Summary. Chicken RBC's in the presence of calcium ion or after treatment with metaperiodate were absorbed by cultured primary and stable line monkey kidney cells and to a lesser extent by rabbit kidney cells. An extensive variety of other primary or stable cell lines of human or animal origin did not adsorb the cells. Treatment of responsive cells with crystallized trypsin or chymotrypsin inhibited the hemadsorption. Apparently the anomalous hemadsorption was unrelated to viral infection. The morphology of attachment varied with cell strain. BSC-1 cells which displayed few cytoplasmic projections adsorbed RBC's through extensive contact in contrast to adsorption by MK-2 cells by microvillae which characterized the surface of these cells.

We wish to acknowledge the excellent technical assistance of Mrs. Joan L. Schick. Raymond Wayne Ziegler prepared the electron micrographs.

1. Vogel, J., Shelokov, A., Science, 1957, v126, 358.
2. Shelokov, A., Vogel, J. E., Chi, L., Proc. Soc. Exp. Biol. and Med., 1958, v97, 802.
3. Marston, R. Q., Vaughan, E. R., ibid., 1960, v104, 56.
4. Rosanoff, E. I., ibid., 1961, v106, 563.
5. Marcus, P. I., Symp. Quant. Biol., 1962, v27, 351.
6. Portella, O. B., Arch. f. Virusforsch., 1964, v14, 19.
7. Oda, M., Virology, 1964, v24, 432.
8. Neuman, R. E., Tytell, A. A., Proc. Soc. Exp. Biol. and Med., 1963, v112, 61.
9. Eagle, H., Science, 1959, v130, 432.
10. Morgan, J. F., Morton, H. J., Parker, R. C., Proc. Soc. Exp. Biol. and Med., 1950, v73, 1.
11. Oyama, V. I., Eagle, H., ibid., 1956, v91, 305.
12. Crystalline Enzymes, J. H. Northrop, M. Kunitz, R. M. Herriott, Columbia Univ. Press, N. Y., 1948, 2nd Ed.
13. Howatson, A. F., Almeida, J. D., J. Biophys. Biochem. Cytol., 1958, v4, 115.
14. Hotchin, J. E., Cohen, S. M., Raska, H., Raska, C., Virology, 1958, v6, 689.
15. Weiss, L., Int. Rev. Cytol., 1960, v9, 187.

Received December 22, 1964. P.S.E.B.M., 1965, v119.

Cytopathogenic Effects in a Cell Culture Infected with a Murine Leukemia Virus.* (30132)

R. L. TYNDALL, J. G. VIDRINE, ERNESTINE TEETER, A. C. UPTON, W. W. HARRIS AND M. A. FINK

Biology Division, Oak Ridge National Laboratory; Oak Ridge Gaseous Diffusion Plant, Oak Ridge, Tenn.; and Laboratory of Viral Oncology, National Cancer Institute, Bethesda, Md.

Quantitative studies of murine leukemia viruses have been hampered by lack of an established cell line in which they may be propagated. The lack of such a culture has likewise hindered the development of an *in vitro* assay based upon cytopathogenic effects (CPE) which might be elicited by these viruses. It is noteworthy, therefore, that Wright and Lasfargues have recently developed a mouse spleen and thymus tissue culture that supports the propagation of several murine leukemia viruses(1). Such infected cultures grown under the conditions described by Wright and Lasfargues (personal communication) have failed, however, to exhibit any obvious CPE. The present report describes conditions in which CPE have been observed in such a culture infected with the Rauscher murine leukemia virus.

Materials and methods. The modified NCTC 109 medium consisted of 2.0 g yeastolate, 0.5 g neopeptone, 6.8 g powdered NCTC 109 medium, 0.1 g each of penicillin and streptomycin, 0.5 g $NaHCO_3$, 250 ml inactivated human serum, 50 ml inactivated calf serum, and 700 ml triple-distilled H_2O. The medium was adjusted to pH 7.0 with NaOH, if necessary, and Seitz filtered. The 4XBME medium consisted of Eagle's basal medium(2) containing 4-fold concentrations of amino acids and vitamins, 0.1 g/l of peni-

* Research sponsored in part by U.S. Atomic Energy Commission under contract with Union Carbide Corp.

cillin and streptomycin, and 10% inactivated calf serum. A continuous cell culture propagating the Rauscher leukemia virus(1) and the noninfected control culture were obtained through the courtesy of Dr. B. S. Wright and Mr. J. L. Davenport.† The infected culture has been designated as JLS V5 and the control uninfected culture as JLS V6 by Dr. Wright.† Cell transfers were made, in both cases, by scraping cell sheets from the glass with rubber policemen. Neither trypsin nor versene was employed.

