Cultivation of Poliomyelitis Virus (Lansing Strain) in Human Embryonic and Placental Tissues.* (17833)

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Recently Enders *et al.*(1,2) have reported the successful cultivation of the Lansing strain of poliomyelitis virus in human embryonic and foreskin tissues. These workers presented the first evidence that the poliomyelitis virus is capable of *in vitro* multiplication "in cells other than those of nervous origin." Prior to these reports Sabin and Olitsky(3) demonstrated multiplication of a monkey-adapted strain of poliomyelitis (MV) in tissue cultures composed of human embryonic brain fragments. Their attempts to propagate this strain in non-nervous tissues were unsuccessful. Although various strains of mouse encephalomyelitis virus multiply readily in animal tissue cultures (4,5) and the chick embryo(6), experiments with the poliomyelitis virus have thus far given negative results.

The present studies were undertaken to confirm the results of Enders *et al.*(1) on the cultivation of the Lansing strain of poliomyelitis in human embryonic tissues. Experiments were also carried out to attempt propagation of the Lansing strain in placental tissue because of the difficulty in obtaining human embryos.

Methods. The technic of Weller and Enders(7) was employed in the preparation of all tissue cultures. Essentially the cultures consisted of tissue fragments suspended in 3 to 20 cc of a mixture of balanced salt solution (3 parts) and ox serum ultrafiltrate (1 part). Tissues of human embryos ranging from 4 weeks to 4 months of age and premature infants approximately 7 months of age were used. Most of the tissues were obtained from embryos 2 to 4 months old. The following tissue fragments were employed: skin, intestine, arm and leg muscle and brain. Both individual and pooled tissue cultures were prepared. The initial inoculum of each tissue culture flask consisted of 0.1 to 0.4 cc of a 10% centrifuged mouse brain suspension of the Lansing strain of poliomyelitis virus(8) depending on the volume of nutrient fluid present. The nutrient fluid was removed as completely as possible and replaced at intervals ranging from 4 to 7 days The presence of virus in the supernatant fluid of the tissue culture was demonstrated by inoculation of Swiss mice (Webster and Plymouth strains) and occasionally rhesus monkeys intracerebrally. Penicillin and streptomycin mixtures (250 units/cc) were added to the tissue cultures to prevent bacterial contamination. If contamination occurred, the cultures were discarded. Tissue cultures were also prepared in the same manner from the chorion, amnion and spongios tissues of both mature and immature human placentas. Cultures were composed of both individual and pooled tissues. The identity of the virus used as the primary inoculum and cultivated was verified by (1) production of typical paraly-

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1. Enders, J. F., Weller, T. H., and Robbins, F. C., *Science*, 1949, v109, 85.

2. Weller, T. H., Robbins, F. C., and Enders, J. F., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 153.

3. Sabin, A. B., and Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1936, v34, 357.

4. Evans, C. A., and Chambers, V. C., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 436.

5. Van Rooyen, C. E., and Rhodes, A. J., *Virus Diseases of Man* (2nd Ed.), N.Y.: Nelson, 1948, p. 911.

6. Riordan, J. T., and Sá-Fleitas, M. J., *J. Immunol.*, 1947, v56, 263.

7. Weller, T. H., and Enders, J. F., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 124.

8. Milzer, A., Oppenheimer, F., and Levinson, S. O., *J. Immunol.*, 1945, v50, 331.

TABLE I.
Multiplication of Poliomyelitis Virus in Various Individual Human Embryonic Tissue Cultures.

Exp. No.	No. supernatant fluid changes	Tissue culture				
		Skin	Intestine	Arm muscle	Leg muscle	Brain
I	3	4/5*	1/6	5/6	1/6	—
	4	5/5	0/5	4/5	1/5	—
	5	3/5	0/6	5/6	1/6	—
II	3	1/6	0/5	0/5	6/6	—
	4	2/6	0/5	1/6	4/6	—
	5	1/6	0/5	0/5	4/6	—
III	3	1/6	—	2/6	0/6	3/5
	4 (contaminated)					
IV	3	2/5	—	2/4	—	—
	4	2/6	—	2/5	—	—
	5	3/5	—	2/5	—	—
V	3	1/5	0/4	1/4	—	0/5
	4	2/4	0/5	0/5	—	0/5
	5	1/5	0/4	0/4	—	0/5
VI	3	4/4	0/4	—	0/6	—
	4	2/4	0/4	—	0/6	—

* Numerator = No. of mice which developed paralysis.
Denominator = No. of mice inoculated.

sis in mice and rhesus monkeys following intracerebral inoculation, confirmed by histopathology and (2) neutralization tests with hyperimmune Lansing monkey antiserum.

