3. Henry, C., Youngner, J. S., *J. Immunol.*, 1956, v78, 273.
4. Wright, B. S., Sagik, B. P., *Virology*, 1958, v5, 573.
5. Simpson, R. W., Hirst, G. K., *ibid.*, 1961, v15, 436.
6. Choppin, P. W., *ibid.*, 1962, v18, 332.
7. Takemoto, K. K., Liebhaber, H., *ibid.*, 1961, v14, 456.
8. ———, *Fed. Proc.*, 1962, v21, 461.
9. Choppin, P. W., Eggers, H. J., *Virology*, 1962, v18, 470.
10. Schulze, I. T., Schlesinger, R. W., *ibid.*, 1963, v19, 49.
11. Liebhaber, H., Takemoto, K. K., *ibid.*, 1961, v14, 502.
12. Youngner, J. S., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 203.
13. Heymann, H., Gulick, Z. R., De Boer, C. J., De Stevens, G., Mayer, R. I., *Arch. Biochem. Biophys.*, 1958, v73, 366.
14. Gerber, P., Dutcher, J. D., Adams, E. V., Sherman, J. H., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 590.
15. Smith, D. B., Cook, W. N., *Arch. Biochem. Biophys.*, 1953, v45, 232.
16. O'Neill, A. N., *J. Am. Chem. Soc.*, 1955, v77, 6324.
17. Gerber, P., Adams, E., *Science*, 1958, v128, 1571.

Received October 7, 1963. P.S.E.B.M., 1963, 114.

Cytopathic Effects of Duck Hepatitis Virus in Duck Embryo Kidney Cell Cultures.* (28807)

J. E. FITZGERALD, L. E. HANSON AND M. WINGARD
(Introduced by C. A. Brandly)
Department of Pathology and Hygiene, College of Veterinary Medicine, University of Illinois, Urbana

Duck hepatitis has been the subject of periodic investigations since it was first described by Levine and Fabricant(1). The virus of duck hepatitis (DHV) has been successfully propagated in tissue cultures but cytopathic effects (CPE) associated with its multiplication in tissue cultures have not been described(2-4). This report describes the cellular alterations associated with the growth of DHV in duck embryo kidney (DEK) cell cultures.

Materials and methods. Virus. Duck hepatitis virus strain R85952(5) which had undergone 18 serial passages in embryonating chicken eggs, 16 passages in chicken embryo liver cells, 4 passages in duck embryo liver cells and 24 passages in duck embryo kidney cells was used.

A twenty-fourth DEK passage cell culture medium with a titer of $10^{5.0}$ chicken embryo infective doses 50 per 0.1 ml served as the source of virus.

Tissue culture. Primary monolayer cultures were obtained by placing trypsin-dispersed DEK cells in 15 × 175 mm rubber stoppered glass tubes containing 11 × 22 mm glass cover slips. Tubes were incubaated in a slanted position at 37°C. The growth medium consisted of 79.5% Hanks' balanced salt solution, 0.5% lactalbumin hydrolysate, 20% sheep serum, and 100 units of penicillin and 100 μg of streptomycin per ml. The same medium except containing 5% instead of 20% sheep serum was used for maintenance of the cultures. When a confluent cell sheet had formed, usually in 3 to 4 days, cultures were inoculated with virus.

Cytologic techniques. For determination of cellular alterations in infected monolayer cultures, cover slips from tubes containing infective and noninfective fluids were removed at 4, 8, 12, 16, 24, 36, 48, 72 and 96 hours after inoculation and were fixed in absolute methanol. Cells on cover slips were stained by the May-Grünwald-Giemsa technique and examined microscopically for evidence of CPE.

Results. Uninoculated control cultures con-

* This project supported by grants from NIH and Illinois Agri. Exp. Station.

sisted of groups of epithelial-like cells interspersed with fibroblasts. The epithelial-like cells were polygonal or elongated with a large round or oval nucleus. One or two vacuoles were often present near the nucleus. By the 6th days after seeding, there was a noticeable increase in fibroblasts and some of the epithelial cells were undergoing degenerative changes or had become necrotic.

