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Tissue Cultures as a More Sensitive Method Than Animal Inoculation for Detecting Equine Encephalomyelitis Virus.

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Cultures consisting of minced chick embryo tissue suspended in Tyrode's solution[1] have been found favorable for the growth of equine encephalomyelitis virus. If it can be shown that cultures are equally or even more effective for revealing minimal quantities of virus in a material, or that cultures can bring to light the presence of virus when animal inoculation fails, a useful method will be available. Equally important is the fact that in a single test inoculum, a larger amount can be introduced into a culture than in an animal, since by intracerebral inoculation* a guinea pig can receive with safety a limiting quantity of only 0.2 cc., and a mouse 0.05 cc.

A comparison was therefore made of the relative sensitivity of cultures and of intracerebral inoculation of mice and guinea pigs for detection of the virus.

In Experiment 1, shown in Table I, a single disc Seitz filtrate of a 10% broth suspension of infected mouse brain tissue was diluted decimally to 10^{-8} dilution. Each of 4 mice received an intracerebral injection of 0.05 cc. from each dilution and a like number of cultures were inoculated with the same quantity. After 72 hours' incubation at 37°C. each culture was tested for the presence of active virus by intracerebral injection of mice with 0.05 cc. of the centrifuged supernatant fluid. Active virus was detected up through 10^{-5} dilution of the Seitz filtrate by animal test, and in 10^{-6} dilution by inoculation of tissue cultures.

In Experiments 1 and 3 (Table I) the cultures revealed virus in dilutions tenfold greater than the limiting dilutions active by direct intracerebral injection of animals.

Both "collar flasks"[2] and 50 cc. Erlenmeyer flasks have been employed as culture containers with equally good results. In large, flat bottomed flasks correspondingly greater quantities of culture suspension and of inocula can be used.

[1] Cox, H. R., Syverton, J. T., and Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 896.

* All such operations were done with the aid of deep ether anesthesia.

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

TABLE I.

Experiment	Substance and final dilution of virus tissue	Quantity inoculated in each culture or animal	Results of inoculation		
			Tissue culture	Animal†	
				Guinea pig	Mouse
		cc.			
1	Filtrate M.B. 10^{-4}	.05	4/4‡	—	4/4
	10^{-5}	.05	4/4	—	4/4
	10^{-6}	.05	2/4	—	0/4
2	Filtrate G.P.B. 10^{-3}	.1	3/3	3/3	—
	10^{-4}	.1	3/3	3/3	—
	10^{-5}	.1	2/3	1/3	—
3	Filtrate G.P.B. 10^{-4}	.1	3/3	3/3	—
	10^{-5}	.1	3/3	3/3	—
	10^{-6}	.1	2/3	0/3	—
4*	Supernatant M.B. 10^{-6}	.05	5/5	—	5/5
	10^{-7}	.05	5/5	—	5/5
	10^{-8}	.05	5/5	—	2/5
5*	Supernatant M.B. 10^{-6}	.05	4/4	—	4/4
	10^{-7}	.05	4/4	—	3/4
	10^{-8}	.05	2/4	—	1/4

M.B. = mouse brain; G.P.B. = guinea pig brain. For mice virus dilutions were prepared in hormone broth of pH 7.6; for guinea pigs in Tyrode's solution.

*Here dilutions were prepared from supernatant fluid obtained by spinning in an angle centrifuge at 4,000 r.p.m. for 30 minutes.

‡The denominator shows the number of cultures inoculated; the numerator the number of cultures showing virus.

†The denominator designates the number of animals receiving same substance as cultures and the numerator the number of animals dead from encephalitis.

Cultures of amounts of virus too small to be detected by direct animal inoculation contained, after incubation, virus in a concentration of at least 1:10,000. The virus present in such cultures had multiplied at least 700,000 fold for when 0.05 cc. of the test material was added to 3.5 cc. of medium, there was an immediate dilution of 1:70. After incubation the culture could be further diluted to at least 10,000 times and 0.05 cc. of the dilution induced lethal encephalitis in mice. Such increases in virus content were demonstrated in all of three cultures titrated.

To conclude, cultures of minced chick embryo tissue suspended in Tyrode's solution can reveal the presence of equine encephalomyelitis virus (Eastern strain) in dilutions which are inactive after intracerebral inoculation of mice and guinea pigs; the virus multiplies rapidly in cultures within 72 hours and, with more medium, a single inoculum larger than that employed in the animals can be tested for virus content.

The culture method has been applied in attempts to detect small amounts of active encephalomyelitis virus in formolized vaccines

which were inactive by animal inoculation. Moreover, multiplication of minute quantities of virus in tissue cultures was not inhibited by the presence in them of formolized vaccines in which the formalin was neutralized by ammonia. It was thus possible to determine that an amount of vaccine (1 cc.)—the immunizing dose—contained no detectable active virus.

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Host Age and Cell Metabolism in Mouse Lymphatic Leukemia.

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During the course of experiments on the relationship of cell virulence to cell metabolism, certain results indicated that the age of the host influenced the metabolism of transmitted leukemia cells. To investigate the effect of age, the following experiments were performed: Mice of 2 age groups (6-8 weeks and 6-8 months) were inoculated with the same transfer cells of a transmission line M-liver. Metabolic measurements, namely Q_{O_2}, $Q_{CO_2}^{O_2}$ and $Q_{CO_2}^{N_2}$ in Ringer's solution[6] were made on the lymph nodes 4 days after inoculation. The mice were of strain C 58, 100% of which develop lymphatic leukemia after inoculation with line M-liver; 90% develop spontaneous lymphatic leukemia after 6 months of age.[1] So far as could be determined, *i. e.,* from palpation and blood counts before inoculation and autopsy, no evidence of spontaneous leukemia was found in mice used. A summary of the metabolic results is presented in Table I. The metabolism was significantly decreased in every characteristic studied. The decrease in Q_{O_2} of the leukemic lymph nodes parallels the decrease found in normal lymphoid tissue previously reported.[2] On the other hand the decrease in $Q_{CO_2}^{O_2}$ and $Q_{CO_2}^{N_2}$ is actually greater than the table indicates, for it has been found that the normal glycolytic rates are higher in 6-8-month-old mice than in 6-8-weeks-old mice. In other words the actual excess glycolysis, both aerobic and anaerobic produced by the leukemia cells is less than the values in the table.

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[6] Warburg, O., *Metabolism of Tumours,* London, Constable & Co., Ltd., 1930.

[1] MacDowell, E. C., and Richter, M. N., *Arch. Path.,* 1935, **20**, 709.

[2] Victor, J., and Potter, J. S., *Brit. J. Exp. Path.,* 1935, **16**, 243.