Results. Evidence of virus multiplication was obtained in 9 of 16 embryos in various tissue cultures consisting of both pooled and individual tissues. In most of the positive pooled tissue cultures virus was demonstrated in the fifth nutrient fluid supernatant change which represents an approximate dilution of the original inoculum of 10^8 times. The LD_{50} titer of the original mouse brain inoculum was approximately $10^{-3.5}$. In another experiment virus was demonstrated in the third supernatant of a transfer of the original culture (5th supernatant) to a second fresh tissue culture representing an approximate dilution of 10^{11} times of the original inoculum.

Two rhesus monkeys inoculated intracerebrally with the third supernatant fluid change of a pooled tissue culture consisting of skin, arm muscle, intestine and brain developed a triplegia and paraplegia after an incubation period of 6 and 14 days respectively. These animals were sacrificed and sections of their cervical and lumbar cord showed pathologic findings typical of poliomyelitis. Neutralization tests were conducted with the fourth supernatant of the same tissue culture. Mice inoculated with tissue culture plus hyperimmune monkey Lansing antisera were protected while control mice developed typical paralysis and died. We have also successfully immunized mice by repeated intraperitoneal inoculations of the active tissue culture virus to subsequent intracerebral challenge inoculation with mouse CNS virus. The degree of such active immunity from the tissue culture virus was considerably less than that usually achieved by mouse CNS virus(8).

Virus multiplication was demonstrated in skin tissue cultures in 6 of 6 attempts; in arm muscle in 5 of 5 attempts; in leg muscle in 2 of 4 attempts; in intestine in 1 of 4 attempts and in brain in 1 of 2 attempts. These results are summarized in Table I.

Attempts were also made to propagate the Lansing strain in tissue cultures prepared from the chorion, amnion and spongios tissues of 11 mature and two immature human placentas. The placenta tissue fragments were washed 3 to 12 times with nutrient

fluid prior to use in order to remove as much gamma globulin as possible. Evidence for virus multiplication was obtained in only 2 instances. One was a pooled chorion and amnion tissue culture in which virus was demonstrated in the third supernatant (original virus dilution approximately 10^5 times) and the other a spongios tissue culture in the fourth supernatant (original virus dilution approximately 10^7 times). Seven attempts to cultivate the Lansing strain in human foreskin tissue cultures have given negative results, although Weller *et al.*(2) have recently reported positive findings. We feel that our experiments are inconclusive since the foreskins were not received in satisfactory condition and merthiolate was used to disinfect the prepuce prior to circumcision. Additional experiments are planned with more suitable preparations.

Comment. Our findings definitely confirm those of Enders *et al.* on the cultivation of the Lansing strain of poliomyelitis in various human embryonic tissue cultures. We were also able to confirm their claim that this virus is capable of multiplying in cultures composed of non-nervous tissues. The multiplication of the poliomyelitis virus in tissue culture is extremely important because it opens up the possibility of employing tissue culture virus for attempts to produce a poliomyelitis vaccine for human immunization. Undesirable reactions due to inoculation of brain tissue vaccines could thus be eliminated.

Evidence of virus multiplication in placental tissue cultures was obtained in only 2 of 13 experiments. Perhaps the failures were due to the presence of traces of gamma globulin in placental tissues even though these tissues were washed repeatedly with nutrient fluid. The difficulty in removing all antibodies from cells by repeated washings is a common laboratory experience. Moreover, gamma globulin has been shown to have a relatively high neutralizing antibody titer for the Lansing strain of poliomyelitis virus(9,10). Possibly no Lansing neutralizing antibodies were present in the two placenta tissue cultures giving positive results.

Summary. The propagation of the Lansing strain of poliomyelitis virus in various human embryonic tissue cultures was confirmed. The virus was also shown to multiply in cultures composed of non-nervous tissues. Optimum results were obtained with embryonic skin and muscle tissues. The identity of the virus cultivated was established by intracerebral inoculation of mice and rhesus monkeys, histopathologic findings, neutralization tests with Lansing hyperimmune serum, and production of specific active immunity. Evidence of virus multiplication in human placental tissue was obtained in only 2 of 13 tissue cultures. It is suggested that the negative results may have been due to the presence of gamma globulin in the placental tissues.

9. Bodian, D., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 259.

10. Levinson, S. O., Milzer, A., and Wolf, A. M., Abraham Levinson, Anniversary Vol., Studies in Pediatrics and Medical History, New York; Froben Press, 1949, p. 131.

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Distribution of Azo-Proteins in the Tissues of the Normal Mouse.* (17834)

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The preparation of a highly colored azo-protein, R-salt-azo-benzidine-azo-ovalbumin (1) provided a chemically labelled antigen which could be detected colorimetrically in

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1. Heidelberger, M., Kendall, F. E., and Soo, C. M., *J. Exp. Med.*, 1933, v58, 137.