The cytopathic effect of DHV was charac-

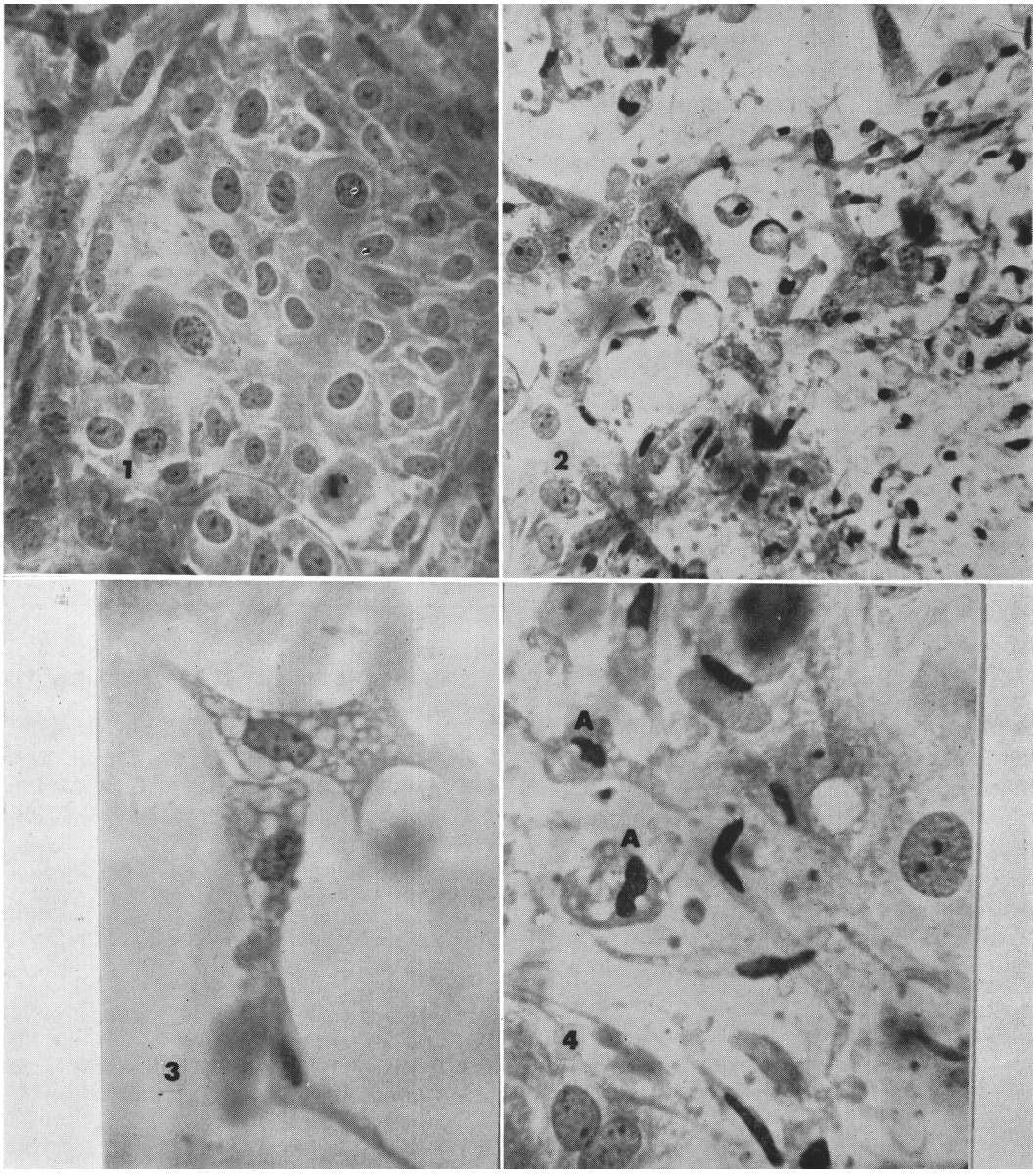

FIG. 1. Normal DEK cell culture. May-Grünwald-Giemsa. ×320.

