permeability detected by T-1824 blueing of the skin, but in animals under barbiturate anesthesia it resulted in cyanosis and death. The reaction was prevented by epinephrine, ether, and Phenergan, and was not evoked by administration of other plasma volume expanders.

An anaphylactoid reaction to dextran has previously been encountered only in the rat. The comparatively innocuous nature of the reaction in the unanesthetized mouse undoubtedly accounts for its having gone generally unnoticed in this animal. We suggest that the mouse is not the most suitable experimental animal for use in evaluating LMWD as a therapeutic agent, as the anaphylactoid reaction may interfere with and confuse the interpretation of any possible therapeutic effects.

1. Anderson, R. A., Hardenbergh, E., Fulmer, J. T., *Fed. Proc.*, 1964, v23, 311.

2. Long, D. M., Jr., Folkman, M. J., McClenathan, J. E., *J. Cardiovasc. Surg.*, 1963, v4, 617.

3. Gelin, L., Ingelman, B., *Acta Chir. Scand.*, 1961, v122, 294.

4. Squire, J. R., Bull, J. P., Maycock, W. d'A., Ricketts, C. R., *Dextran, Its Properties and Use in Medicine,* Charles C Thomas, Springfield, Ill., 1955.

5. Voorhees, A. B., Baker, H. J., Pulaski, E. J., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 254.

6. Hanna, C. H., Marshall, L. H., *Am. J. Physiol.*, 1957, v191, 615.

7. Gözsy, B., Kátô, L., *Rev. Canad. Biol.*, 1960, v19, 425.

8. Halpern, B. N., Briot, M., *C. R. Soc. Biol.*, 1954, v148, 1046.

9. Morrison, J. L., Richardson, A. P., Bloom, W. L., *Arch. Int. Pharmacodyn.*, 1951, v88, 98.

10. Goodman, L. S., Gilman, A., *The Pharmacological Basis of Therapeutics,* Macmillan Co., N. Y., 1955.

Received July 3, 1964. P.S.E.B.M., 1964, v117.

Cytopathic Effects of Normally Non-Cytopathogenic Viruses in Tissue Cultures Held Under Increased Oxygen Pressure.* (29639)

DIEGO SEGRE

Department of Pathology and Hygiene, College of Veterinary Medicine, University of Illinois, Urbana

The virus of hog cholera (HC) has been propagated in tissue cultures(1-6), but with one exception(7) it has not induced cytopathic effects (CPE). Lack of cytopathogenicity in a virus so virulent for its animal host as HC is a rather surprising finding which, however, would be explained if the environment in which cells are held in tissue cultures were unfavorable to viral synthesis. Whereas a highly efficient mechanism for oxygen transport, the erythrocyte, is operative in the animal, no such mechanism is available in cell cultures. It seemed possible, therefore, that the quantity of oxygen available to cell cultures would not be sufficient for maximal viral synthesis. If this were the case, increasing the oxygen concentration in the medium bathing HC-infected tissue cultures should result in increased viral synthesis and, possibly, in CPE.

Materials and methods. Trypsinized kidney and testicle cells of swine and rabbit origin were seeded in cotton-plugged, slanted tubes and incubated at 37°C in a moist atmosphere of 95% air and 5% CO_2. The tissue culture medium consisted of 79.5% Hanks' balanced salt solution, 0.5% lactalbumin hydrolysate, and 20% sheep serum, and contained 100 units of penicillin and 100 μg of streptomycin/ml. When a cell monolayer had formed, cultures were inoculated with materials derived from HC-infected swine. The virulent materials were either heparinized whole blood or 10% spleen extract in Hanks' balanced salt solution, and had been

* This work was supported in part by a Cooperative Agreement between U. S. Dept. of Agriculture, Agri. Research Service, and Board of Trustees, Univ. of Illinois, and by a grant from the Hog Cholera Fund, National Hog Farmer, Grundy Center, Iowa.

