., *J. Exp. Med.*, 1930, **52**, 361.

[7] Dochez, A. R., Mills, K. C., and Kneeland, Y., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 513.

[8] Powell, H. M., and Clowes, G. H. A., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 332.

[9] Muckenfuss, R. S., and Rivers, T. M., *J. Exp. Med.*, 1930, **51**, 149.

[10] Muckenfuss, R. S., *J. Exp. Med.*, 1931, **53**, 377.

[11] Eagles, G. H., and McClean, *Brit. J. Exp. Path.*, 1929, **10**, 35.

glass in 10 cc. of Tyrode as described earlier.[12] Three cc. portions of the suspension were incubated in pyrex test tubes under sterile oil at 37.5° for the intervals stated. Control consisted of 2 cc. of the same suspension in a Carrel D5 flask. Immediately after preparation and at intervals of one hour 0.2 cc. portions were removed after mixing and planted in Carrel D3 flasks in 1 part heparinized rabbit plasma and 2 parts tissue extract, a medium well known to promote rapid growth. Some of the same suspension was incorporated into a tissue culture clot, fixed, sectioned and stained for microscopic examination. Only the results on planted suspensions are reported in this preliminary note.

In general it has been found that incubation under oil as described causes serious damage to the tissues as expressed by an increase in the latent period of growth and a decrease in the vigor of the growth. Finally there is complete failure of growth.

In the control cultures at the beginning the latent period may be as short as 1½ hours and after growing for 48 hours the growth is dense. In the cultures from under oil the increase in latent period may be detected by the 4th or 5th hour and by the 12th hour there is serious impairment of growth. The impression gained is that failure of growth occurs not later than 16-20 hours. Control cultures from the Carrel flask at 24 hours showed practically no difference as compared with that planted at the beginning.

It is realized that the ability to grow is not the only criterion that may be utilized in determining the condition of cells in these preparations. Correlation of the histological and hydrogen ion changes is being made.

[12] King, Joseph T., *Arch. f. Exp. Zellforsch.*, 1930, **10**, 341.