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Determination of Circulating Red Cell Volume by Radioactive Chromium[1]

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Previous investigators (*3–9*) have reported accurate methods of measuring the circulating red blood cell volume by the injection of red cells labeled with radioisotopes. Radioactive iron and radioactive phosphorus were available for these studies. The preparation of red cells tagged with radioactive iron necessitates the administration of radioiron for several weeks to volunteer donors who incorporate it into the hemoglobin of their red cells, which must then be transfused into the experimental subject (*3–5*). Radioactive phosphorus (*6–9*) may be used to label a small volume of the experimental subject's own red cells *in vitro*. However, the phosphate exchanges rapidly between the red cells and plasma, causing the radioactivity of the tagged cells to fall significantly after 1–3 hr (*6–9*).

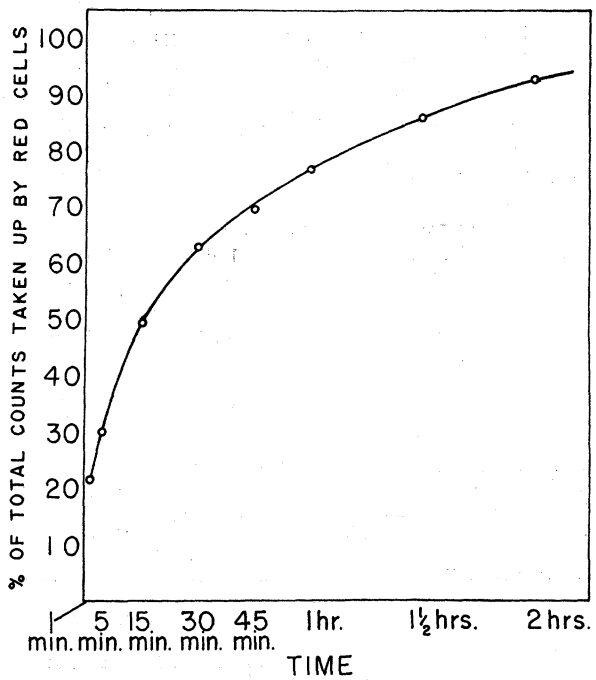

FIG. 1. Uptake of Cr^{51} as $Na_2Cr^{51}O_4$ by human red blood cells; 86 μg chromium added to 2 ml packed red cells suspended in saline at 24° C.

The present study concerns a new biological tracer, radioactive chromium (Cr^{51}), with a half-life of 26.5 days. When this isotope is added to blood *in vitro* as $Na_2Cr^{51}O_4$, it is taken up avidly by the red cells, which retain their radioactivity without significant loss for periods of 1 day or more after injection into experimental animals. The uptake of Cr^{51} by human red cells is illustrated in Fig. 1, demonstrating the marked affinity of red cells for $Na_2Cr^{51}O_4$. Since the exchange of Cr^{51} between red cells and plasma is negligible for 24 hr, this isotope appears ideal for the tagging of red blood cells and the measurement of the circulating red cell volume.

Approximately 50 ml of whole blood was withdrawn from a normal mongrel dog and heparinized. Cr^{51} (7–26 μc),[3] as $Na_2Cr^{51}O_4$, was added to whole blood or a red cell suspension in saline. After an hour, the red cells were washed with either physiological saline or plasma and then resuspended in plasma from the same dog. The plasma radioactivity was less than 1% of the radioactivity of the red cells.

A 4-ml aliquot was removed for counting, and the remaining measured volume of blood containing tagged red cells was injected intravenously into the dog. After allowing time for mixing in the circulation, 4-ml samples were withdrawn for counting at intervals varying from 10 min to 24 hr.

The injected blood and all subsequent blood samples were centrifuged in 4-ml hematocrit tubes, and the plasma was separated. One milliliter of each plasma sample was pipetted into a weighed planchet, dried overnight at 60° C (to constant weight) and counted with a Geiger-Müller counter. The plasma, regardless of the time interval, had no significant counts above background. The packed red cells were dried overnight at 60° C, then ground to a fine powder and counted in weighed planchets, preferably in duplicate. Since Cr^{51} is a soft x-ray emitter, self-absorption corrections were applied to all samples. All counts were corrected for radioactive decay.