Results. Both the infected culture and the noninfected control culture were initially propagated in our laboratory at 36°C ± 0.5 using NCTC 109 medium formulated by Dr. Wright (personal communication), as described above. Under these conditions no gross differences were detectable between the two cultures. When they were subcultured directly on 4XBME medium, however, the cells of the infected cultures showed an abrupt rounding after several days, whereas those of the uninfected culture remained unaltered (Fig. 1-2). The abruptness and synchrony of the rounding effect in all the infected cultures prompted an attempt to restore their appearance to normal by replacing the 4XBME with fresh 4XBME. This measure was only partially corrective, *i.e.*, the rounded cells responded by proliferating to form a semiconfluent sheet, but on successive transfers it became apparent that the culture remained grossly altered. Increased viscosity of the overlying medium in such cultures was also apparent. The abnormalities evident microscopically at this time were dissociation of cells, accentuation of their spindle-shaped processes, loss of contact inhibition, and proliferation of the cells in a crisscross fashion (Fig. 3). Such cells tended to form multilayered cultures. Cells of the infected culture also exhibited a marked increase in cytoplasmic eosinophilia, the dye being present in granules or large inclusion bodies (Fig. 4). Many cells likewise showed marked cytoplasmic vacuolization (Fig. 5). The nuclei of such cells were highly variable in size, shape, and intensity of staining (Fig. 4-5). On subsequent passages these abnormalities became more severe. In association with the progression of the CPE, there was a decline in viability of the culture, resulting ultimately in death of the cells. Severely affected cultures could be restored to normal growth and appearance, however, by reculturing on modified NCTC 109 medium. When such normal-appearing cultures were again subcultured on 4XBME medium, the CPE recurred.

Preliminary examination of the infected culture has been carried out with the use of fluorescein-labeled rabbit anti-Rauscher virus antisera, according to methods described by Fink and Malmgren(5). Results of such studies to date indicate fluorescence of cytoplasmic and nuclear inclusions in cells of the infected culture (Fig. 7), which varies markedly in degree and is not detectable in all cells. No fluorescence has been detected in cells of uninfected control cultures.

Electron microscopic examination of supernatant fluids from cultures exhibiting CPE revealed large numbers of tailed particles (Fig. 6) indistinguishable from the Rauscher leukemia virus(3).

Altered infected cells washed 3 times with 4XBME were highly leukemogenic upon intraperitoneal inoculation into suckling RF mice. The resultant reticulum cell sarcomas in the leukemic mice were preceded by palpable spleens in all inoculated mice 3 weeks after inoculation. Histopathological examination of spleens at this time revealed the erythroblastosis which typically precedes development of leukemia infection with the Rauscher leukemia virus(4).

Discussion. Although the cell cultures exhibiting CPE have been shown to be highly leukemogenic, the CPE cannot as yet be unequivocally ascribed to the Rauscher virus. Fluorescence of such cells with fluorescein-labeled antiserum, presumed to be specific for the Rauscher virus(5), suggests an association between the observed inclusion bodies and the Rauscher virus.

Unlike the conversion toward immaturity in white blood cells of chick embryo cultures infected with avian myeloblastosis virus(6), the CPE observed in our cultures propagating the Rauscher leukemia virus resembles

† Chas. Pfizer Co., Maywood, N. J.

that observed in cells transformed by tumor viruses(7-9). This type of CPE is of interest since some of the leukemia agents have been isolated from transplantable tumors(10-12). It is noteworthy that the *in vitro* cell transformation by Rous sarcoma virus likewise depends upon the composition of the overlying medium(13,14). An association between CPE and the Rauscher virus is of further interest in suggesting the possibility

