New York Section.

New York Academy of Medicine, January 20, 1932.

5917

Studies of Yellow Fever Virus in Tissue Culture.

E. HAAGEN* AND M. THEILER. (Introduced by J. H. Bauer.)

From the Yellow Fever Laboratory, International Health Division, Rockefeller Foundation, New York.

The virus of yellow fever, like most invisible agents of infectious diseases, has not been cultivated on any of the usual bacteriological media. After it had been established that this virus belongs to the group of filterable infectious agents, it seemed worth while to attempt its cultivation *in vitro* by methods successfully developed for other filterable viruses. A strain of yellow fever virus highly pathogenic for mice made it possible for the infectivity of cultures to be tested repeatedly and accurately on a large scale.

Of the several methods of growing viruses in tissue cultures, the use of Carrel dishes has given the best results. Only after repeated attempts were we finally able to carry the virus through the first subculture. The culture medium chiefly used has been a mixture of normal monkey serum and Tyrode solution in which were suspended small fragments of fresh tissue. Many kinds of tissue were used, principally minced kidneys and testicles of guinea pigs and rabbits or minced chicken embryos 8 to 10 days old. The minced embryos have been used almost entirely in the more recent experiments because the virus appeared to remain more uniformly virulent in the cultures containing these tissues.

The virus for the primary culture was prepared by grinding infectious mouse brain in a mortar with 9 times its weight of Tyrode solution and centrifuging the suspension for about 10 minutes at 3000 revolutions per minute. One part of the supernatant fluid was added to 4 parts of the culture medium, and the mixture was distributed in several Carrel dishes, about 2.0 cc. in each. Taking

* Reichsgesundheitsamt, Berlin.

the concentration of infected mouse-brain tissue as one, the final dilution of virus would be 1:50. The cultures and subcultures were always tested for bacteriological sterility. Neither on bacteriological culture medium nor in smears or sections of the tissue cultures was any visible organism apparent. Three to 4 days is the most favorable time between transfers. Subcultures were made in the following manner: The fluid portion of the previous culture was allowed to stand in conical centrifuge tubes to permit as much as possible of the old autolysed tissue to settle. Of the supernatant fluid 0.5 cc. was added to 2.0 cc. of freshly prepared medium in each of several Carrel dishes. The dilution factor of the virus in each subculture, in relation to the virus content of the previous culture, was therefore 5. It is estimated that in the 10th subculture the original virus was diluted about one-half of a billion (5×10^8) times, in the 20th subculture about 5 million billion (5×10^{15}) times. The fact that the last passages were just as infectious as the original culture shows not only that the virus had been preserved but that in all probability multiplication had taken place. The incubation temperature used for the cultivation was 37.5°C.

The infectivity tests were always done in mice. All mice inoculated intracerebrally with the 22nd subculture showed typical symptoms of encephalitis with paralysis. Sections of brain from these mice revealed characteristic perivascular round cell infiltrations and acidophilic changes in the nuclei of the ganglion cells.

From these experiments it appears that the virus of yellow fever like other filterable viruses needs living cells for growth *in vitro*. This virus is extremely labile and is changed or destroyed by the most minute and undetermined changes in the culture medium.

It is therefore possible to grow yellow fever virus indefinitely by a method which has been used successfully for the continuous cultivation of other viruses.

---

[1] Stokes, A., Bauer, J. H., and Hudson, N. P., *Am. J. Trop. Med.*, 1928, **8**, 103.

[2] Theiler, M., *Ann. Trop. Med. and Parasit.*, 1930, **24**, 249.

[3] Haagen, E., *Arch. Exp. Zellforsch.*, 1929, **8**, 499, and **12**, in press.