Technical Papers

Influenza Virus Proliferation in Hypoxic Mice[1]

Seymour S. Kalter and Jay Tepperman

Departments of Bacteriology and Parasitology and Pharmacology, State University of New York Medical Center, and the Virus Laboratory, Bureau of Laboratories, Syracuse

It has been demonstrated that experimental manipulation of the rate of protein anabolism in the host cell is reflected by alterations in rate of influenza virus growth. When protein anabolism is stimulated by the administration of testosterone or pituitary growth hormone to the host, virus growth is enhanced (*1*). On the other hand, when the dynamic equilibrium of protein metabolism is disturbed in favor of catabolic processes, as in castration of male mice and in ACTH and cortisone administration, the rate of virus growth is diminished (*2*).

In order to study further the impact of alterations in the metabolism of the mammalian host cell on virus proliferation, experiments have been done on the rate of growth of influenza virus in mice rendered hypoxic in a decompression chamber. It is well known that the synthesis of tissue protein requires an expenditure of energy, and that if the energy-yielding oxidative reactions in cells are acutely compromised, tissue catabolism exceeds anabolism and an excess of nitrogen is excreted in the urine. If the intricate syntheses involved in virus reproduction require coupled host cell oxidative mechanisms as a source of energy, a sudden impairment in the efficiency of those mechanisms would interfere with the ability of virus particles to reproduce. The present study is concerned with an exploration of this possibility.

Groups of 10 mice were inoculated intranasally with approximately 1000 LD_{50} of influenza virus under light ether anesthesia. Upon recovery from the anesthetic the mice were separated into two groups of 5; one group was kept in a small cage at sea level barometric pressure, and the other was placed in a well-ventilated vacuum desiccator type of decompression chamber. Water and food pellets were given to both groups *ad lib*. Within 15 min after virus inoculation the decomposition chamber was evacuated to a simulated altitude of about 30,000′, which is approximately equivalent to an oxygen partial pressure of 6.5% (*3*). At the end of the designated time for each experiment the pressure in the chamber was permitted to rise, the mice were sacrificed, and their lungs removed for estimation of virus growth.

In a preliminary series of experiments done on pooled lungs of groups of 5 mice exposed to low barometric pressure for 3, 6, 9, 12, 15, 18, 21, and 24 hr after inoculation it was found that the experiments performed at the 15-hr interval most consistently showed a difference in virus growth between the hypoxic groups and the control groups. Therefore, the experiments reported here were done at 15 hr after virus inoculation.

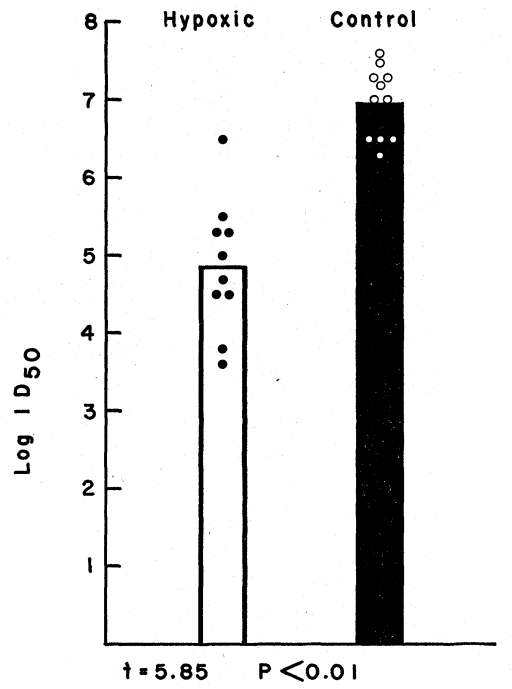

FIG. 1. The growth of influenza virus in hypoxic and control mice after 15 hr.

The data shown in Fig. 1 were obtained on two occasions when 11 mice were made hypoxic and 11 served as controls. Only one of the hypoxic mice failed to survive decompression. Each of the remaining 21 is considered as an individual, for each pair of lungs was removed, homogenized in a chilled Waring blendor, and titrated *in ovo* (*4*). At least 4 eggs per dilution were used for each pair of mouse lungs. The presence or absence of virus was determined by the ability of the allantoic fluid of inoculated eggs to agglutinate erythrocytes. The ID_{50} was determined by the method of Muench and Reed (*5*).

The results indicate a growth inhibition of about two logarithmic intervals in the animals subjected to decompression; the mean log ID_{50} for the controls was $6.97 \pm SE\ 0.143$, and that for the exposed mice was $4.87 \pm SE\ 0.27$. A statistical evaluation of this mean difference revealed a t value of 5.85, and the probability that this result could be due to chance is, therefore, much less than 0.01.

The relationship between oxygen lack and virus proliferation in tissue culture has been known for some time. Zinsser and Schoenbach (*6*) demonstrated that

[1] Aided by a grant from the Hendricks Research Fund.

the rate of Western equine encephalitis virus growth in tissue culture was greatest during maximal oxygen consumption by the host cells. Magill and Francis (7) found that influenza virus in tissue culture did not multiply under anaerobic conditions. The data reported here show that the rate of proliferation of influenza virus is low in the intact hypoxic animal as compared with the normally oxygenated one. Further studies are being done on possible mechanisms involved in this response.