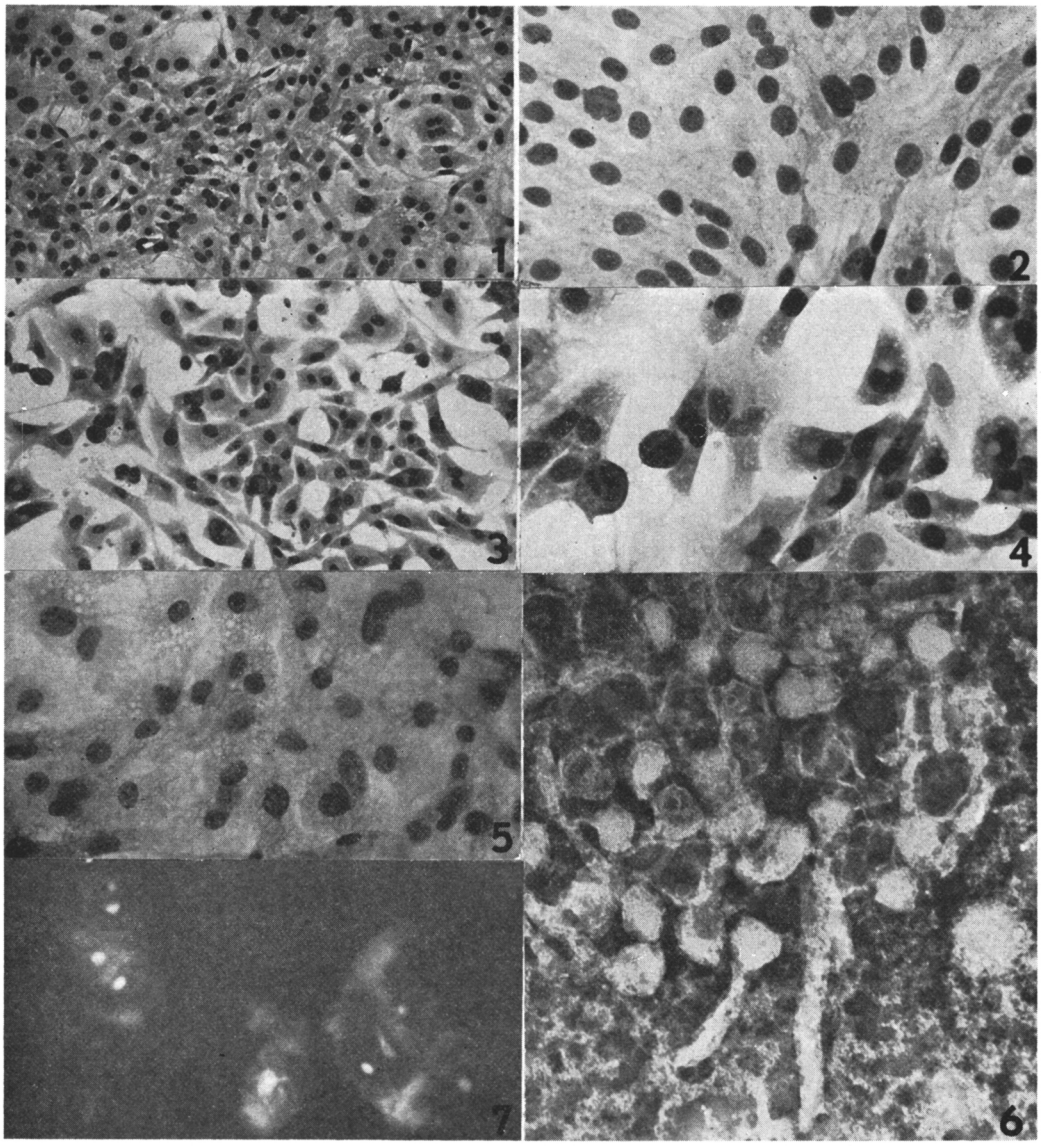

FIG. 1. Cells of uninfected control cultures grown on coverslip. H & E (× 125).
FIG. 2. Uninfected control culture. H & E (× 320).
FIG. 3. Cells of infected culture, showing dissociation and accentuation of spindle-shaped processes or rounding. H & E (× 125).
FIG. 4. Cells of infected culture, showing eosinophilic cytoplasmic granules and inclusion bodies, and pleomorphism and hyperchromatism of nuclei. H & E (× 320).
FIG. 5. Cells of infected culture, showing cytoplasmic vacuolation and pleomorphism of nuclei. H & E (× 320).
FIG. 6. Cluster of tailed particles from infected culture. PTHA (× 70,000).
FIG. 7. Cells from infected culture showing cytoplasmic and nuclear fluorescence with fluorescein-labeled rabbit anti-Rauscher virus antiserum. Counterstained with rhodamine (× 500).

of an *in vitro* assay for this and other murine leukemia viruses.

