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THE USE OF Cr-51 IN THE DETERMINATION OF RED CELL VOLUMES IN EXPERIMENTAL ANIMALS

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Introduction

THE measurement of red cell volume by tagging of the erythrocytes with Cr–51 was first described by Gray and Sterling (1). Since then various modifications of the technique have been suggested (2, 3). The present report deals with a technique for use in sheep; the method was developed in an attempt to measure small changes in circulating blood volume. The technique was found to be equally suitable with rabbits. From the toxicological viewpoint, measurement of red cell volume is useful in the calculation of inhibition of red cell enzymes (e.g. cholinesterase, carbonic anhydrase).

Materials and Methods

The Cr–51 was obtained as sodium chromate solution in isotonic saline. The specific activity varied from 30–100 mC/mg Cr. Adult female sheep ranging in weight from 41–67 kg, and Old English and Albino rabbits of both sexes varying from 1.5–2.3 kg in weight were used.

Technique for investigations in sheep

20 ml of blood was withdrawn from the jugular vein into an all glass syringe and transferred to a sterile bottle containing 20 mg of heparin powder (Pularin Evans). The plasma was separated by centrifuging for three minutes at 600 g and then diluted with 0.9% saline to form a 3% plasma-in-saline solution for washing cells after activation. 40 C of Cr–51 in 1.0 ml of saline (1 μg $Na_2 CrO_4$/ml 0.9% NaCl) was added to the packed cells, and this mixture incubated at room temperature for 30–45 minutes.

After incubation the cells were centrifuged three times in 3% plasma-in-saline and were then resuspended to 25 ml in the same solution. 20 ml of the suspension was drawn up into the syringe and well mixed; 1.0 ml was expelled for use as a weighed standard. The syringe was weighed, its contents injected into the jugular vein of the same sheep and the empty syringe reweighed. The remaining 5 ml of suspended cells was centrifuged and the supernatant used to estimate the extracellular Cr–51.

8 ml of blood was withdrawn from the opposite jugular vein 20 minutes after the injection of labelled cells. In some experiments samples were taken at other time intervals (10 minutes–3 hour safter injection). In addition the rate of elution of Cr–51 from the red cells was determined for a period of up to eight days, by taking daily blood samples.

5 ml of each blood sample withdrawn was lysed in 5 ml of water and its Cr–51 content determined in a scintillation counter (Ekco N559). Haematocrits were determined in quadruplicate on each sample, using a capillary-tube technique: approximately 50 µl of blood was drawn into a 1 mm constant-bore capillary tube (MSE) and the end was flame-sealed. The set of tubes was then spun for 30 min. at approx. 600 g (3,000 rpm @ 7 cm radius). The haematocrit was read below the buffy layer. No correction was made for trapped plasma.

Technique for rabbits

The above mentioned method was modified in the following manner when applied to rabbits. 2 ml of blood was withdrawn from the ear, heparinised, and activated with 20 µC of Cr–51. After washing and resuspending the erythrocytes 1 ml of blood was injected into the marginal vein of the ear. A 1.5 ml blood sample was taken from the opposite ear 20 minutes after injection, and 1 ml of each sample was diluted to 10 ml with water for measurement of radioactivity. In two rabbits blood samples were also taken at daily intervals for 11 days and the Cr–51 content estimated as above in order to determine the rate of elution.

Additional experiments in vitro

(a) *Decay of physical solution.* To study the rate of radioactive decay of Cr–51, two types of standard were used. One was prepared as an aqueous solution from which 10 ml aliquots were taken. The other was made as a 3% gel in agar in a polythene counting cup. Both standards were counted daily.

(b) *Effect of sodium chromate on sheep cells.* 0.01–16 mg of sodium chromate was added to 2 ml of heparinised sheep blood, left at room temperature for two hours, centrifuged at 200 g for 15 minutes and examined for the usual signs of haemolysis.

Calculation of red cell volume. The red cell volume was calculated as follows:

$$\text{Red cell volume} \times \text{Cr–51 activity per unit volume} = \text{Total Cr–51 injected.}$$

$$\therefore \text{Red cell volume} = \frac{\text{Total counts injected}}{\text{Count per ml. of red cells recovered}}$$

$$= \frac{\text{Count per ml of cell suspension injected} \times \text{injected volume} \times \text{haematocrit (fractional)}}{\text{Count per ml of blood recovered}}$$

Also, Calculated blood volume = (red cell volume)/haematocrit (this measure although simpler, is subject to theoretical objection; see Discussion).

