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SURVIVAL OF ^{50}Cr-LABELED ERYTHROCYTES AS STUDIED BY INSTRUMENTAL ACTIVATION ANALYSIS

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Gray and Sterling (*1*) first developed a method of using hexavalent radioactive ^{51}Cr as a red blood cell tag, enabling a quantitative determination of erythrocyte survival. This procedure has since been perfected to the extent that it has become a routine clinical practice. However, in certain clinical populations, such as children and pregnant females, the use of a radioactive isotope may be undesirable. Recent developments in neutron activation analysis have provided a means whereby the label need not be radioactive but can be used in a stable form and subsequently activated following recovery from the patient. Using ^{50}Cr labeling followed by neutron activation with conversion of the stable isotope to ^{51}Cr, Donaldson *et al* (*2,3*) showed that the survival values of human red cells were comparable with those obtained by standard ^{51}Cr procedures. However, their approach suggested the necessity of extensive chemical isolation procedures for critical quantitation of the element. The present study was designed to further investigate the applicability of ^{50}Cr tagging of red blood cells and to attempt to accurately quantitate millimicrogram amounts of tracer ^{50}Cr bound to erythrocytes without having to resort to a chemical separation.

MATERIALS AND METHODS

The studies were performed on six healthy young adult male rhesus monkeys (Macaca mulatta). The animals weighed approximately 10 lb with the exception of one 5-lb monkey. The chromium half-life of autologous erythrocytes from each monkey was determined first by ^{50}Cr-neutron activation analysis using high resolution Ge(Li) gamma spectrometry and 3 months later using ^{51}Cr tagging followed by NaI(Tl) scintillation gamma spectrometry.

Sodium chromate was prepared from $^{50}Cr_2O_3$ by alkali fusion. The resultant solution contained 182.1 μg chromium and 8.9 mg NaCl/ml with a pH of 7.2.

Twelve-milliliter samples of blood were withdrawn from the saphenous vein of each subject and transferred to sterile plastic (Unitag)* bags containing 2-ml acid citrate dextrose solution and ^{50}Cr-Na_2CrO_4 solution. The volume of the latter ranged from 0.43 to 0.47 ml, an amount calculated to yield a concentration of less than 20 μg elemental chromium per milliliter of red blood cells. After a 30-min incubation period at room temperature, 25 mg ascorbic acid were added and incubated for an additional 10 min. The mixtures were then injected intravenously, each monkey receiving its autologous erythrocytes.

Samples of blood were withdrawn 24 hr, 6, 10, 15, 20, 27, and 31 days postinjection. Duplicate 1-ml aliquots were transferred to quartz vials for neutron bombardment. After 24 hr of neutron irradiation at a thermal flux of 3×10^{13} neutrons/cm^2/sec the samples were processed and counted using a lithium-drifted germanium detector and a multichannel analyzer. The stepwise procedure used in the activation analysis of ^{50}Cr has been described (*4*) and is not included here. The method essentially consists of drying the blood samples, of neutron irradiation followed by a 3-week decay period, and of gamma-spectrometric analysis.

The ^{51}Cr half-life of autologous labeled erythrocytes was determined in the same animals. The procedures and duration of incubation period were identical with that used for ^{50}Cr tagging. Fifteen microcuries of ^{51}Cr-Na_2CrO_4 (specific activity 236.7 μCi/μg) served as the source of the label for each 10 ml of blood (0.016 μg/ml red cells). Hemoglobin values were measured at the onset of the investigation

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TABLE 1. COMPARISON OF ^{50}Cr AND ^{51}Cr $T_{1/2}$ VALUES FOR RHESUS MONKEY ERYTHROCYTES

Monkey	$T_{1/2}$ (days)	
	^{50}Cr	^{51}Cr
1	18.25	16.00
2	16.75	17.25
3	18.25	16.50
4	16.25	18.25
5	18.35	17.25
6	19.00	17.25
Mean	17.8 (s.d. ± 1.1)	17.1 (s.d. ± 0.8)

as cyanmethemoglobin; the levels were within normal limits and remained stable throughout the study.

RESULTS

The mean chromium $T_{1/2}$ of ^{50}Cr-labeled autologous erythrocytes in the rhesus monkey determined by neutron activation analysis is 17.8 (s.d. ±1.1) days, a value which is in close agreement with the ^{51}Cr half-life of 17.1 (s.d. ±0.8) days calculated for the same subjects. Figure 1 compares the rate of disappearance of ^{50}Cr- and ^{51}Cr-tagged erythrocytes from the peripheral blood. The initial samples were obtained 24 hr after injection and designated as 100% activity; the half-life spans were considered as those points at which the radioactivity had fallen to 50% of the level at zero time. Table 1 lists the half-lives obtained for each individual subject using both the stable and radioactive labels. A comparison of the chromium $T_{1/2}$ values determined by the two methods reveals that the best correlation was 0.5 days while the poorest was 2.25 days. Neither approach was found to yield consistently higher or lower survival times.