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[3] The radioactivity dosage required for this method is well within the tolerance limits for human use. From the disintegration scheme of H. Bradt *et al.* (*1*), the administration of 42 μc of Cr^{51} per kg body wt approximates a total dose of 1 rep (*2*), on the conservative assumptions that there is no excretion and that radiation is localized in the red cells. The amount of chromium injected per kg body wt varied from 1 μg to 20 μg, depending upon the specific activity of the sample. This is well below the toxicity level.

In order to obtain the high specific activity required for this research, samples of chromium, enriched in Cr^{50}, were bombarded in the nuclear reactor. We wish to express our thanks to the Isotopes Division of the Atomic Energy Commission for making this material available.

The counts per ml of packed red cells were obtained by computing the counts in 0.35 g of dried ground red cells, since this value was found to be equivalent to 1 ml of packed red cells.

The circulating red cell volume of the animal is given by the formula:

Red cell volume in ml =

$$\frac{\text{total counts injected}}{\text{counts per ml packed red cells in sample}}. \quad (1)$$

The numerator of this fraction is obtained from the counts per ml of packed red cells, the volume of whole blood injected, and the hematocrit.

TABLE 1

DETERMINATION OF RED CELL VOLUME WITH CHROMIUM AS A RADIOACTIVE TRACER

Dog No.	Sex	Wt (in kg)	Red cell volume in ml*				Mean (in ml)	Standard deviation (in %)
			10–30 min	20–60 min	30 min–3 hr	18–24 hr		
6	♀	11.6	332	330	345	344	338	2.3
10	♀	17.5	477	450	495	503	481	4.9
12	♂	14.0	442	442	436	—	440	0.8
13	♀	13.2	446	436	441	458	445	2.1
15	♂	9.0	353	368	372	380	368	3.1

* Determinations based on consecutive blood samples.

Table 1 lists the measurements of red cell volume obtained from consecutive blood samples after allowing time for mixing of the tagged cells. The values are in close agreement with standard deviations of 0.8%–4.9% for periods of 18–24 hr. The activity of the red cell samples declined gradually thereafter. The plasma continued to show no significant activity.

The accuracy of the method was demonstrated by a second determination after transfusion or hemorrhage of a known volume of red cells. The dogs were transfused with approximately 400 ml of their own untreated blood withdrawn earlier. Additional blood samples were obtained following the transfusion, and the activity of the circulating red cells, now diluted by the addition of the untagged blood, was determined. The circulating red cell volume was then calculated according to equation (1).

After hemorrhage, however, the radioactivity of the circulating red cells remained unaltered, since hemorrhage represents the removal of an aliquot of blood containing an unaltered proportion of tagged and untagged red blood cells. Consequently, a second injection of tagged red cells was necessary to verify the red cell volume after hemorrhage. As before, this is given by the formula:

Red cell volume in ml =

$$\frac{\text{total counts in circulation}}{\text{counts per ml packed red cells in sample}}. \quad (2)$$

The numerator here represents the sum of the counts of the two injections of tagged cells minus the counts removed by the hemorrhage.

TABLE 2

RED CELL VOLUME DETERMINATION BY Cr^{51} BEFORE AND AFTER HEMORRHAGE OR TRANSFUSION

Dog No.	Cr^{51} Determinations		Calculated change in red cell volume (in ml)	Measured hemorrhage or transfusion (volume of whole blood × hematocrit) (in ml)	Difference (in %)
	Initial red cell volume (in ml)	Final red cell volume after hemorrhage or transfusion (in ml)			
7	382	228	−154	−148	4.1
8	300	480	+180	+171	5.3
9	360	538	+178	+179	0.6
11	678	466	−212	−215	1.4
14	386	527	+141	+141	0

Table 2 shows five such experiments in which the changes in red cell volume, as determined by the Cr^{51} method within a 24-hr period, were in good agreement with the actual volumetric measurement of the hemorrhage or transfusion with a difference of 5.3% or less.

Determination of the circulating red cell volume by Cr^{51} shares with the P^{32} method the advantage that a small sample of the subject's own blood may be tagged rapidly *in vitro* and then reinjected. In contrast to P^{32} tagging, however, the Cr^{51}-tagged cells retain their activity without loss to the plasma for periods of 1 day or longer after injection, which fact should prove advantageous for clinical studies. After 3 days, the red cell activity decreases by approximately 15%; a similar fall is observed within 3 hr when P^{32}-tagged red cells are used (*6–9*).

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