17085. Failure of Human Convalescent and Hyperimmune Monkey Poliomyelitis Serums to Neutralize "Egg Adapted" Lansing C (M) Variant.

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Enright and Schultz[1] reported the successful propagation of the Lansing C (M) strain of poliomyelitis virus in the embryonated hen's egg. Their conclusion was based on the production of typical lesions and paralysis in monkeys with early egg-passage material only. In addition, neutralization was accomplished by anti C (M) rabbit serum, but not by antiserums against the murine SK and Theilers GD VII strains, although some cross-reaction was observed with the former. Unreported observations by Schultz[2] have since indicated that the so-called Lansing variant is more closely related to the murine SK strain than to the original Lansing C (M) virus. More recently, Powell and Jamieson[3] have reported the isolation of an apparently similar variant in embryonated eggs. They subsequently proved by mouse protection tests that it was serologically distinct from the original Lansing CM virus and similar to the MM and murine SK strains. Previous unsuccessful or questionable attempts to propagate the Lansing strain to eggs have been reported by Riordan and Sa-Fleitas[4] and Gard.[5]

The egg-adapted variant mentioned above was received from Dr. Edwin W. Schultz in 1948 and readily multiplied in chick embryos.[1] The range of infectivity for mice by the intracerebral route was between 10^{-5} and 10^{-7}. Preparations from the first and subsequent passages made in this laboratory were also infectious for mice by the intraperitoneal, subcutaneous, intravenous, intranasal, and occasionally by the oral route. Seven and a half times the intracerebral dose was necessary for infection intraperitoneally. The regularity with which paralysis occurred following intraperitoneal injection suggested the possibility of using this means for human serum neutralization tests. For this purpose, 58 specimens of serum from convalescent cases of poliomyelitis were studied. Of these, 33 were taken within 6 months after the onset of the disease, 10 between 6 months and 5 years, and 11 between 5 and 13 years. The time of onset in the other cases was unknown. Immune guinea pig serum was prepared against the Schultz variant for use as a control.

Methods. Neutralization tests were performed in the following manner. The virus was prepared so that 0.1 ml contained 50 LD_{50} as titrated by the intraperitoneal route. The convalescent serums were diluted either 1:5 or 1:10, and mixed with equal volumes of the virus suspension. Extract broth was used as the diluent in both cases. The mixtures were incubated in a 37°C water-bath for 2 hours, and 0.2 ml inoculated intraperitoneally into 3-week-old Swiss mice. Controls with homologous immune serum, normal guinea pig serum, and in some cases monkey serum were run simultaneously with each series. Six to 8 mice were used for each serum tested. During the course of experiments, three separate lots of egg-passage material were utilized, each being previously standardized for the LD_{50} dose. Animals dying within the first 24 hours following injection were discarded; death or paralysis after this period was taken as the end-point and usually occurred during the first week. All experiments were terminated at the end of 2 weeks. Irregular results in the control animals were obtained occasionally. These were found to be due to the use of animals older than four weeks, rather than to deterioration of the virus, which was stored at –6°C.

Results. A total of 58 human convalescent

1 Enright, J. B., and Schultz, E. W., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 541.

2 Schultz, E. W., personal communication.

3 Powell, H. M., and Jamieson, W. A., *J. Infect. Diseases*, 1948, **83**, 238.

4 Riordan, J. T., and Sa-Fleitas, M. J., *J. Immunol.*, 1947, **56**, 263.

5 Gard, S., *Nature*, 1943, **152**, 660.

poliomyelitis serums were tested. Of these, 56 showed no neutralization whatsoever. Only 2 gave slight but definite protection. With 50 LD_{50} of virus, the 50% end-point with these serums did not exceed 10^{-1}. In several experiments complete protection was never obtained with 50 LD_{50} of virus, but in two experiments complete protection against 25 LD_{50} was provided by a 1:5 dilution of the serums. Of interest is the fact that the serum of one patient was obtained 13 years after the clinical disease, whereas the other was drawn 3 weeks after the onset of paralysis.

Similar tests were carried out using potent hyperimmune monkey serums* against the Lansing, Kotter (Brunhilde group) and Brockman strains of human poliomyelitis virus. None of these serums protected against the Schultz variant. Experiments in which 10, 25, and 50 LD_{50} of virus were used, together with a 1:5 serum dilution, showed no significant difference between the hyperimmune and pooled normal monkey serums. On the other hand, the "variant" immune guinea pig serum completely protected against 50 LD_{50} of MM virus.†

Summary. The egg-adapted variant originally obtained by Enright and Schultz has not proved to be of value as a means of measuring antibody response in serums from hyperimmune monkeys and convalescent human poliomyelitis cases. The results support the conclusions of Schultz, and of Powell and Jamieson that the egg-adapted variant is serologically related to the MM-SK group of murine encephalomyelitis viruses, rather than to human and monkey-adapted strains.

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† Obtained from Dr. C. A. Evans.

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17086. Electron Microscopy of Tissue Cultures Infected with the Virus of Eastern Equine Encephalomyelitis.*

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During the past 2 years we have been studying the growth of several viruses in tissue culture with particular emphasis on the use of the electron microscope. The cellular destruction and overwhelming growth of the virus of eastern equine encephalomyelitis are here reported.

Successful electron microscopy of virus infected cells has been limited to studies on "virus induced" tumors[1,2] and has not been applied to small rapidly multiplying viruses.

Our technic is essentially that of Porter[3] except that cells are usually fixed for only ten minutes with osmic acid vapor.[4]

Chick embryo tissue was cultured for 6

TABLE I.
Equine Encephalomyelitis in Tissue Culture. Titration on 10-day Embryos of Fluid Removed from Tissue Cultures.

Exp. No.	Original inoculum	Titer of virus in tissue culture fluid
1	10^{-2}	$10^{-5.5}$
2	10^{-3}	$10^{-6.5+}$
	10^{-3}	$10^{-6.5+}$
3	10^{-4}	10^{-7}

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1 Claude, A., Porter, K. R., and Pickels, E. G., *Cancer Research*, 1947. **7**, No. 7.

2 Porter, K. R., and Thompson, H. P., *J. Exp. Med.*, 1948, **88**, 15.

3 Porter, K. R., Claude, A., and Fullam, E. F., *J. Exp. Med.*, 1945, **81**, 233.

4 Bang, F. B., and Gey, G. O., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 86.