FIG. 2. 48 hr after inoculation. Marked necrosis of cell sheet is evident. May-Grünwald-Giemsa stain. ×320.

FIG. 3. 16 hr after inoculation. Early degenerative change in cytoplasm characterized by vacuolization. May-Grünwald-Giemsa stain. ×725.

FIG. 4. 48 hr after inoculation. Necrosis of cells as evidenced by pyknotic nuclei and cell disintegration (A). May-Grünwald-Giemsa stain. ×725.

terized by marked degeneration and necrosis of monolayer cultures. Multiple small foci of degeneration and necrosis were observed as early as 16 hours post inoculation. These focal areas enlarged until most cells were affected by 72 hours after exposure to the virus.

Early cellular changes consisted of rounding of cells with condensation of the cytoplasm. These changes were often accompanied by vacuolization or ballooning of the cytoplasm and shrinkage of the nucleus. Next, necrosis, as evidenced by pyknosis of the nucleus and lysis of the cell, occurred rapidly. Many of the pyknotic nuclei assumed a rod or horseshoe shape. Neither intranuclear nor intracytoplasmic inclusions were observed.

Discussion. Duck hepatitis virus had been propagated in cell cultures derived from chicken embryos but cytopathic changes were not observed(2-4). CPE was first noticed in our experiments during the 8th passage of DHV in DEK cell cultures. Cytopathic effects occurred consistently from the 16th through the 26th passage in this system and usually appeared 24 to 36 hours after inoculation with virus.

Cell culture fluid containing infectious virus produced gross lesions of DHV in chicken embryos(5). Duck serum, known to contain antibodies against DHV, inhibited the production of CPE in DEK cell cultures and prevented occurrence of lesions in chicken embryos.

In other experiments, ducks inoculated with 2nd egg passage origin DHV exhibited gross and microscopic lesions of duck hepatitis(6). A liver suspension obtained from one of the affected birds was inoculated into DEK cell cultures. Focal areas of cytopathic changes similar to those described above have been sporadically observed during 5 passages of the liver material in DEK cell cultures. Fluid from the 25th DEK cell passage containing infectious virus and showing CPE failed to produce cytopathic changes in 2 passages in chicken embryo kidney cell cultures prepared in the same manner and with the same nutrient medium used for preparation of DEK cultures. These findings would suggest that the host cell is of prime importance in determining the nature and degree of cytopathic changes resulting from infection by DHV.

Summary. The cytopathic effect of duck hepatitis virus on duck embryo kidney cell cultures was characterized by marked degeneration and necrosis of the cells of the monolayers. Cytopathic effects occurred consistently through several viral passages and were usually apparent within 24 to 36 hours after inoculation. Inclusion bodies were not observed in the infected cells.

1. Levine, P. P., Fabricant, J., *Cornell Vet.*, 1950, v40, 71.

2. Pollard, M., Starr, T. J., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 521.

3. ———, *J. Bact.*, 1960, v80, 571.

4. Kaberle, M. L., Drake, J. W., Hanson, L. E., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 755.

5. Hanson, L. E., Alberts, J. O., *J.A.V.M.A.*, 1956, v128, 37.

6. Fabricant, J., Richard, C. G., Levine, P. P., *Avian Dis.*, 1957, v1, 256.

Received October 7, 1963. P.S.E.B.M., 1963, v114.

Immediate and Prolonged Effects of Hydrocortisone on the Free Amino Acids of Rat Skeletal Muscle.* (28808)

WAYNE L. RYAN AND MICHAEL J. CARVER (Introduced by I. C. Wells)
Department of Biochemistry, Creighton University School of Medicine and Nebraska Psychiatric Institute, University of Nebraska College of Medicine, Omaha

The facilitation of the transport of glucose from the extracellular to the intracellular space by insulin may be typical of the manner by which hormones control cellular me-

* This investigation was supported in part by grant from Nat. Inst. Health.