Results

Sheep. The withdrawal of blood and its activation with Cr–51 caused no visible haemolysis or clotting. Sheep blood clotted in acid-citrate-dextrose U.S.P., but not in heparin. Deliberate incubation of sheep erythrocytes with 0.08% Na_2 CrO_4, a concentration 800 times that used for the activation, produced no haemolysis. The proportion of the Cr–51 added to the blood which remained on the red cells after washing was 75–80%. The activity of the suspending medium for re-injection was very low, in all cases less than 0.5% of that in the suspended cells.

The blood samples withdrawn from the sheep showed no signs of haemolysis. The plasma contained no significant radioactivity (less than 1% of the cell activity). The red cell activity was approximately 5–10 times background (depending on the weight of the animal), so that no special counting precautions were necessary.

The quadruplicate haematocrit determinations on each sample were internally consistent to within 1% (e.g. Haematocrit=0.391, 0.389, 0.389, 0.385). The variation of haematocrit between blood samples from the same animal was up to 5% (Table I).

TABLE I

Characteristics of consecutive blood samples taken from a single sheep

Time after injection (hr)	*Haematocrit (fraction)*	*Red-cell volume (ml)*
0.3	0.283	776
0.7	0.273	804
1.0	0.285	796
1.3	0.269	794
3.0	0.271	793

There was only a very small systematic decrease in the activity of the blood samples up to 3 hours after injection (Table I). Thereafter, however, the activity decreased to reach a fairly steady level, 30% of the original after 5 days (Fig. 1). Both the red cell volume (calculated from the 20 minute value) and the blood volume varied considerably as a fraction of the body weight (Table II).

Rabbits. The activity of the suspending medium was less than 0.3% of that in the cells. The plasma of the blood samples withdrawn after the Cr–51 injection contained no observable activity, and the red cell activity was readily countable.

The internal consistency of the haematocrits was similar to that for the sheep, but the haematocrit fell markedly (by up to 15%) with repeated blood sampling, so that

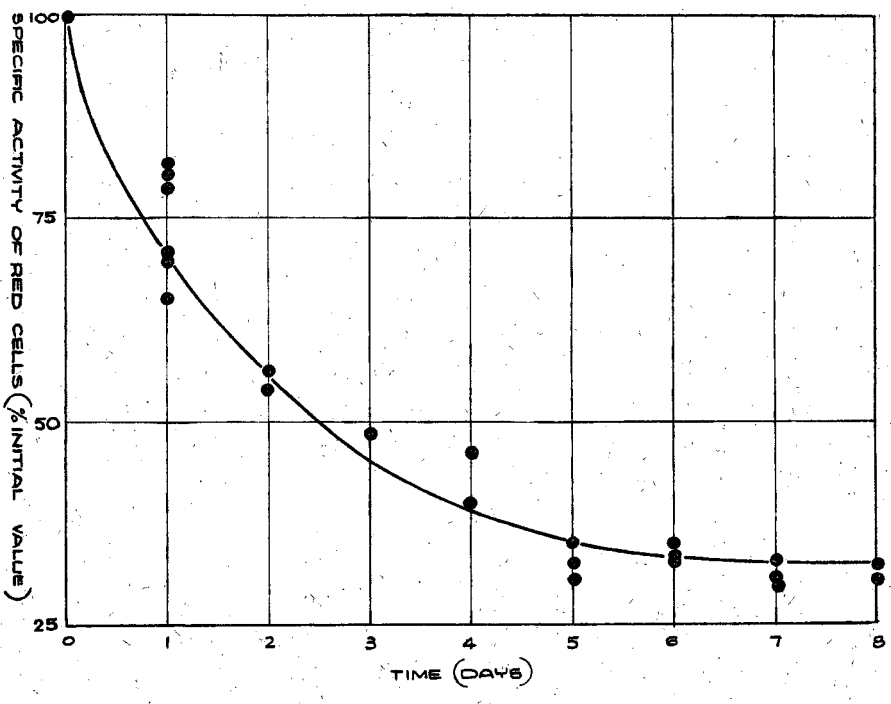

Figure 1. *Elution of Cr-51 from the Red Cells of five Sheep*

TABLE II
The red cell volume and calculated blood volume of sheep

Weight (kg)	*Red cell volume (ml/kg)*	*Blood volume (ml/kg)*
42	23	66
48	29	73
60	26	69
56	19	50
60	27	63
64	16	51
67	21	50
68	24	48
mean	23.3	58.8
S.E. mean	1.5	3.7