In addition, the data obtained from each monkey were subjected to a nonlinear least-squares analysis. In this instance the mean fitted curves still approached identity with a chromium $T_{1/2}$ of 17.1 (s.d. ±1.4) and 17.9 (s.d. ±2.7) days, respectively, for ^{51}Cr and ^{50}Cr.

DISCUSSION

In the analysis of ^{50}Cr in whole blood, several theoretical sources of error can be postulated. Among these is the $^{54}Fe(n,\alpha)^{51}Cr$ reaction as well as the $^{50}Cr(n,\gamma)^{51}Cr$ reaction from naturally occurring iron and chromium in the blood. According to Bowen (5) and Šimková and Křivánek (6) the average iron and chromium content of blood are 480 μg/ml and 3×10^{-2} μg/ml, respectively. Of the latter, only 4.31% of the naturally occurring element is the ^{50}Cr nuclide. Taking into account these data as well as the thermal and fast neutron flux of the irradiation facility, the maximum theoretical ^{51}Cr activity from these sources was less than 0.1% for any given determination. When a sample of blood containing ^{50}Cr is irradiated, along with the formation of ^{51}Cr, several other stable nuclides in blood undergo nuclear transmutations producing radioactive isotopes. Elements such as carbon and hydrogen present in large quantities in biological material do not present any problem because of their extremely low neutron capture cross section. However, elements such as chlorine, sodium, potassium, phosphorus, and sulfur produce radioactive isotopes which cause significant interferences in the detection of ^{51}Cr. Of these, short-lived isotopes such as ^{38}Cl, ^{24}Na, and ^{42}K can be allowed to decay during an appropriate time period between irradiation and counting. However, the long-lived isotope ^{32}P generated from ^{32}S, ^{31}P, and ^{35}Cl does present problems in detection and quantitation of ^{51}Cr. One method of resolving this problem would be to chemically isolate the chromium from the sample (*3,7*). Another alternative is to use a detector system capable of resolving the 0.320-MeV gamma peak of ^{51}Cr from the bremsstrahlung produced by beta radiation from ^{32}P and other isotopes in the matrix. The lithium-drifted germanium detector provides a system with a very fine resolution for gamma spectrometry, and, in the present study, has proven to be highly suitable for the analysis of chromium in blood without the necessity of resorting to chemical separation techniques. Figure 2 compares the gamma-ray spectra of a ^{51}Cr standard with that of an irradiated blood sample containing ^{50}Cr as recorded by the Ge(Li) detector. The same samples produced the spectra shown in Fig. 3 when a NaI(Tl) detector was used. It is apparent that the ^{51}Cr photopeak is poorly defined by the sodium iodide detector whereas it is sharply demarcated by the germanium crystal.

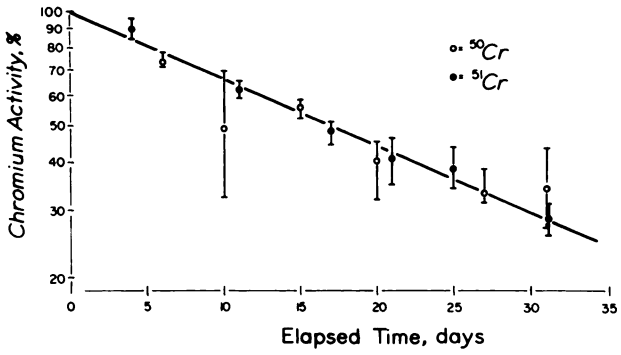

FIG. 1. Comparison of rate of disappearance from peripheral blood of ^{50}Cr and ^{51}Cr-labeled erythrocytes. Each point represents mean value of six pairs of samples. Bars represent minimal and maximal range of activity.

TABLE 2. MEAN QUANTITIES OF CHROMIUM (mμg) DETERMINED FROM DUPLICATE 1-ml ALIQUOTS

	Elapsed at time of sampling (days)						
Monkey	0	6	10	15	20	27	31
1	187	146 ± 1	131 ± 13	103 ± 1	85 ± 6	72 ± 4	62 ± 3
2	374	284 ± 12	199 ± 13	196 ± 13	143 ± 6	119 ± 10	101 ± 5
3	231 ± 4	168 ± 3	109 ± 6	125 ± 3	73 ± 6	74 ± 4	78 ± 3
4	241 ± 4	173 ± 5	95 ± 3	134 ± 1	96 ± 12	76 ± 3	105 ± 5
5	268 ± 12	193 ± 1	87 ± 1	158 ± 9	116 ± 3	87 ± 4	90 ± 3
6	437 ± 13	311 ± 6	232 ± 10	256 ± 12	187 ± 10	144 ± 23	146 ± 1

The difference from the mean of the individual samples is expressed as a ± value.

