enhancing enzyme activity is not clear. It has been recently shown that it has a similar enhancing effect on the activity of several other oxidases in the liver(6).

The absence of L-gulonolactone oxidase activity in the liver of guinea pig and amphibians, and its presence in frog kidney as determined by other technics(1) offer an excellent biological control for the histochemical method by the use of which we found an identical general distribution and which provided the advantage of histological localization of activity.

Summary. A histochemical method was described which localized the activity of L-gulonolactone oxidase, the final oxidative step in synthesis of ascorbic acid from glucose. The enzyme was found to be active in the centrilobular cells of the rat liver and in selected portions of the mesonephric tubules of the frog kidney. It was absent from guinea pig kidney and liver, and from frog liver.

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Received November 21, 1960. P.S.E.B.M., 1961, v106.

Latent Viral Infection of Cells in Tissue Culture. IX. Abortive Infection with Psittacosis Virus.* (26320)

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A latent infection of L cells with psittacosis virus has been described which is induced by nutritional depletion of cells with a balanced salt solution (BSS) for 2 days prior to infection(1). Following entry into the cell the virus cannot be detected by infectivity tests in chick embryos, but addition of a synthetic medium containing amino acids and vitamins results in reappearance of infectious virus(2). The specific amino acid requirements for conversion of the latent infection to an active one have been delineated(2) and deletion of a single essential amino acid from the medium was shown to be sufficient to render L cells incapable of supporting psittacosis virus propagation and to establish a latent infection.

Since L cells survive longer on a medium deficient in a single amino acid than on BSS alone, studies were initiated to determine if the duration of the latent infection could be extended to permit a more detailed study of its nature.

Materials and methods. Psittacosis (6BC strain) virus stocks were prepared as previously described(3) and diluted in the appropriate amino acid-deficient culture medium for inoculation of the cell cultures. The single dilution method of Golub(4) was used for virus assay in which 0.25 ml of a 10^{-1} dilution of culture fluid was inoculated into the yolk sac of a dozen 7-day-old chick embryos. Virus titers were expressed as the $\log_{10}LD_{50}$ per ml. Cultures of L cells were grown in T-15 flasks in Parker's medium #199 supplemented with 10% horse serum. When a uniform sheet of cells had grown over the glass surface, the growth medium was replaced with a synthetic medium which was deficient in either phenylalanine or isoleucine. Cultures containing approximately 2×10^6 cells were exposed to approximately 10^5 LD_{50} of psittacosis virus after 2 or 3 days' mainte-

* Supported by research grants from Nat. Cancer Inst., U.S.P.H.S., and N. B. Delavan Fund for Virus Research.

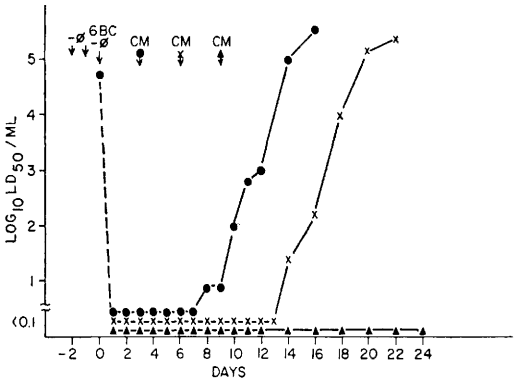

FIG. 1. L cells were maintained on phenylalanine-deficient medium ($-\phi$) for 2 days before infection with 6BC psittacosis virus. At intervals after infection, a complete medium was added and culture fluids were tested for recovery of virus.

nance on the deficient medium. At intervals after infection, the complete synthetic medium (CM), containing amino acids, vitamins, glutamine, glucose, and inorganic salts (2), was added. Culture fluids were tested daily for virus infectivity and the morphological condition of the cells was recorded. Cells were examined for content of virus following lysis by freezing and thawing and inoculating the lysate into eggs as in the technic used for titration.

Results. L cells which were maintained on a phenylalanine-deficient medium for 2 days prior to infection did not support psittacosis virus propagation. If such infected cells were lysed by freezing and thawing 24 hours after inoculation with virus, no virus was detectable by infectivity tests in chick embryos. The possibility that small amounts of virus might be present and would not be sufficient to kill the chick embryos on initial inoculation was eliminated by preparing yolk sac suspensions from test chick embryos which were injected into additional embryos which also survived. When the complete synthetic medium (CM) was added to the L cell cultures 3 days after infection, virus reappeared in the culture fluids within a few days and degenerative changes typical of virus action were eventually observed (Fig. 1). If a 6-day interval between infection and addition of complete medium was allowed, a longer time was required for virus to become detectable in the culture fluids. By the 9th day after infection, cells maintained on phenylalanine-deficient medium had become rounded and some loss of cytoplasm was noted. A few cells had disengaged from the glass surface, but this occurred also in noninfected controls. Although addition of the complete medium brought about a morphological recovery of the cells, virus was not released into the culture fluids and no cytopathic effects were noted in a 19-day observation period. When these recovered cultures were reinfected, the virus grew readily and produced complete disintegration of the cultures.

