these cells to the level of cortical steroids present in the blood must be affected by the neurohumor presumed to be secreted by the hypothalamus. Our data do not permit us to decide which of these possibilities is correct.

Conclusions. The compensatory adrenal hypertrophy which normally follows unilateral adrenalectomy is suppressed in rats with diabetes insipidus produced by hypothalamic lesions.

1. McCann, S. M., *Am. J. Physiol.*, 1952, v171, 746.
2. Hume, D. M., *Ann. Surg.*, 1953, v138, 548.
3. Anand, B. K., Raghunath, P., Dua, S., and Mohindra, S., *Indian J. Med. Research*, 1954, v42, 2.
4. McCann, S. M., and Brobeck, J. R., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 318.
5. McCann, S. M., and Sydnor, K. L., *ibid.*, 1954, v87, 369.
6. Gemzell, C. A., Van Dyke, D. C., Tobias, C. A., and Evans, H. M., *Endocrinol.*, 1951, v49, 325.
7. Sydnor, K. L., and Sayers, G., *ibid.*, 1954, v55, 621.
8. Tepperman, J., Engel, F. L., and Long, C. N. H., *ibid.*, 1943, v32, 373.
9. Ganong, W. F., *ibid.*, 1954, v55, 117.
10. Ganong, W. F., and Hume, D. M., *ibid.*, 1954, v55, 474.

Received July 27, 1955. P.S.E.B.M., 1955, v90.

Plaque Formation of Poliomyelitis Viruses on Human Amnion Cell Cultures.* (21944)

JØRGEN FOGH AND ROSEMARY O. LUND. (Introduced by W. M. Stanley.)
From the Virus Laboratory, University of California, Berkeley.

In a previous publication(1) human amnion cells were reported to support propagation of poliomyelitis viruses, and it was indicated that tissue cultures obtained from this readily available tissue could provide a source for the large scale production of poliomyelitis virus.

In the present work plaque formation in monolayer tissue cultures of amnion cells by the 3 types of this virus was obtained, similar to the plaque formation by this virus in monkey kidney and testis cultures(2) and human cancer (HeLa) cell cultures(3). It is, therefore, possible to employ this tissue also for the quantitative assay of poliomyelitis virus.

Material and methods. Virus. Poliomyelitis viruses, type 1, strain Mahoney; type 2, strain MEF-1; type 3, strain Saukett, obtained from Dr. Jonas Salk in 1952, were passed several times in monkey kidney cells, 3-4 times in HeLa cells and finally 1 or 2 times in monkey kidney cells. The final virus preparations were stored at −60°C. *Cells.* Monolayer cultures of human amnion cells were prepared in 50 mm petri dishes as previously described(1). After centrifugation the cell pack was diluted 1:120 and the cultures were grown at 37°C in a 3% CO_2-atmosphere in a medium consisting of 20% ox serum and Earle's balanced salt solution containing 0.5% lactalbumin hydrolysate (Nutritional Biochemicals). The culture fluid was replaced by fresh medium after incubation for 4-5 days. The cell sheet was usually confluent after 10-14 days. *Infection and overlay.* The cultures were washed 3 times with buffer solution at 37°C after removal of the nutrient fluid and inoculated with 0.3 ml of various dilutions of the virus preparation. After permitting adsorption of the virus for at least 30 minutes at 37°C, the plates were overlayed with 3-4 ml of melted agar containing nutrient fluid. 100 ml nutrient fluid consisted of 55 ml 1% Difco yeast extract in distilled water, 6 ml 5% bovine albumin powder V (Armour) in Hanks' solution, 9 ml 5% $NaHCO_3$ in distilled water and 30 ml 10 times concentrated Hanks' solution without phenol red(4). One part of 3% agar was melted and kept in a water bath at 44°C. One part of the nutrient fluid was mixed with an equal volume of dis-

* Aided by a grant from the American Cancer Society and a grant from the National Foundation for Infantile Paralysis.

