Cultivation of Measles Virus in Stable Line of Human Amnion Cells. (23865)

JACK W. FRANKEL AND MARGARET KING WEST (Introduced by Bettylee Hampil)
Merck Institute for Therapeutic Research, West Point, Pa.

Changes which occur in primary trypsinized tissue cultures of human amnion cells infected with the Edmonston strain of measles virus are the formation of stellate-shaped cells and syncytial masses or "giant multinuclear cells" containing eosinophilic intranuclear and intracytoplasmic inclusions. Eventually, necrosis occurs and the entire culture disintegrates(1). The latter phenomenon is a slow one. Three or 4 weeks may be required for cytolysis to be complete. Occasionally the cytopathogenic changes are difficult to discern since the changes induced may be limited to the formation of 3-4 nuclei in only a few cells.

The following report describes the cultivation of measles virus in stable human amnion cells(2), and the differences in the cytopathogenic changes produced in comparison with those observed in primary human amnion cells.

Methods. Fifty thousand stable human amnion cells suspended in medium 199 containing 10% heated calf serum were inoculated into roller tubes. Within 3-4 days, the cell sheets were established and the cultures re-fed with medium 199 or Eagle medium(3), with or without glutamine, containing 10% heated calf serum. The cultures were inoculated with the Edmonston, Philadelphia 6 and Philadelphia 26(4) strains of measles virus. Infected tissue culture fluids from sheets of primary trypsinized monkey kidney epithelial (5), primary trypsinized human amnion and Hep-2 cells(6) were employed. Whole blood and mucous membrane washings from patients with measles (previously shown to contain measles virus) were used also. All tubes were rolled at 36.5°C and observed daily for evidence of cytopathogenic changes.

Results. The prominent change observed in stable human amnion in contrast to that occurring in primary amnion cultures was necrosis which became apparent 2-3 days following infection. Syncytial masses or "giant multinuclear cell" areas, though far less numerous than necrotic areas, could be seen at this time also, particularly at the periphery of the cell sheets (Fig. 1). These cytopathogenic changes were observed in all cultures inocu-

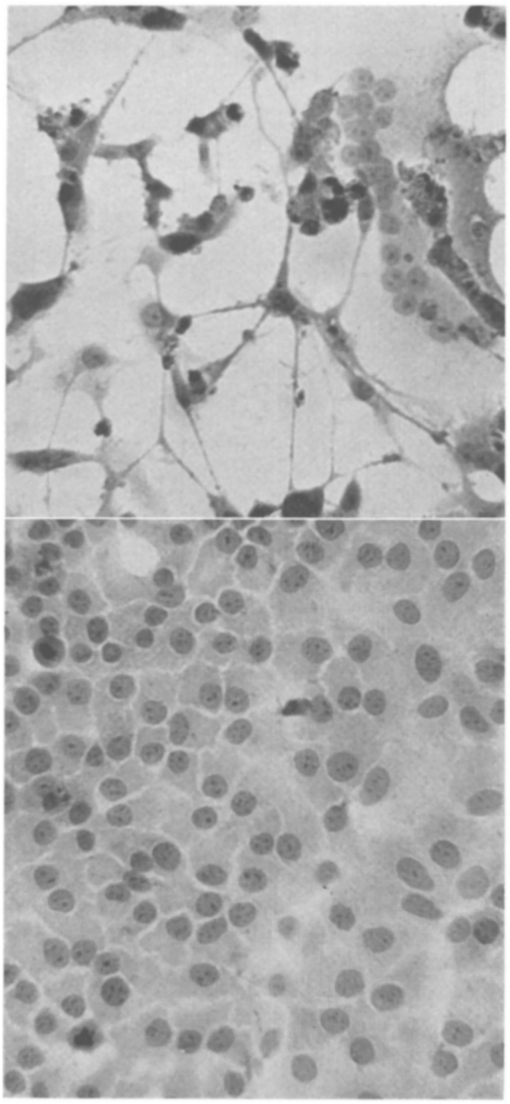

FIG. 1 (top). Stable human amnion. Culture infected with measles virus (Phila. 26 strain) 72 hr after infection. Note extensive necrosis and giant cell. Hematoxylin and eosin stain, about 150×.

