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The Use of ^{51}CR for Sheep Red Blood Cell Survival Studies (38634)

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The usefulness of ^{51}Cr-tagged autologous erythrocytes for determining red cell survival is well documented. The procedure has been established as one of the foremost methods for determining changes in erythrocyte survival rates in man, and certain experimental animals (1–3).

While conducting a series of studies designed to define the biologic significance of low (in relation to normal humans) glucose-6-phosphate-dehydrogenase activity in sheep erythrocytes, we attempted to measure red cell survival time in these animals, using the ^{51}Cr technique. Our initial studies and one previous report revealed biphasic disappearance curves (4). There was very rapid loss of ^{51}Cr activity from the circulation in the first few days followed by a slower decay curve, yielding an apparent red cell half-life which was significantly shorter than would be predicted from work using *in vivo* cohort labelling with isotopic iron (4, 5).

The present study was, therefore, undertaken to further evaluate the ^{51}Cr technique as a method for studying erythrocyte survival in sheep.

Materials and Methods. ^{51}Cr Survival Studies. Under atraumatic, sterile conditions, 30 ml of blood was drawn from the jugular vein of each of five mature ewes and mixed with 10 ml of acid–citrate–dextrose solution (UNITAG Centrifuge Bag with A-C-D solution, Abbott Laboratories) (6). One hundred microcuries of sodium chromate (RACHROMATE-51, specific activity 200 μCi/mg Abbott Laboratories) was added and the samples were incubated at 23° for 30 min, with gentle agitation. Following this, 100 mg of ascorbic acid was added to each sample with gentle mixing. The cells were washed three times with 0.9% saline at 23°, and resuspended to the original bag volume with 0.9% saline. Thirty ml of the suspension was injected into the jugular vein of the autologous animal, and the remainder used to calculate dosage administration.

Blood samples (5–6 ml) were collected at 30 min, 1, 2, 3, 4, 5, and 6 hr, and then at 8, 10, 12, 16, 20, 24 and 36 hr postinjection. Samples were then collected daily for 1 wk and then three times weekly for the next 3 wk. Both whole blood and plasma samples were counted during the first 48 hr post-injection. Thereafter, whole blood was counted. The 24 hr sample was designated as time zero for the survival studies. All samples were counted in a Nuclear-Chicago Auto Gamma Spectrometer with a 3 in. thallium activated well-type sodium iodide crystal. Samples were counted simultaneously at the end of the study to avoid correction for radioactive decay.

Total urine volume was collected from each animal during the first 48 hr after injection of labelled cells. The urines were collected using anchored Foley catheters (Bardex, C. R. Bard, Inc.) and attached plastic bags which were preplaced 24 hr before erythrocyte labelling. The urine samples were collected and volumes recorded at the same intervals as blood samples during the first 48 hr. One milliliter aliquots from each urine sample were saved for counting.

The ^{51}Cr survival study was repeated on sheep No. 52 and No. 64 approximately 6 mo after the initial study.

"In vitro" ^{51}Cr study. Two 10 ml blood samples were removed from the jugular vein of each of two adult ewes. Using the techniques described in the ^{51}Cr survival study, one sample from each animal was mixed with 3 ml acid–citrate–dextrose solution, incubated with 30 μCi of sodium chromate and washed three times. Thirty milligrams of ascorbic acid was added at the end of the labelling procedure. The other sample from

each animal was used as an unlabelled control and handled identically otherwise. Under sterile conditions the labelled and nonlabelled samples were then suspended in 30 ml of autologous heparinized plasma to which dextrose had been added to a concentration of 250 mg/100 ml. The samples were incubated at 38.5° for 24 hr using mechanical agitation to mimic circulatory trauma. Samples were removed at 0, 8, 16, and 24 hr for determination of free plasma ^{51}Cr activity, erythrocyte ^{51}Cr activity, free plasma hemoglobulin activity, and total hemoglobulin content (7).