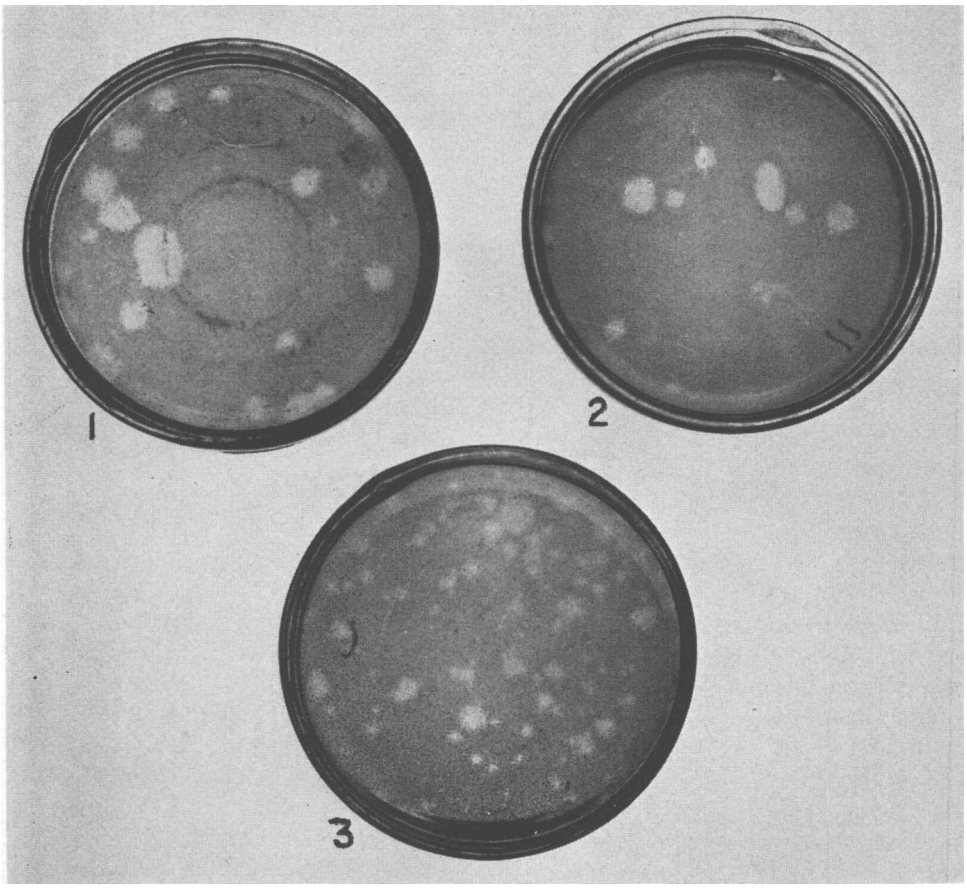

FIG. 1. Plaques of poliomyelitis viruses on human amnion cell cultures 72 hr after infection. 1: Type 1, strain Mahoney; 2: Type 2, strain MEF-1; 3: Type 3, strain Saukett.

tilled water and added to the agar. After 2½ days of incubation at 37°C the plates were stained with 0.3 ml of 1:4000 neutral red in phosphate buffer solution.

The monkey kidney plate cultures used for comparative assays were prepared by the technique devised by Dulbecco and Vogt(2) and grown in a medium consisting of 2% ox serum and Earle's balanced salt solution with 0.5% lactalbumin hydrolysate. The plates were infected, overlayed and incubated as were the amnion plates.

Results. Fig. 1 shows the plaques produced by inoculating suitable dilutions of the 3 types of poliomyelitis virus on monolayer cultures of first generation human amniotic cells. The plaques were almost round areas which could be easily seen and counted without magnification. The size of the plaques was somewhat variable and increased with time of incubation until the plaques became confluent. At an early time when the plaques were still small it was possible to count up to approximately 200 plaques in one plate, since they were very easily discernible.

Table I shows a comparison of titers obtained for 4 different samples assayed on monkey kidney and human amnion plate cultures, at the same time under identical conditions. It can be seen that the number of plaque forming units per ml of Mahoney and MEF-1 virus estimated from the amnion plates was consistently higher than that from the monkey kidney plates. This difference was not observed for the single Saukett virus assay. More detailed comparison of the titers obtained in the 2 tissue culture systems is being made in this laboratory.

