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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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

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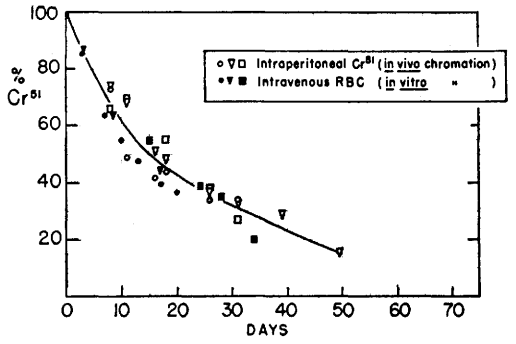

FIG. 1. Hamster red blood cell survival using 30 μc Cr^{51} ($Na_2Cr^{51}O_4$).

scintillation counter (sodium iodide thallium-activated crystal in an Atomic Ins. Co. scintillation head). Photoelectric hemoglobin determinations were performed on each counted sample using a Coleman 6 spectrophotometer. Chromium^{51} counts were computed in terms of specific hemoglobin activity *i.e.*, counts per minute per mg of hemoglobin. The erythrocyte survival curve in 3 hamsters was constructed by plotting % of Cr^{51} against time.

The erythrocytes were sedimented from the plasma in 4 hamsters and each of these portions was counted separately. Blood samples from animals injected intravenously with $Na_2Cr^{51}O_4$ were also divided into plasma and cellular portions before counting.

Results. Chromium^{51} counts varied from 250 to 12,000 per 0.1 ml whole blood per minute, with background counts of 130 to 150 per minute. Radioactivity was detectable in the circulating plasma as well as the red blood cells from 4 to 6 days following intraperitoneal injection, after which significant counts were present exclusively in the erythrocytes. A similar loss of plasma counts was seen following intravenous injection of sodium chromate^{51}. For this reason the fourth day was chosen as a reference point and the curves were extrapolated from this point to the time of intraperitoneal injection. The resulting mean erythrocyte survival curve in normal hamsters is seen in Fig. 1. The half period of red cell radioactivity ranged from 12 to 20 days and the extinction point, representing total red cell longevity, was extrapolated to be from 60 to 70 days. Uptake of Cr^{51} by circulating RBC following intraperitoneally injected $Na_2Cr^{51}O_4$ was in the range of 14 to 20%. Labelling of circulating red cells was completed within a period of 72 hours, as indicated by the fact that no subsequent variations in individual survival curves were encountered.

Discussion. The erythrocyte survival curve (Fig. 1) obtained after intraperitoneal injection of Cr^{51} is quite similar to that obtained by intravenous injection of red cells labelled *in vitro* by the technic of Ebaugh *et al.*(1). However, uptake of Cr^{51} by hamster erythrocytes *in vitro* was significantly less than that which is usually obtained under similar conditions with human erythrocytes, and relatively higher specific radioactivity in the erythrocyte mass could be obtained by chromation *in vivo*.

Preliminary studies in other animals suggest that this simplified technic of erythrocyte chromation *in vivo* may also be applicable to mice, rats, and other small laboratory animals. It has been successfully employed in the dog(7). Its usefulness in other large animals remains to be explored.

Conclusion. 1. Intraperitoneal administration of sodium chromate^{51} results in the effective chromation *in vivo* of red cells in the golden hamster. 2. With use of this labelling technic the erythrocyte survival of the normal golden hamster has been shown to be between 60 to 70 days. 3. The advantages of tagging erythrocytes *in vivo* include relative ease of administration of chromium and elimination of the necessity of obtaining a large initial blood sample.

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