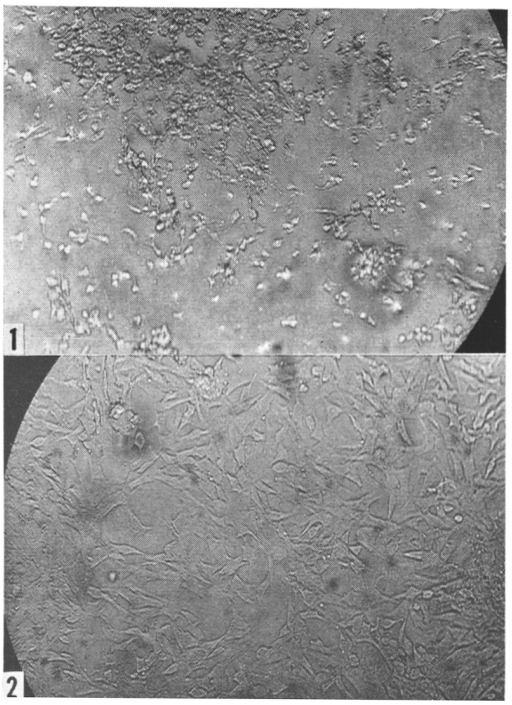

FIG. 1. Rabbit testicular cell culture 24 hr after inoculation with hog cholera virus. The culture was held under 10 p.s.i. pressure of a mixture of 95% O_2 and 5% CO_2. Note complete destruction of cell sheet.

FIG. 2. Uninoculated rabbit testicular cell culture held for 24 hr under 10 p.s.i. pressure of a mixture of 95% O_2 and 5% CO_2.

kept at —25°C for several weeks prior to inoculation into tissue cultures. Following inoculation, the tissue cultures were placed into an airtight metal container.† Uninoculated tissue cultures were included as controls. A mixture of 95% O_2 and 5% CO_2 was introduced into the container from a compressed gas cylinder until a pressure of 10 lb per square inch was reached. The container was then placed at 37°C.

Results. Examination of the cultures revealed extensive CPE as early as 24 hours after inoculation of HC virus-containing materials (Fig. 1). The cells had lost their cytoplasmic processes and appeared rounded and dark. Many cells had become detached from the glass of the tube. CPE was especially evident in swine and rabbit testicular cell cultures, but it was also seen in the kidney cell cultures. The uninoculated control cultures appeared normal after 24 hours of incubation under increased O_2-CO_2 tension (Fig. 2). After 4 or 5 days of incubation, granules were found in the cytoplasm of many cells in the uninoculated cultures. These changes were attributed to toxic effects of the increased oxygen concentration and were readily differentiated from the changes attributed to HC virus. Among the cultures used, rabbit testicular cell cultures seemed to be least affected by the toxic changes attributed to increased oxygen tension.

Inoculation of tissue cultures with the fluid harvested from HC-infected cultures resulted in the appearance of CPE. However, it was not possible to carry out more than 4 serial passages in tissue cultures, since CPE was not seen in later passages. Infectivity titrations were done on several HC virus-containing spleen extracts, which were found to contain from 10^9 to 10^{10} 50% tissue culture infectious doses ($TCID_{50}$) per milliliter. The infectivity titer decreased on serial passage. When HC antiserum‡ was incubated with the virulent materials for 30 minutes prior to inoculation of tissue cultures, CPE was not observed. One-tenth of a milliliter of HC antiserum neutralized 10^7 $TCID_{50}$ of HC virus. Normal swine serum had no neutralizing effect.

Propagation of the virus of transmissible gastroenteritis of swine (TGE) in tissue cultures has not been reported. It was therefore decided to inoculate swine testicular cultures with extracts of the intestinal mucosa of baby pigs which had died of this condition. Some of the inoculated cultures were stoppered with rubber stoppers and incubated under normal atmospheric pressure. Other cultures were incubated under 10 p.s.i. of the O_2-CO_2 mixture. CPE was observed

† All-American Pressure Cooker, manufactured by Wisconsin Aluminum Foundry Co., Inc., Manitowoc, Wis. The control valve was replaced by a gas-type cock. A tight seal was effected by lining the rim of the cover with masking tape.

