ERYTHROCYTE SURVIVAL IN THE MONGOLIAN GERBIL

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Erythrocyte survival studies using ^{51}Cr- and $DF^{32}P$-labeling procedures were performed on the Mongolian gerbil, a "recently discovered" rodent used for medical research. Various isotopic techniques were performed and evaluated.

The Mongolian gerbil (Meriones unguiculatus) has been recently recognized as a valuable medical research animal. Several unique biochemical and physiologic characteristics contribute to the value of this "recently discovered" rodent in biologic research. A high degree of heat tolerance and capacity for temperature regulation (*1*), unique water metabolism (*2*), resistance to x-irradiation (*3*), and sex-determined circulating leukocyte levels (*4*) are among the distinguishing traits of this species. Since only a limited body of definitive hematologic data are available regarding the gerbil, it was considered appropriate to conduct an isotopic study of the survival of the erythrocytes in this species. Using ^{51}Cr- and $DF^{32}P$-labeling techniques the $^{51}CrT_{1/2}$ of chromium-labeled erythrocytes, the mean life span of ^{32}P-tagged cells, and the chromium elution rate from chromium-tagged red cells were investigated. Several techniques for ^{51}Cr labeling were employed in order to allow a basis for comparison, evaluation, and determination of the most suitable approach for specific investigations.

MATERIALS AND METHODS

The studies were performed on healthy, sexually mature Mongolian gerbils (Meriones unguiculatus). The body weights ranged from approximately 60–90 gm.

Chromium-51 labeling studies. Four groups of 8–11 gerbils were studied. Each group involved a different approach to the labeling of erythrocytes with $Na_2{}^{51}CrO_4$ (Mallinckrodt Nuclear).

Group A. Nine animals, lightly anesthetized with ether, were injected intraperitoneally with 20 μCi ^{51}Cr (100 μCi/ml). Blood samples were withdrawn from the right retro-orbital sinus 24 hr after injection (Day zero) and every 3–6 days thereafter for 1 month. Two calibrated microhematocrit capillary tubes were used for each sampling. The pipets were sealed, centrifuged, and the height of the column of red cells was measured. The volume of packed erythrocytes was then determined (1 mm = 0.001045 ml). The ^{51}Cr activity was quantitated in a well gamma ray spectrometer (Packard 3003). The samples of blood were counted while still in their pipets, eliminating the loss of radioactivity by spillage or other manipulations. The amount of radioactivity for each sampling was converted to counts per milliliter packed red blood cells. The activity of the erythrocytes at various intervals was expressed as the percentage of the activity of the initial bleeding for each animal (Day zero = 100% activity). The data were subjected to a mathematical analysis for least-squares fit to a linear equation. The log activity percent was plotted versus time and that point at which the initial radioactivity fell to 50% of zero time was considered the half-life span.

Group B. Blood (0.2–0.6 ml) was withdrawn from the right jugular vein of eight gerbils and incubated for 30 min in a mixture containing 25 μCi ^{51}Cr, 0.3 ml ACD solution, and 0.5 ml physiologic saline. At the end of this period 25 mg ascorbic acid were added to the suspension. After 5 min the labeled blood was reinjected into the animals through the left jugular vein, each gerbil receiving its autologous erythrocytes.

Group C. The labeling technique for 11 gerbils was identical to that of Group B. In this instance,

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TABLE 1. COMPARISON OF $^{51}CrT_{1/2}$ VALUES DERIVED BY VARIOUS TECHNIQUES

Group	Method	$^{51}CrT_{1/2}$* (days)	Standard deviation	Comparison with standard technique† (Student's t-test)
A	i.p. injection of label	8.0	1.55	Significantly different
B	i.v. injection of labeled cells	13.5	1.55	—
C	i.p. injection of labeled cells	13.0	1.41	No significant difference
D	i.v. injection of label	13.7	1.52	No significant difference

* Calculated per regression analysis.
† In vitro labeling, intravenous injection.

however, the suspensions of labeled cells were injected intraperitoneally.