References

1. KALTER, S. S., STUART, D. C., JR., and TEPPERMAN, J. *Proc. Soc. Exptl. Biol. Med.*, **74**, 605 (1950).
2. KALTER, S. S., *et al. J. Exptl. Med.*, **93**, 529 (1951).
3. VAN LIERE, E. J. *Anoxia: Its Effect on the Body*. Chicago: Univ. Chicago Press (1942).
4. HIRST, G. K. *J. Immunol.*, **45**, 285 (1942).
5. REED, L. J., and MUENCH, H. *J. Hyg.*, **27**, 493 (1938).
6. ZINSSER, H., and SCHOENBACH, E. B. *J. Exptl. Med.*, **66**, 207 (1937).
7. MAGILL, T. P., and FRANCIS, T., JR. *Ibid.* **63**, 803 (1936).

Manuscript received December 20, 1951.

Assay of Uranium-bearing Ores by Fission Analysis[1]

W. W. Happ and J. L. Horwood

Radioactivity Division, Mines Branch, Department of Mines and Technical Surveys, Ottawa, Canada

The common methods of assaying uranium ore by physical means require a knowledge of the equilibrium condition of the sample, since an error may be introduced because of the partial or complete absence of some of the daughter elements in the radioactive series. An error may also be introduced by radiation caused by thorium or potassium.

The possibility of assaying uranium ore by measuring the fissionable content with neutron-induced fissions was investigated by using a 50 mc Ra-Be neutron source[2] and standard alpha scintillation detection equipment. It was assumed that the fission particles, because of their greater energy, could be distinguished from α-particles by counting only scintillation pulses of large amplitude. In this way a direct indication of the U^{235} content of the ores could be obtained. Although the method was found to be too complicated for routine analysis, it seems worth while to record some of the results obtained.

Observations on the unknown ore samples were carried out with the following arrangement. Neutrons from the Ra-Be source were slowed down by a 3″ wall of paraffin and impinged on the sample, which was mounted directly below the photocathode of a 931A photomultiplier. The only one of several methods of preparing the sample which proved satisfactory consisted of mixing a 50-mg sample of ore containing about 40% uranium oxide with an equal amount of Patterson D phosphor. The mixture was spread as a thin uniform layer on a microscope slide and mixed with a binder of amyl acetate.

The zinc sulphide phosphor is relatively insensitive to β- and γ-radiation, so that the background count above which the fission products had to be detected was due mainly to the α-particles emitted by all the uranium daughter elements in the course of their natural decay. The contribution to the total background due to the phototube dark current and to γ-sensitivity was found to be negligible. It was important to allow a sufficiently long period for the phosphor to reach a steady state after exposure to daylight. The main difficulty in evaluating measurements of this type arose from the fact that the pulse amplitude distribution from the α-particles and fission pulses turned out to be much alike, and that the fission pulse counting rate was rather low because of the weak neutron source used.

Fig. 1 shows the counting rate observed with and without exposure to the neutron source, and with and without a sample. The curves indicate that the change in counting rate observed as a result of fission detection is more pronounced at higher discriminator settings. This difference, though significant and measurable, is rather small considering the high uranium content of the sample.

Some observations were also carried out to check the time dependence of the fission counts, and these results are summarized in Fig. 2, which shows the pulse distribution for various times of irradiation relative to the α-pulse distribution. There was a grad-

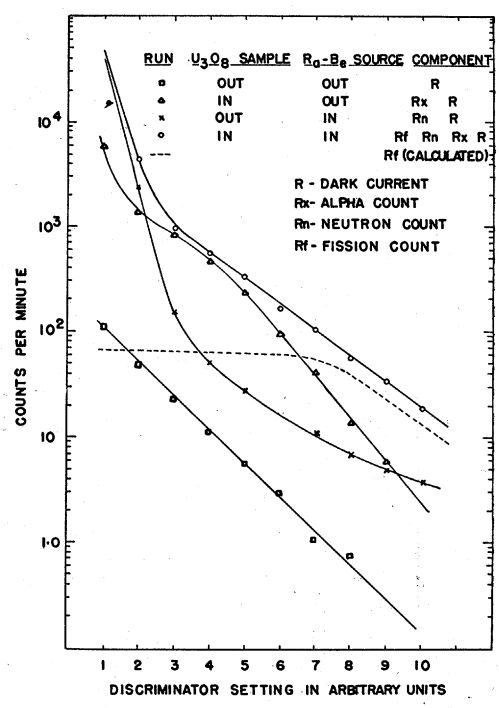

FIG. 1. Scintillation response from uranium-bearing mixture as a result of exposure to the neutron source.

[1] Permission for publication of this work has been granted by the director general of scientific services, Department of Mines and Technical Surveys, Ottawa, Canada.

[2] The loan of the neutron source by the Eldorado Mining and Refining (1944) Ltd., is gratefully acknowledged.