‡ HC antiserum was obtained from a specific-pathogen-free pig which had been vaccinated with attenuated HC virus followed by challenge with virulent HC virus.

24 hours later only in the cultures held under increased gas pressure. Uninoculated control cultures remained normal during a 5-day period of observation.

Discussion. The adverse effect of lowering oxygen tension on growth of viruses in tissue cultures has been reported. Baron *et al*(8) found that different viruses formed plaques in tissue cultures at different depths of agar overlay. Incubation of the cultures in an atmosphere of pure oxygen rather than in air increased the depth at which plaques were formed. Conversely, addition of reducing substances to the medium decreased the depth of plaque formation. A correlation was found between depth of plaque formation and sensitivity to interferon: viruses with high sensitivity to interferon had high oxygen requirements(9). Reduction of available oxygen resulted in increased production of interferon by infected tissue cultures. Conversely, incubation of the cultures in an atmosphere of pure oxygen resulted in decreased production of interferon(9). Mice exposed to influenza virus and held in an atmosphere of 50% oxygen and 50% nitrogen died earlier and in greater numbers than infected mice held in air (20% oxygen)(10). Conversely, mice adapted to high altitude were comparatively resistant to influenza virus(11).

Isaacs(12) postulated that interferon acts by uncoupling oxidative phosphorylation which would be needed for the energy metabolism of the cell during viral synthesis. The results reported here are consistent with the hypothesis that active aerobic energy metabolism is required for viral synthesis by the host cell. Research is now in progress to determine whether the increased susceptibility of tissue cultures to HC and TGE viruses under conditions of increased oxygen tension is mediated by a decrease in production of interferon.

The failure of obtaining CPE upon serial passages of the virus in tissue cultures held under increased oxygen pressure is not well understood. Neutralization by HC antiserum indicates that CPE was caused by the virus. It is possible, however, that infectious virus was not produced by the cells under the conditions of the experiment.

Summary. The viruses of hog cholera and of transmissible gastroenteritis of swine were propagated in tissue cultures in an atmosphere of 95% oxygen and 5% carbon dioxide at a pressure of 10 lb per square inch. Under these conditions, cytopathic effects were observed in the tissue cultures 24 hours after virus inoculation. Conventional tissue cultures in rubber-stoppered tubes did not undergo cytopathic changes following inoculation with the 2 viruses. The cytopathic effects were neutralized by antiviral serum but were not affected by normal serum. Uninoculated tissue cultures were not affected by increased oxygen concentration.

1. Hecke, F., *Centr. of Bakt.*, Abt., Orig., 1932, v1, 517.

2. TenBroeck, C., *J. Exp. Med.*, 1941, v74, 427.

3. Boynton, W. H., *Vet. Med.*, 1946, v41, 346.

4. Dunne, H. W., Luedke, A. J., Reich, M. S., Hokanson, J. F., *Am. J. Vet. Res.*, 1957, v17, 502.

5. Kumagai, T., Shimizu, T., Ikeda, S., Matumoto, M., *J. Immunol.*, 1961, v87, 245.

6. Gustafson, D. P., McKissick, G. E., *Fed. Proc.*, 1963, v22, 675.

7. Gillespie, J. H., Sheffy, B. E., Baker, J. A., PROC. SOC. EXP. BIOL. AND MED., 1960, v105, 679.

8. Baron, S., Porterfield, J. S., Isaacs, A., *Virology*, 1961, v14, 444.

9. Isaacs, A., Porterfield, J. S., Baron, S., *ibid.*, 1961, v14, 450.

10. Sawicki, L., Baron, S., Isaacs, A., *Lancet*, 680, Sept. 23, 1961.

11. Trapani, I. L., *Fed. Proc.*, 1964, v23, 138.

12. Isaacs, A., *Adv. Virus Res.*, 1963, v10, 1.

Received July 20, 1964. P.S.E.B.M., 1964, v117.