Group D. The right jugular vein was exposed in 11 gerbils and 25 μCi $Na_2{}^{51}CrO_4$ were injected directly into the vessel. The blood samples were treated as described in Group A.

DF^{32}P labeling studies. Forty-five microcuries ^{32}P-labeled diisopropylphosphorofluoridate dissolved in propylene glycol (Amersham/Searle) was administered intraperitoneally to each of ten unanesthetized gerbils. Samples of blood were withdrawn from the retro-orbital sinus using capillary pipets 24 hr after injection and approximately every 10 days thereafter for a period of 50 days. Following determination of the packed cell volume, the erythrocytes were washed three times in physiologic saline. After the last washing, the cells were hemolyzed by the addition of 1.4 ml 4% saponin solution. The hemolysate was centrifuged and a 1.0-ml aliquot was transferred to a glass scintillation vial. This solution was bleached by the addition of 1 ml 61% perchloric acid and 2 ml 30% hydrogen peroxide followed by a 24-hr incubation period at 75°C. After bleaching, 20 ml dioxane base scintillation fluid was added to the solution and the B^- activity determined by liquid scintillation spectrometry (Packard Tricarb Spectrophotometer 3320). The scintillation activity of the samples was corrected for quenching by using a channels ratio correction curve. The mean life span of the circulating erythrocytes was determined by the derivation of a regression curve by the method of least squares.

Twelve gerbils were injected with 6 μCi DF^{32}P in a manner identical with that previously described. Four animals died within the following week. The findings at autopsy, except for one case of peritonitis, were noncontributory; the other animals remained in good health. Blood sampling was performed 24 hr after injection and thereafter every 7 days for a period of 50 days. Following centrifugation and determination of packed cell volume, the erythrocytes were washed three times in physiologic saline. Following the last washing, the volume was reconstituted to 1 ml with saline and transferred in toto to metal planchettes and allowed to dry at room temperature. The B^- activity was determined in a low beta proportional counter (Beckman 1600) and the mean life span of the red cells was determined as described earlier.

RESULTS

Chromium-51 labeling studies. The half-life spans of ^{51}Cr-labeled gerbil erythrocytes obtained for all groups of animals as well as their statistical comparison with the standard labeling technique (in vitro labeling-intravenous injection, group B) are presented in Table 1. The $^{51}CrT_{1/2}$ values as calculated by regression analysis ranged from 8.0 to 13.7 days, depending on the method of labeling and associated experimental procedures. The shortest half-life span (8.0 days, s.d. $\pm$ 1.55) was obtained with direct intraperitoneal injection of the radioactive nuclide (Group A). The longest half survival time (13.7 days, s.d. $\pm$ 1.52, Group D) was obtained with in vivo labeling effected by intravenous injection of the isotope (Fig. 1). Comparable values were also derived from in vitro labeling followed by intraperitoneal injection of the tagged erythrocytes (13.0

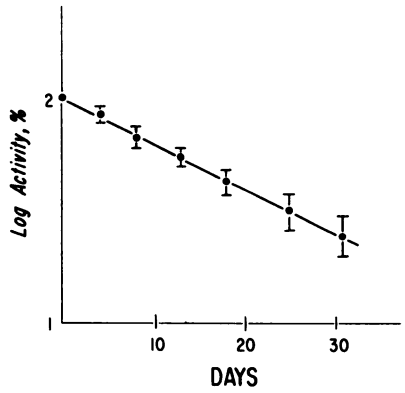

FIG. 1. Disappearance of ^{51}Cr-labeled erythrocytes from Mongolian gerbil. Erythrocytes were tagged by direct intravenous injection of $Na_2{}^{51}CrO_4$. Vertical bars represent 1 s.d. Curve fitted by least-squares analysis, $y = 0.21x + 1.98$.

days, s.d. ± 1.41, Group C) and from in vitro labeling and intravenous injection (13.5 days, s.d. ± 1.55, Group B).