The approach presented in this investigation allowed measurement of concentrations as low as 62 mμg ^{50}Cr/ml of blood and can be used to determine ^{50}Cr concentrations in the range of 25–35 mμg/ml. Table 2 lists the quantities of elemental chromium determined in the paired 1-ml samples obtained at each venesection. Of the total 40 pairs of samples studied, the mean difference between duplicate specimens was ±4.4% (s.d. ±3.1) with deviations in excess of 10% observed in only two instances. In addition, the reproducibility of the final sampling did not differ significantly from that of the initial specimens. This high degree of sensitivity allows blood samples as small as 1 ml to be satisfactorily analyzed, and suggests that initial tagging concentrations as low of 10 μg ^{50}Cr/ml red cells may be feasible. Further modifications in technique such as separation of the phosphorous-rich plasma before irradiation, performed by Johnson et al (*2*) should be helpful in this regard.

It is significant to note that the labeling concentrations of chromium per milliliter of red blood cells used here ranged from 0.016 μg/ml for ^{51}Cr to 20 μg/ml for ^{50}Cr. The latter level has been generally accepted as a level that does not adversely affect erythrocyte survival (*8,9*). Because the half-life values derived by the two methods are nearly identical in the same animals, it would appear this is true at least for the red cell of the species studied here.

The application of stable isotopes as substitutes for, or in conjunction with, radioactive tracers holds considerable potential. It can allow the determination of the chromium half-life of erythrocytes without any radiation penalty, or it can enhance the accuracy of double tagging procedures by permitting the simultaneous tagging of two erythrocytic populations in a given subject with labels having identical physiological characteristics. It should be recognized, however, that this method is more time consuming and does not lend itself to body-surface scanning. In the present study the individual analytic data points for ^{50}Cr also demonstrated a greater scatter than did those of ^{51}Cr. This is attributable to the increased complexity of activation analysis compared with direct spectrometry. Nevertheless, when all ^{50}Cr and ^{51}Cr data for any given individual monkey were subjected to the least-squares analysis, a non-biased mathematical formulation, the resultant survival times were consistently similar.

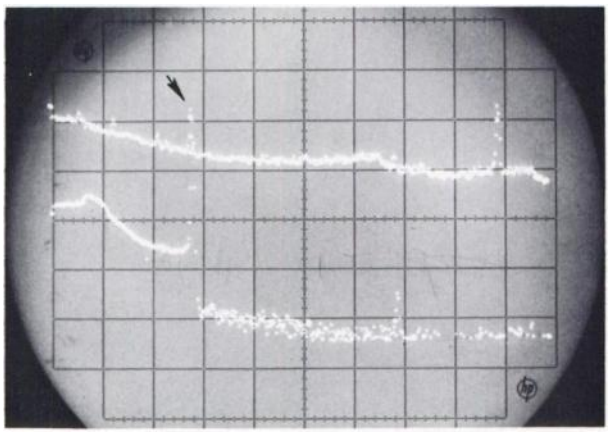

FIG. 2. Gamma-ray spectra recorded by Ge(Li) detector of standard solution of ^{51}Cr (lower spectrum) and 1-ml sample of blood containing ^{51}Cr from ^{50}Cr (n,γ)^{51}Cr reaction (upper spectrum). Arrow indicates ^{51}Cr 0.320-MeV photopeak.

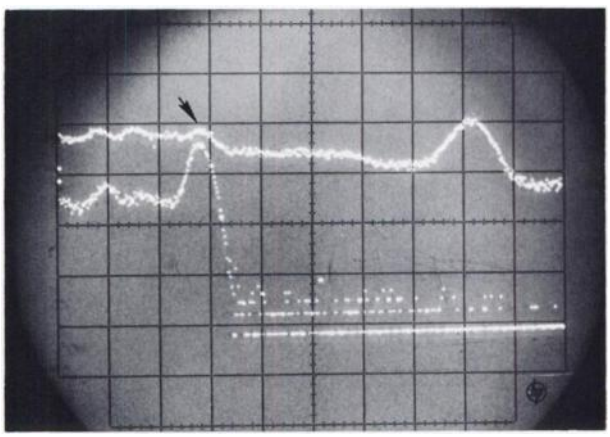

FIG. 3. Gamma-ray spectra recorded by NaI(Tl) detector of same standard (lower spectrum) and blood sample (upper spectrum) used in Fig. 2. Arrow indicates ^{51}Cr 0.320-MeV photopeak.

SUMMARY

The tagging of erythrocytes with stable isotope ^{50}Cr followed by instrumental activation analysis is a satisfactory method of monitoring the survival of erythrocytes. The method discussed is highly sensitive and reproducible, moreover, it allows critical quantitation without resorting to chemical separation of the label from the blood. The ^{50}Cr half-life of autologous erythrocytes in the rhesus monkey is 16.25–19.0 days with a mean value of 17.8 (s.d. ±1.1) days compared with 17.1 (s.d. ±0.8) days derived by ^{51}Cr tagging and gamma-ray spectrometry.

ACKNOWLEDGMENT

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