Although 3 days' maintenance on isoleucine-deficient medium was necessary before addition of virus to establish a latent infection, the resulting phenomena were similar to those described for phenylalanine depletion. Isoleucine-deficient cells retained their normal morphological characteristics longer than cells on phenylalanine-deficient medium, but the latent infection with the virus could not be extended beyond 8 days. After this time cultures fed with complete medium produced no virus, but on reinfection supported viral multiplication and were destroyed.

Discussion. Extension of the interval between infection and the addition of virus-stimulating culture medium to amino acid-depleted L cells *in vitro* did not result in extension of the period of latency beyond 6 to 8 days. The previous report(3) of a latent period lasting as long as 14 days after infection of depleted chick embryo tissue fragments is probably a reflection of a slower diffusion of nutrients from the cells in the fragments and possibly a relatively lower metabolic turnover, since a 13-day depletion period was required before the latent state could be established in such preparations.

It is apparent that the virus does not recover from its latent phase with extended metabolic depletion of L cells and therefore that the fate of the infecting particle depends upon the metabolic activity of the host cell. Amino acid deficiency in L cells prevents completion of the infectious cycle of psittacosis virus with establishment of a latent state for up to 8 days and eventual cure of infection, and survival of cells which retain

their susceptibility to reinfection. The possibility that all of the cells initially infected had lysed leaving only uninfected survivors is improbable, since toxic reactions of L cells to high multiplicities of psittacosis virus have not been noted in the absence of virus multiplication(5) and the slight decrease in infected cells during phenylalanine or isoleucine depletion was not observably different from that seen in noninfected cultures.

Summary. L cells which are maintained for 2 or 3 days on phenylalanine- or isoleucine-deficient medium do not support the growth of psittacosis virus and a latent infection is established. Virus can be recovered from these cultures only after addition of a complete medium, but extension beyond 8 days of the interval between infection and addition of complete medium results in a permanent disappearance of infectious virus. Recovered cultures are susceptible to reinfection with psittacosis virus.

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Received November 28, 1960. P.S.E.B.M., 1961, v106.

RBC Survival in Hamsters Using Intraperitoneal $Na_2Cr^{51}O_4$.* (26321)

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The use of radioactive chromium (Cr^{51}) for determination of red cell longevity has been well established both in man and animals. The technic usually employed for labelling erythrocytes involves incubation of blood *in vitro* with $Na_2Cr^{51}O_4$(1,2,3). The intravenous route of administration has commonly been used to return the labelled red cells to the circulation, in expectation of obtaining the most predictable and highest level of specific radioactivity(4,5,6).

Performance of erythrocyte survival studies in small animals is relatively more difficult than in larger animals or in humans because of limitations imposed by small circulating blood volume and the technical difficulty of returning labelled red cells intravenously. Accordingly, in our studies of the anemia of tumor bearing hamster, another method of erythrocyte chromation was tested, namely *in vivo* chromation with intraperitoneally administered $Na_2Cr^{51}O_4$. This method has proved eminently satisfactory and is the basis for this report.

Materials and methods. Golden hamsters (*Misocricetus auratus*) weighing 60-80 g were fed Purina Laboratory Chow and water *ad lib.* and kept in an air-conditioned animal room.

An aqueous solution of $Na_2Cr^{51}O_4$† with specific activity of 0.25 to 1.0 millicuries per mg of chromium metal was diluted with an appropriate volume of normal saline just before injection, so that an injectable dose of 30 μc of Cr^{51} was contained in approximately 0.3 ml of final solution. This dose of radioactive chromium was injected intraperitoneally into each of 7 hamsters. Twenty-four hours later and thereafter at 2 to 7 day intervals 0.1 ml of whole blood was obtained by cardiac puncture using a 22-gauge needle and a tuberculin syringe. This sample was placed in 0.5 ml of normal saline in a graduated centrifuge tube and the volume adjusted to 1.0 ml by addition of normal saline. The samples were then counted in a well-type

* Supported in part by Grant from Nat. Cancer Inst. N.I.H., U.S.P.H.S. and by Am. Cancer Soc. (Mass. Div.).

† The authors are grateful for technical assistance rendered by Dr. Belton Burrows and Dept. of Radioisotopes, Mass. Memorial Hospitals.