DF^{32}P labeling studies. The mean life span of DF^{32}P-labeled erythrocytes as determined by liquid scintillation spectrometry was 42.6 days (s.d. ± 3.05). When the survival time was determined by proportional counting of dried samples of erythrocytes, the mean life span as calculated by regression analysis was 47.0 days, s.d. ± 1.4 with a range of 46.5–49 days.

The elution rate of chromium from the ^{51}Cr-labeled erythrocytes as based on the ^{51}Cr data (Group B) and DF^{32}P (liquid scintillation) data was 2.77% per day (s.d. ± 0.44). When calculated on the basis of ^{32}P activity as quantitated by proportional counting (dry sample), the elution rate was 3.01% per day (s.d. ± 0.6).

DISCUSSION

The results of this investigation support the concept that the survival of circulating erythrocytes in the gerbil can be effectively monitored by labeling with ^{51}Cr. Although the time required for the disappearance of one half of the chromium-tagged erythrocytes did display minimal individual variations with different techniques, the derived half-life spans in all but one group were not significantly different. It appears, therefore, that the labeling technique can be determined by the requirements of specific investigations. The comparatively low $^{51}CrT_{1/2}$ of 8 days obtained by direct intraperitoneal injection of the isotope is most likely due to the fact that this route lends itself to the diffuse labeling of lymph and peritoneal fluid proteins as well as plasma proteins, marrow cells, and circulating erythrocytes. The data are thus based on a relatively unstable pool of isotopically labeled elements. Nevertheless, if the survival time is based on counts beginning with Day 4 or 5 after injection, the derived value is in accord with those of the other groups. These observations are in excellent agreement with those of Rigby, et al (*5*) who demonstrated that in vivo and in vitro labeling of hamster erythrocytes were similarly effective means of studying erythrocyte longevity particularly if a 4-day period was allowed for plasma clearance of the label in the former procedure. It should be noted that several advantages can be afforded by the intraperitoneal technique. These include the opportunity to use unanesthetized subjects and the avoidance of withdrawing blood from a small rodent for the initial labeling procedure.

The B^- activity of DF^{32}P-labeled red cells was monitored in two ways: proportional counting of dried samples and liquid scintillation spectrometry. The former approach proved to be the better of the two methods. The drawbacks of liquid scintillation counting included a greater number of manipulations and the problem of sample discoloration with subsequent quenching of the scintillation. Although a quench correction curve was used to rectify this feature, it is likely that it did not totally eliminate the error. This suggestion is supported by the fact that not only was the apparent life span shorter when it was based on liquid scintillation data (42.6 compared with 47 days) but, in addition, the standard deviation was also greater for this technique (± 3.05 compared with ± 1.4).

The $^{51}CrT_{1/2}$ of 13.0–13.7 days obtained for three independently studied groups of animals (Groups B, C, D), because of their similarity and reproducibility, most likely approximate the actual half-life of chromium-labeled gerbil erythrocytes. The only other reported value for the gerbil was that published by Womack (*6*), who observed a mean half-life span of 9.9 days. The reason for this lack of correlation is not readily apparent. It is also interesting to note that direct intrajugular injection of $Na_2{}^{51}CrO_4$ did not yield results different from the more conventional approach of in vitro labeling and intravenous injection. Since the data points fitted the calculated regression line particularly well (Fig. 1), it would appear that this close graphic concordance was due, at least in part, to the blunting of the initial rapid rate of disappearance of chromium seen in chromium survival studies by the concomitant introduction into the blood of ^{51}Cr-labeled erythrocytes from the bone marrow. In addition, the elimination of an initial labeling phlebotomy prevented any loss of blood as well as avoiding any potential damage to the erythrocytes while being tagged with the isotope.

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