the embryonic mouse brain tissue was not infected by either test virus, the tissue promoted the growth of the equine virus in such a way that when the supernatant fluid of these cultures, after 48 hours of incubation, was inoculated into chick embryo tissue cultures, the tissue became infected and failed to grow when it was plasma-patched.

The sensitivity of the *in vitro* titration method presented here was compared with intracerebral mouse inoculation. Soon after the serially diluted virus was inoculated into tissue culture, 0.03 cc of the material from each dilution was inoculated intracerebrally into mice. The results are shown in Table 1. It can be seen that the titer obtained in the tissue culture is the same as that of the intracerebral mouse inoculation test. The test was repeated 3 times with approximately similar results.

The method herein presented is rather complicated and can hardly be applied for mass study. However, it suggests the possibility of detecting virus in tissue culture if a highly susceptible tissue is employed. Another method which obviates this complex procedure has been developed and will be reported separately.[5]

*Conclusion.* Both the St. Louis encephalitis virus and the Jungeblut-Sanders mouse virus have been successfully titrated *in vitro* by the use of embryonic mouse brain tissue and with the help of another system consisting of chick embryo tissue and Western strain of equine encephalomyelitis virus.

[5] Huang, C. H., PROC. SOC. EXP. BIOL. AND MED., in press.

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## A Visible Method for Titration and Neutralization of Viruses on the Basis of pH Changes in Tissue Cultures.*

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The advantages of determining virus potency and of demonstrating neutralizing antibodies of the Western strain of equine encephalomyelitis (W.E.E.) virus in tissue culture as presented in previous papers[1,2] include (a) reduction of cost of experiments; (b) removal of the variable of individual animal reactivity; and (c) the possibility that the tissue culture method is a more sensitive indicator of the presence of virus than animal inoculation. However, the application of the method to other viruses was limited, since the titration and neutralization of the W.E.E. virus depended upon growth, or absence of growth of tissue. Such a test could not be applied to many viruses because of the low susceptibility of the fibroblasts to virus. Thus, the St. Louis encephalitis virus and the Jungeblut-Sanders mouse virus[3] could not be titrated in chick embryo tissue culture and a more complicated procedure had to be developed when embryonic mouse brain, the most susceptible tissue, was used.[4] This complicated procedure was introduced because of the difficulty of growing the mouse brain tissue

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[1] Huang, C. H., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 396.

[2] Huang, C. H., *J. Exp. Med.*, 1943, **78**, 111.

[3] Jungeblut, C. W., and Sanders, M., *J. Exp. Med.*, 1940, **72**, 407.

[4] Huang, C. H., PROC. SOC. EXP. BIOL. AND MED., in press.

in plasma patched preparations. To obviate the complex procedure, a simplified method has been developed.

During an investigation concerned with metabolism of infected and non-infected tissue, it was found[5] that the degree of glycolysis in the tissue infected with the W.E.E. virus as measured in the respirometer of a type described by Victor,[6] was much lower than that of non-infected tissue. On the basis of this observation, the present method for titration and neutralization of several viruses has been developed. The method does not require transfer of tissue into Carrel dishes and the whole experiment can be carried out in the same tube. The method is based on the fact that the products of glycolysis are acid in reaction. In preliminary experiments, it was found that when non-infected tissue was present, the pH of the culture medium shifted to the acid side and in the presence of phenol red, the color of the medium changed from red to yellow within 2 hours at 37.5°C in partial vacuum (a desiccating jar evacuated for one hour by water suction). Conversely, when tissue was heavily infected with virus for a sufficiently long period of time, there was either very little change or no change in the pH of the medium.

In order to obtain the greatest change in the pH of the medium, it was necessary to substitute a Ringer's solution containing 0.1% glucose and 5 mg % phenol red for the serum ultrafiltrate medium after the tissue cultures containing virus had been incubated for several days. The change of the medium was essential since diluted serum ultrafiltrate is a well-buffered medium and does not reflect pH changes as readily as the Ringer's solution. In this way, the potency of and neutralizing antibodies against the W.E.E. virus in chick embryo tissue culture could be calculated by making use of visible changes in the fluid medium. In the same manner, the St. Louis encephalitis virus and the Jungeblut-Sanders mouse virus could be titrated in embryonic mouse brain tissue culture in a relatively short period of time. There is evidence that the method is practicable and is accurate when compared with animal inoculation.

*Method.* Ten-fold dilutions of virus were made in a physiological solution "$Z_2$."[7] 0.1 cc of the material from each dilution was inoculated into tissue cultures consisting of a series of tubes each containing 15 to 20 pieces of minced embryonic tissue (mouse embryonic brain tissue for Jungeblut-Sanders mouse virus and St. Louis encephalitis virus; chick embryonic skeletal tissue for equine virus) and 1.5 cc of diluted serum ultrafiltrate.[7] The different mixtures were incubated at 37.5°C for different periods of time depending on the particular virus used (2-3 days if Jungeblut-Sanders mouse virus and equine virus were tested and 6-7 days when St. Louis encephalitis virus was used). After incubation, supernatant fluid of each tube was discarded and the tissue was washed twice with 5 cc of modified Ringer's solution. The washed tissue was finally suspended in approximately 0.3 cc of the modified Ringer's solution. The entire preparation was then kept in a desiccating jar and the air inside the jar was evacuated by water suction pump for 1 hour. After this period of evacuation, the desiccating jar containing the cultures was kept at 37.5°C and the readings were made in 2 to 4 hours.

[5] Victor, J., and Huang, C. H., unpublished data.

[6] Victor, J., *Am. J. Physiol.*, 1935, **111**, 477.

[7] Simms, H. S., and Sanders, M., *Arch. Path.*, 1942, **33**, 619.