FIG. 2 (bottom). Stable human amnion. Culture inoculated with mixture of measles virus (Phila. 26 strain) and measles-immune serum. One wk after inoculation. H and E stain, about 150×.

lated with measles virus, either from tissue culture fluids or clinical materials, but were not observed in tubes inoculated with mixtures of measles virus and measles-immune sera (Fig. 2). Within 7-14 days, necrosis was virtually complete even when dilute inocula were employed. Syncytial formation was almost completely suppressed by the addition of freshly prepared glutamine to the medium. The latter effect is similar to that reported in Hep-2 cultures infected with measles virus to which glutamine had been added(7).

Eosinophilic intranuclear and intracytoplasmic inclusions have been observed as early as 3 days following inoculation of large quantities of virus. Complement fixing antigen (1:8) was detected on the 4th-5th day in tissue culture fluids harvested from roller tube cultures containing 2.0 ml of medium. The average infectious titer per 0.1 ml of tissue culture fluid was 10^{5} TCD_{50}. Twenty-one serial passages of measles virus have been made in stable amnion cultures.

The relative ease not only in the preparation of stable human amnion cultures but also in the early recognition of specific cytopathogenic effects suggests that these cells be used in preference to primary trypsinized human amnion cultures for measles virus studies. In our hands, these cells are preferable to either monkey kidney or Hep-2 cells in performing serum neutralization tests. They have been used also in the preparation of standard antigens for the complement-fixation test. Further work would be required to determine their general usefulness in the isolation of measles from clinical materials.

Summary. Cytopathogenic changes induced by measles virus propagated in a stable line of human amnion cells is described. The prominent change is necrosis in contrast to formation of "giant multinuclear cells" occurring in primary human amnion cultures infected with measles virus. Preliminary studies indicate the general utility of this line of cells for investigations on measles virus.

1. Milovanovic, M. V., Enders, J. F., Mitus, A., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 120.
2. Fogh, J., Lund, O. L., *ibid.*, 1957, v94, 532.
3. Eagle, H., *Science,* 1955, v122, 501.
4. Frankel, J. W., Burnstein, T., Deviney, J. T., West, M. K., *Bact. Proc.,* 1957, 76.
5. Enders, J. F., Peebles, T. C., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 277.
6. Black, F. L., Reissig, M., Melnick, J. L., *ibid.,* 1956, v93, 107.
7. Reissig, M., Black, F. L., Melnick, J. L., *Virology,* 1956, v2, 836.

Received January 22, 1958. P.S.E.B.M., 1958, v97.

Effect of Reserpine on Prolactin Content of Rabbit Pituitary.* (23866)

JOSEPH MEITES†
Department of Physiology and Pharmacology, Michigan State University, East Lansing

A single intravenous injection of reserpine into mature female rabbits induced a limited degree of lactation after prior treatment with estrogens or at the end of pseudopregnancy (1-3). Administration of reserpine(3) and chlorpromazine(4-7) have also been reported to occasionally initiate lactation in women patients. These results, as well as the finding that reserpine may prolong luteal function in the rat(8), suggests that this drug may stimulate production and release of prolactin from the pituitary. The present experiment demonstrates that reserpine can increase the prolactin content of the pituitary of rabbits.

Methods and results. Twenty-two mature female rabbits, predominantly New Zealand White in origin, were divided into 4 groups of 5 to 6 each. The rabbits averaged 6-9 pounds in body weight and were fed a standard rabbit diet. Treatment of the 4 groups was as follows: 1, controls; 2, a single intravenous

* Published with approval of director of Mich. Agric. Exp. Station as Journal Article No. 2195.

† Reserpine was kindly provided by Dr. Robert Gaunt of Ciba Pharmaceutical Products, and by Dr. Russel Kraay of Eli Lilly and Co.