tolic and diastolic pressures distal to the constriction, and the development of a systolic murmur, (b) a rise in maximal and initial tensions in the right ventricle, and (c) an elevation of right atrial pressures. In some instances, relative tricuspid insufficiency developed.

The elevation of central venous pressure is obviously associated with translocation of a large volume of blood to the venous side of the circulation. Evidence is presented that a part of this transfer occurs from the pulmonary vascular bed, although it is admitted that a shift may also occur within the systemic circuit itself toward the central veins.

Received August 19, 1949. P.S.E.B.M., 1949, **72.**

Cultivation of Poliomyelitis Virus in Cultures of Human Foreskin and Embryonic Tissues.* (17359)

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Recently, the propagation *in vitro* of the Lansing strain of poliomyelitis virus in human embryonic tissues was reported and evidence presented that this virus is capable of multiplying in cells other than those of nervous origin.[1] These experiments have been continued and this agent now has been carried for a total period of 224 days through 13 serial cultures in which the tissue consisted of mixed human embryonic skin and muscle. This strain has also been maintained for 173 days in two lines of 11 serial cultures each and composed respectively of human embryonic intestine and brain.

Additional experiments described here in a preliminary manner are reported. Two objectives were in mind: (a) to determine whether the Lansing strain was capable of multiplying in completely differentiated non-nervous tissue as well as in embryonic tissue; (b) to determine whether the Brunhilde strain of poliomyelitis virus—a strain immunologically distinct from the Lansing group[2] and not adaptable to rodents—could, like the Lansing strain, be cultivated in non-nervous human embryonic tissues.

Propagation of the Lansing strain in human foreskin tissue. As a source of completely differentiated non-nervous tissue fragments of human foreskin were employed. The use of this tissue was suggested by the report of Blank, Coriell, and Scott,[3] who explanted it on the chorioallantoic membrane according to the method of Goodpasture. The material was derived from patients between 4 and 11 years of age. Each prepuce was sufficient for the preparation of at least 8 cultures and before mincing was washed 2 or 3 times in nutrient fluid[4] containing 50 units each of streptomycin and penicillin per ml. The fluid phase of the cultures, which contained the same concentration of antibiotics was removed and replaced at intervals of 4 days. The original set of cultures was inoculated with 0.1 cc of a 10% suspension of pooled mouse brains infected with the Lansing strain. As inoculum for subcultures 0.1 ml of the pooled

* Aided by a grant from the National Foundation for Poliomyelitis, Inc.

† Work done while a post doctorate Fellow of the United States Public Health Service.

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1 Enders, J. F., Weller, T. H., and Robbins, F. C., *Science*, 1949, **109**, 85.

2 Bodian, D., Morgan, I. M., and Howe, H. A., *Am. J. Hyg.*, 1949, **49**, 234.

3 Blank, H., Coriell, L. L., and Scott, T. F. McNair, PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 341.

4 Weller, T. H., and Enders, J. F., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 124.

TABLE I.
Multiplication of Lansing Poliomyelitis Virus in Human Foreskin Tissue.

Culture set	No. of nutrient fluid changes prior to subculture	Day of incubation subculture done	Mouse LD_{50} of pooled fluids used to inoculate subcultures	Calculated dilution of original inoculum at time of subculture
Original*	4	20	$10^{-1.0}$	10^{-6}
1st subculture	3	16	$10^{-1.7}$	10^{-11}
2nd "	3	16	$10^{-1.2}$	10^{-16}
3rd "	4	20	$10^{-0.0}$	10^{-22}
4th "	3	16	$10^{-1.6}$	10^{-27}
5th "	(in progress)			

* The LD_{50} of the infected mouse brain used in the original inoculum was $10^{-3.7}$; 0.1 cc of a 1:50 dilution of brain was employed and the calculated mouse LD_{50} titer of the fluid phase immediately after inoculation was equal to $10^{-0.5}$.

centrifuged fluids removed on the 16th or 20th day of cultivation from the preceding set of cultures was used.

In the experiment summarized in Table I the virus was maintained for 88 days through 5 serial cultures of human foreskin. During this interval the fluid phase was removed and replaced 17 times. Pooled centrifuged supernatant fluids removed from the 4th culture series on the 20th day of cultivation when inoculated intracerebrally produced paralysis in a rhesus monkey. Histological changes consistent with a diagnosis of poliomyelitis were observed in the monkey's cord. Supernatant fluids removed from each serial culture produced paralysis in mice on intracerebral inoculation. Mice injected with fluids from uninoculated control cultures of tissues from the same sources remained well. It was calculated that during the 88-day period of cultivation the suspension of virus originally inoculated had undergone a dilution of at least 10^{-27}. It would appear, therefore, that the Lansing strain is capable of multiplication in the presence of well differentiated skin and subcutaneous tissue and in the absence of intact nerve cells.