^{59}Fe Survival Study. Isotopic iron (Ferrous citrate-^{59}Fe, Abbott specific activity of 23 mCi/mg) was diluted in a 0.1% sodium citrate solution to a concentration of 5 μCi/ml. Each of six adult sheep received 50 μCi of ^{59}Fe intravenously in a volume of 10 ml. Blood samples (3–4 ml) were collected at 15 min, 1, 2, 3 and 4 hr postinjection. Blood samples were then collected twice weekly for the next 4 wk. The average life spans were calculated (8). All samples were counted simultaneously at the end of the study to avoid correction for radioactivity decay in a Nuclear-Chicago Auto Gamma Spectrometer with a 3-in. thallium activated well-type sodium iodide crystal.

"In conducting the research described in this report, the investigators adhered to the 'Guide for Laboratory Animal Facilities and Care,' as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences—National Research Council."

Results. In the five animals used in the ^{51}Cr study, approximately 21% of the total injected label was excreted in the urine during the first 8 hr postinjection. At the end of 24 hr, 33.6% of the label had been excreted, and at 48 hr, 36.2% had been excreted. All urines were negative for both red cells and free hemoglobin. During this period an equally rapid loss of ^{51}Cr activity from the circulation was noted. Figure 1 shows the urinary appearance of label paralleling the loss of circulating label. The corresponding plasma samples revealed an insignificant amount of radioactivity with counts ranging

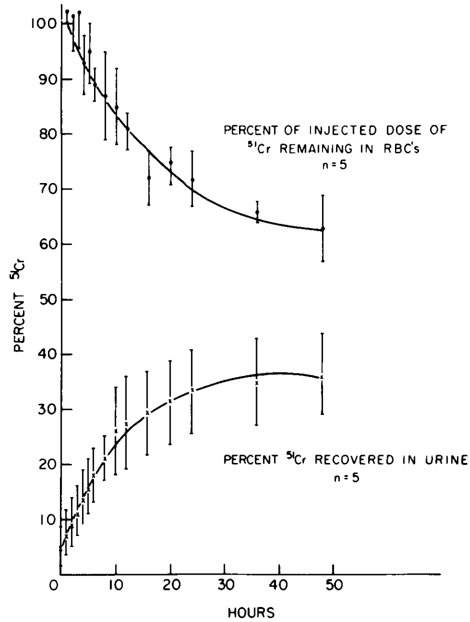

FIG. 1. Percent loss of label from circulation and excretion into urine during first 24 hr after injection of labelled cells.

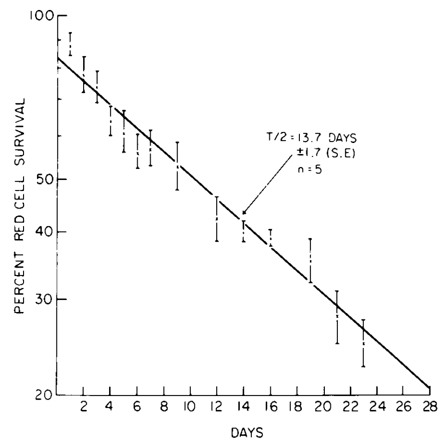

FIG. 2. Mean half-life of ^{51}Cr-tagged autologous erythrocytes in the five sheep.

from 0 to 0.004% of the total injected label.

The mean half-life of ^{51}Cr-tagged autologous erythrocytes in the five sheep was 13.7 (SE $\pm$ 1.7) days (Fig. 2). The respective half-life values for the five animals were 14.0, 20.0, 12.0, 10.5, and 12.0 days. The 24 hr sample was used as the time zero sample.

The ^{51}Cr survival time when repeated in sheep No. 52 (12 days) and No. 64 (14 days) approximately 6 months after the first study

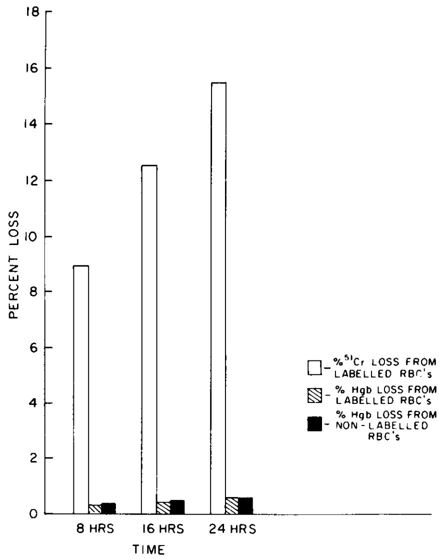

FIG. 3. Percent *in vitro* loss of ^{51}Cr and hemoglobin into the plasma from labelled and nonlabelled erythrocytes.

revealed comparable values of 11 days and 13 days for erythrocyte half-life.