Summary. Cell cultures grown in modified NCTC 109 medium and infected with the Rauscher leukemia virus showed CPE when subcultured in Eagle's basal medium. Non-infected control cultures treated in a similar manner showed no CPE. The CPE in infected cultures included increased cytoplasmic eosinophilia in the form of either eosinophilic granules or inclusion bodies, cytoplasmic vacuolization, and marked variation in the size, shape, and intensity of staining of cell nuclei.

The authors acknowledge the encouragement and advice of Dr. F. J. Rauscher throughout this study

1. Wright, B. S., Lasfargues, Jennie C., Proc. Am. Assn. Cancer Res., 1964, v5, 70.
2. Eagle, H. S., Science, 1955, v122, 501.
3. Ziegel, R. F., Rauscher, F. J., J. Nat. Cancer Inst., 1963, v30, 207.
4. Rauscher, F. J., ibid., 1962, v29, 515.
5. Fink, M. A., Malmgren, R. A., ibid., 1963, v31, 1111.
6. Baluda, M. A., Goetz, I. E., Virology, 1961, v15, 185.
7. Kilham, L., Herman, C. M., Fisher, E. R., Proc. Soc. Exp. Biol. and Med., 1953, v82, 298.
8. Black, P. H., Rowe, W. P., ibid., 1963, v114, 721.
9. Jensen, F. C., Girardi, A. J., Gilden, R. V., Koprowski, H., Proc. Nat. Acad. Sci. U. S., 1964, v52, 53.
10. Moloney, J. B., J. Nat. Cancer Inst., 1960, v24, 933.
11. Graffi, A., Ann. N. Y. Acad. Sci., 1957, v68, 540.
12. Friend, C., J. Exp. Med., 1957, v105, 307.
13. Manaker, R. A., Groupé, V., Virology, 1956, v2, 838.
14. Rubin, H., ibid., 1960, v12, 14.

Received January 4, 1965. P.S.E.B.M., 1965, v119.

Effects of Sugars on Potential Difference Across Wall of Small Intestine of Rodents. (30133)

TOMOAKI ASANO (Introduced by Morris Pollard)
Lobund Laboratory, Department of Biology, University of Notre Dame, Notre Dame, Ind.

It seems well established that the potential difference (PD) across the wall of the small intestine depends on the presence of glucose on the mucosal side(1,2,3). Furthermore the short-circuit current across the intestinal wall was also dependent upon the mucosal glucose, and the results imply that the active transport of glucose is intimately related to the electrical phenomena, which, in turn reflect the active transport of Na ions(4). However these observations were made only with the small intestine of the rat. In view of the fact that there is a difference among species of animals with regard to intestinal handling of different sugars, it seemed worthwhile to investigate the effect of various sugars on the PD with different animal species. For this purpose the small intestines of the rat, the golden hamster, and the guinea pig were tested with glucose, galactose, fructose, sorbitol, and 3-0-methyl glucose in relation to PD.

Method. Experimental animals used were female Wistar strain rats which weighed between 100 and 180 g, female golden hamsters which weighed between 80 and 110 g, and guinea pigs of either sex which weighed between 300 and 500 g. Animals were fasted overnight prior to experiment except that water was available *ad libitum*.

The experimental procedure was similar to that described previously(2), except that a Keithley electrometer amplifier (#603) was used for determination of PD and the experiments were carried out at 33°C. The animal was anesthetized lightly with ether and a portion of the middle segment of the small intestine (10 to 15 cm in length) was excised and made into an everted sac. Glucose free Ringer's fluid (0.6 to 1.0 ml) was deposited in the sac (serosal side) and this fluid remained therein throughout the experiment. The sac was immersed in about 250