Propagation of the Brunhilde strain in human embryonic tissues. Two series of experiments were initiated utilizing the same technic. In one, the cultures consisted of mixed skin, muscle and connective tissue from the extremities of human embryos of 3 to 4 months gestation, and in the other of brain tissue from the same embryos. In each, the original set of cultures was inoculated with 0.1 cc of a 10% suspension of monkey cord infected with the Brunhilde strain of poliomyelitis virus, supplied through the kindness of Dr. H. A. Howe. The skin and muscle series was subcultured three times to fresh tissues and the brain series twice.

Following intracerebral inoculation into rhesus monkeys of pooled centrifuged supernatant fluids of the skin-muscle series on the 12th and 21st days, respectively, from the second and third subcultures, muscular weakness of the lower extremities was observed. Histologic examination of the cords from these animals revealed changes consistent with infection by the virus of poliomyelitis. Fluids from the second subculture of the series prepared with brain tissue also produced paralysis on intracerebral inoculation into a rhesus monkey. No illness resulted after intracerebral inoculation of a portion of the same pooled supernatant fluids, from the 3rd subculture into Swiss white mice.

These findings indicate that the Brunhilde strain had been maintained for 73 days in the skin-muscle cultures and 39 days in those of brain. It was calculated that during the longer period the original inoculum of virus had been diluted 10^{-22} times as a result of subculture and changes in the fluid phase.

According to Bodian[5] the usual titer of monkey cord infected with this strain for the rhesus monkey is 10^{-6}. From the calculated dilution of the primary inoculum of the tissue cultures, it may, therefore, be inferred that this strain, like the Lansing virus, also multiplies *in vitro* in the presence of non-nervous tissue. Whether or not it can be propagated

[5] Bodian, D., *Am. J. Hyg.*, 1949, **49**, 200.

indefinitely under these conditions must await further investigation.

We are grateful to members of the Staff of the Boston Lying-in Hospital and the Children's Hospital for supplying the tissues. We also acknowledge with thanks the indispensable assistance in the experimental work of Marguerite Buckingham and Jeanette Levens.

Received August 24, 1949. P.S.E.B.M., 1949, **72.**

Fluorocardiography (Electrokymography) During Normal Respiration. (17360)

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Previous studies[1,2] described an apparatus, utilizing our modification of the Henny-Boone method of electrokymography,[3,4] and the typical patterns of tracings recorded over the major cardiovascular and pulmonary structures. Fluorocardiography was suggested as more descriptive than previously employed names for the method.

In previous studies, the tracings were recorded during voluntary apnea in an intermediate phase of respiration. Comparative observations showed that the pulsations of the lung (and, to a lesser extent, those of the hilar shadows and the pulmonary artery) were greater during inspiration than in sustained expiration. For practical purposes, an intermediate phase was chosen; a few words of instruction usually sufficed to teach the patient to hold his breath without excessive inflation or deflation of the lungs. This procedure was followed without demonstrable disadvantage in our entire series of clinical cases during 1947 and 1948. On the other hand, it was found difficult to obtain reliable tracings in children and patients with chronic diseases of the lungs who were unable to control their respiration because of age or dyspnea. For this reason, an attempt was made to overcome this difficulty by technical means.

Technical aspects. The problem was presented to Maurice B. Rappaport, E.E. He suggested that a filter may be introduced to minimize respiratory effects though with the introduction of some error.

Wandering of the base line caused by respiration may be reduced considerably by altering the electrical time constant of the apparatus. When this constant is shortened, there is a proportionally greater attenuation of the coarse as compared with the more rapid waves. Respiration is represented by extremely slow waves in the fluorocardiogram, far coarser than any of those caused by cardiac action. A suitable condenser, or filter, introduced between the fluorocardiograph and the recording galvanometer, may modify the time constant of the apparatus; the smaller the condenser, the lesser is the electrical constant. Condensers of various size, which may be introduced by means of a switch, permit selection of the optimum time constant, namely the minimum value producing a fluorocardiogram that does not wander off the paper. The reduction of the electrical time constant of the apparatus does introduce a certain degree of error in the fluorocardiogram which is dependent upon the amount of reduction. The error presents itself in two forms: one is a slight error in the phase (or actual time) of registration of the component waves; the other is a slight change in their configuration. However, there is no elimination of the essential component waves present

[1] Luisada, A. A., Fleischner, F. G., and Rappaport, M. B., *Am. Heart J.*, 1948, **35**, 336.

[2] Luisada, A. A., Fleischner, F. G., and Rappaport, M. B., *Am. Heart J.*, 1948, **35**, 348.

[3] Henny, G. C., and Boone, B. R., *Am. J. Roentgen.*, 1945, **54**, 217.

[4] Henny, G. C., Boone, B. R., and Chamberlain, W. E., *Am. J. Roentgen.*, 1947, **57**, 409.