TABLE II.
Average Concentration of Fat in the Livers of Patients with Gastro-intestinal Carcinoma Who Were Fasted and of Those Given Various Dietary Supplements Preoperatively.

No. of patients	Preoperative supplement	Avg conc of hepatic fat, g per 100 g wet tissue	% reduction of hepatic lipid effected by supplement
28	None	16.4	—
11	8 g lipocaic	8.0	51
7	3 g choline chloride	11.2	39
8	1200 mg inositol	6.9	58
10	280 " "	8.2	50

logic effects of the lipocaic, another group of 10 patients with carcinoma of the gastro-intestinal tract was given during the last 10 preoperative hours only that amount of inositol (280 mg) contained in the effective dose of lipocaic. The clinical and chemical methods used have been described in a preceding communication of this series.[1] The results obtained from the ingestion of the smaller amounts of inositol indicate that in the patients studied, the lipotropic properties of the lipocaic could be accounted for by its content of inositol alone (Tables I and II). Of the values of hepatic lipid, only one was significantly elevated, and the average of the group was only 0.50 that of the control group.

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Titration of St. Louis Encephalitis Virus and Jungeblut-Sanders Mouse Virus in Tissue Culture.*

C. H. HUANG.† (Introduced by Murray Sanders.)

From the Departments of Medicine and Bacteriology, College of Physicians and Surgeons, Columbia University, New York City.

Following successful titration and neutralization of the Western strain of equine encephalomyelitis virus in chick embryo tissue culture,[1,2] attempts were made to see whether or not the same method could be applied to other viruses. The St. Louis encephalitis virus and the Jungeblut-Sanders mouse virus[3] were chosen for study.

The principle on which titration of the equine virus in tissue culture is based, is the finding that tissue fails to grow when it is heavily infected with virus. Thus, in the earlier studies when St. Louis encephalitis virus and Jungeblut-Sanders mouse virus were tested in chick embryo tissue cultures, it was found that potency could not be determined as simply as with the equine virus. There was no appreciable difference in the growth of infected and non-infected tissue when the tissue was subsequently patched with plasma.

Since it was felt that the failure to note a difference in growth of infected and non-infected tissue treated with St. Louis encephalitis and Jungeblut-Sanders mouse viruses was probably due to low susceptibility of chick embryo tissue to these 2 viruses,[2] it became of interest to determine whether or not a difference in the growth of infected and non-

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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.

TABLE I.
Comparison of *in vitro* and *in vivo* Titration of St. Louis Encephalitis Virus and Jungeblut-Sanders Mouse Virus.

Virus used	Titrated in	Virus dilution tested: No virus	10-2	10-3	10-4	10-5	10-6	10-7	10-8	10-9
St. Louis encephalitis virus	Mice	A A	4 4	4 4	5 5	5 6	A A	A A	A A	
		A A	4 5	4 4	5 5	6 A	A A	A A	A A	
	Tissue culture	OOOO	GGGG	GGGG	GGGG	GGGG	OOOO	OOOO	OOOO	
		OOOO	GGGG	GGGG	GGGG	GGGG	OOOO	OOOO	OOOO	
Jungeblut-Sanders mouse virus	Mice	A A	2 2	2 2	3 3	3 3	3 3	3 4	A A	A A
		A A	2 2	3 3	3 3	3 3	3 4	4 4	A A	A A
	Tissue culture	OOOO	GGGG	GGGG	GGGG	GGGG	GGGG	GGGG	OOOO	OOOO
		OOOO	GGGG	GGGG	GGGG	GGGG	GGGG	GGGG	OOOO	OOOO

A = Mouse remained alive 8 days.
Each number indicates day of death of the animal after the infection and presumably represents a virus death.
G = Growth of fibroblasts from explants noted.
O = No growth from the explants.
Each "G" or "O" represents growth activity of a single explant. Eight explants were used for each plasma preparation.

infected tissue could be demonstrated if a more susceptible tissue were employed. Embryonic mouse brain tissue was therefore chosen since adult mice succumbed regularly to intracerebral infection with these 2 viruses in high dilution. As the growth of brain tissue in plasma preparation has not been satisfactory, the following method has been introduced.

Mouse brain tissue containing the virus (either St. Louis encephalitis virus or Jungeblut-Sanders mouse virus) was diluted serially in physiological solution "Z_2."[4] One drop of the material from each dilution was inoculated into tissue cultures consisting of a series of tubes each containing 10 pieces of minced embryonic mouse brain tissue (the embryo measured 8-10 mm in length) and 1 cc of diluted serum ultrafiltrate.[4] The entire preparation was incubated at 37.5°C for 4 days in the case of the Jungeblut-Sanders mouse virus and 6 days when St. Louis encephalitis virus was tested. After incubation, one drop of 10 M.L.D. of Western strain of equine encephalomyelitis virus was introduced into each tube which was then re-incubated. At the end of 48 hours, one drop of the supernatant fluid from each tube was inoculated into separate tubes each containing 10 pieces of minced skeletal muscle of 9-day developing chick embryo and 1 cc of serum ultrafiltrate diluted in a physiological solution. They were then kept at 37.5°C. After 48 hours of incubation, pieces of tissue from each tube were transferred and patched with plasma in separate Carrel dishes. Readings were made under the low power microscope after 24 hours of incubation.

It was thus found that embryonic mouse brain tissue infected with either the St. Louis encephalitis virus or the Jungeblut-Sanders mouse virus incubated for 6 and 4 days respectively, failed to support the growth of 10 M.L.D. of Western strain of equine encephalomyelitis virus. This is shown by the fact that the equine virus disappeared from these cultures after 48 hours of incubation. Consequently, when the supernatant fluid from these cultures was subsequently inoculated into chick embryo tissue cultures, the chick embryo tissue remained uninfected by equine encephalomyelitis virus and was able to proliferate when the tissue was patched with plasma. Since it has been found that both the St. Louis and the Jungeblut-Sanders mouse viruses have no influence on the growth of chick embryo tissue and therefore they do not interfere with the test, no attempt was made to see whether or not these viruses were still present in these cultures after the introduction of equine virus. Conversely, when

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

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.*

C. H. HUANG.† (Introduced by Murray Sanders.)

From the Departments of Medicine and Bacteriology, College of Physicians and Surgeons, Columbia University, New York City.

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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