TABLE I. Number of Plaques and Calculated Plaque Forming Units (PFU) per ml for 4 Samples of Poliomyelitis Virus Assayed on Monkey Kidney and Human Amnion Plates in Various Dilutions.

Virus samples	Dilution	Cell layer from Monkey kidney Plaques/plate	Monkey kidney PFU/ml	Human amnion Plaques/plate	Human amnion PFU/ml
Mahoney	$10^{-5.5}$	19, 20, 21, 19	2.1×10^{7}		
	10^{-6}	9, 7, 5, 5	2.2×10^{7}	25, 24	8.2×10^{7}
	10^{-7}			0, 6	1.0×10^{8}
"	10^{-6}	38, 41, 43	1.4×10^{8}		
	$10^{-6.5}$	24, 17, 15	2.0×10^{8}		
	10^{-7}	2, 5, 4	1.2×10^{8}	26, 26	8.7×10^{8}
	$10^{-7.5}$			8, 11, 11	1.1×10^{9}
MEF-1	10^{-6}	25, 25, 34	8.9×10^{7}	113, 115	3.8×10^{8}
	$10^{-6.5}$	10, 10, 7	9.6×10^{7}		
	10^{-7}	2, 4, 3	1.0×10^{8}	10, 4	2.3×10^{8}
Saukett	10^{-4}			217, 189	6.8×10^{6}
	10^{-5}	88, 107, 90	3.2×10^{7}	85, 68	2.6×10^{7}
	$10^{-5.5}$	31, 32, 31	3.3×10^{7}		
	10^{-6}	3, 11	2.3×10^{7}	3, 5	1.3×10^{7}

Summary. Plaque formation of all 3 types of poliomyelitis virus has been obtained in monolayer cultures of human amnion cells. The plaque titer obtained on amnion was 3-6 times higher than on monkey kidney cell cultures for strains Mahoney and MEF-1.

1. Zitcer, Elsa M., Fogh, J., and Dunnebacke, Thelma H., *Science*, 1955, v122, 30.

2. Dulbecco, R., and Vogt, Marguerite, *J. Exp. Med.*, 1954, v99, 167.

3. McClain, Mary E., and Schwerdt, C. E., *Fed. Proc.*, 1954, v15, 505, abstract.

4. Youngner, J. S., 1955, personal communication.

Received July 27, 1955. P.S.E.B.M., 1955, v90.

Propagation of Newcastle Disease Virus in Ehrlich Ascites Cells *In vitro* and *In vivo*.* (21945)

ARTHUR D. FLANAGAN, ROBERT LOVE, AND WALTER TESAR.
(Introduced by Herald R. Cox.)

From Viral and Rickettsial Section, Research Division, American Cyanamid Co., Pearl River, N. Y.

Mengo encephalitis virus has been cultivated in the Ehrlich ascites carcinoma cell *in vitro*(1). While no cytopathogenic effect of Mengo virus—in spite of its multiplication—was visible in the test tube, its oncolytic property was readily demonstrated in the ascites cells *in vivo*. Some workers have reported the inhibition of Ehrlich ascites tumor growth when the inoculum was first mixed with Newcastle disease virus (NDV) either just prior to or at the time of inoculation(2). However, sustained multiplication of the NDV in the Ehrlich cells could not be demonstrated(3).

It seemed of interest to ascertain whether this virus could be adapted to grow in the Ehrlich ascites tumor and to determine whether viral multiplication would result in oncolysis *in vivo*.

Material and methods. Virus. The Newcastle disease virus was originally isolated in Massachusetts in 1945 by Dr. Van Roekel, and is designated MD20Z. It was received by Dr. Floyd Markham from the United States Bureau of Animal Industry as the fifth chick embryo passage of the original strain.

* The authors wish to thank Dr. Hilary Koprowski for his helpful suggestions, and Donald Hendrickson for his valuable technical assistance.