The mean erythrocyte survival time as calculated by *in vivo* ^{59}Fe-labelled erythrocytes in six mature sheep was 111.7 (SE $\pm$ 8.4) days. The individual life span values were 96.5, 102.4, 102.8, 107.1, 108.6 and 152.9 days.

The *in vitro* incubation of labelled erythrocytes from two adult sheep in glucose-rich plasma revealed a mean loss of 8.9%, 12.5% and 15.5% of the label from the red cell at 8, 16, and 24 hr after labelling. Minimal release of hemoglobin into the plasma occurred during the incubation, with no significant difference observed between the labelled and nonlabelled samples (Fig. 3). The mean hemoglobin concentration in the plasma of all samples at time zero was 3.91 mg/100 ml and the concentration at 24 hr was 19.9 mg/100 ml in the labelled samples and 19.5 mg/100 ml in the nonlabelled samples. In the labelled samples only 0.58% of the total erythrocyte hemoglobin was released into the plasma at the end of 24 hr, whereas, 15.5% of the erythrocyte ^{51}Cr was lost into the plasma. In the unlabelled controls, 0.57% of total erythrocyte hemoglobin was released into the plasma at 24 hr.

Discussion. This study confirms our earlier observations on the short apparent red cell half-life when ^{51}Cr is used to label sheep erythrocytes. The rapid loss of ^{51}Cr activity from the circulation appears to be due primarily to rapid elution of the isotope from the labelled cells. Approximately one-third of the injected label disappears from the circulation and appears in the urine during the first 24 hr. The urinary ^{51}Cr-activity is not associated with hematuria or hemoglobinuria, suggesting that label elutes rapidly from the erythrocytes and is cleared and excreted by the kidneys. Our *in vitro* incubation study lends further support to the notion that elution of label from the red cells is the single most important determinant of ^{51}Cr disappearance. In the *in vitro* incubations we noted elution of 15.5% of label into the incubation medium over 24 hr in the absence of any significant hemolysis.

In man, ^{51}Cr elution from red cells is also a major determinant of the ^{51}Cr-disappearance rate (2). The elution rate is slower than we have observed here in sheep, yielding ^{51}Cr red cell half-lives of 28–32 days (7). Total red cell life span, as determined by cohort isotopic iron labelling, is similar for both humans and sheep, measuring about 110–120 days (4, 7). The molecular events which account for the elution of ^{51}Cr from red cell binding sites remain undefined. In human erythrocytes, the major binding of ^{51}Cr is at residue 93 on the β-chain (9). Additional binding also occurs to glutathione and other low molecular weight proteins (10–12). It is not clear from which pool the ^{51}Cr loss occurs. In certain hemolytic states, and with certain hemoglobinopathies, the elution rate is sufficiently variant from normal to yield false estimates of actual red cell life span (13).

In sheep erythrocytes, the ^{51}Cr binding sites remain to be determined. It may be that no β-chain binding occurs, and the rapid elution occurs because of this. We are planning to explore this possibility.

We conclude that there is very rapid elution of ^{51}Cr from sheep red cells. Since it is not clear what the determinants of this elution rate are, the use of ^{51}Cr to evaluate sheep red blood cell life span in experimental or pathological states should be approached with caution.

Summary. The red blood cell half-life as determined by ^{51}Cr-labelled autologous cells in five adult sheep was 13.7 days. In contrast, the red blood cell life span measured by cohort ^{59}Fe-labelling in six adult sheep was 111.7 days. The rapid loss of ^{51}Cr-activity from the circulation appears to result from rapid elution of the label from the circulating red cells. ^{51}Cr does not appear to be a suitable isotope for erythrokinetic studies in sheep.

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