frequent sampling was abandoned. There was no observable elution of Cr–51 between samples taken at 20 minutes and 150 minutes after injection. The subsequent loss of activity was slower than in sheep, but did not reach a plateau (Fig. 2). The calculated blood volumes of the female animals was a very constant fraction of the body weight, and a significant difference was observed between sexes (Table III); the difference is significant at the 5% level by Student's test. The haematocrit did not vary systematically with sex. The red cell volume accordingly showed the same difference as the blood volume, but was more variable, so that the difference was not significant. The variation in all three parameters was much greater in males than in females (Table III).

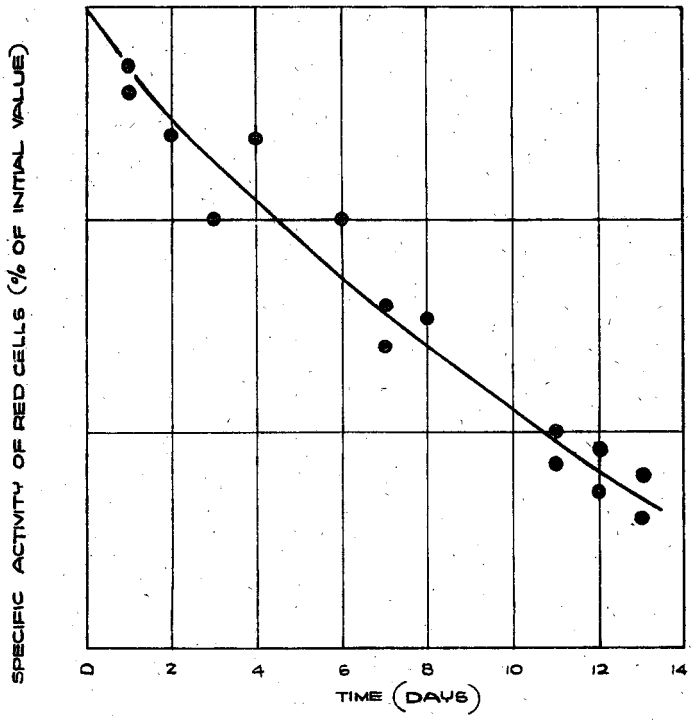

Figure 2. Elution of Cr-51 from the Red Cells of two Rabbits

Decay of standard solutions of $Na_2 Cr0_4$

A dilute solution of Na_2Cr0_4 (0.3×10^{-9} Na_2Cr0_4/ml) kept as a counting standard lost activity more rapidly than predicted from the radioactive decay of Cr–51. On the other hand a gel of the same solution decayed at the correct rate, and this was, therefore used as the standard (Fig. 3).

Discussion

The use of chromium labelling to measure blood volume is now a routine clinical procedure (1–4) and has been used extensively in animals (5–8, 16–20). We have

TABLE III

The red-cell and calculated blood volumes of rabbits

Type	*Body weight (kg)*	*Haematocrit (fraction)*	*Red-cell volume (ml/kg b.w)*	*Blood volume (ml/kg b.w)*
A. Male				
1 Albino	1.7	0.36	18	50
2 „	2.1	0.45	24	54
3 „	2.0	0.36	20	54
4 „	2.0	0.32	20	60
5 „	1.9	0.30	14	47
6 „	2.3	0.34	14	43
7 Old English	1.9	0.38	20	53
8 „	1.5	0.43	21	49
mean	1.92	0.368	18.9	51.2
S.E mean		0.018	1.2	1.8
B. Female				
1 Albino	2.1	0.34	15	48
2 „	2.3	0.33	15	46
3 „	2.0	0.39	18	45
4 „	2.1	0.36	15	44
5 „	2.1	0.37	16	46
6 Old English	1.6	0.39	18	46
7 „	1.6	0.40	19	48
mean	1.97	0.369	16.6	46.1
S.E mean		0.010	0.7	0.6

attempted here to use this experience, and our own experimental work, to describe some of the errors inherent in the method and the accuracy to be expected from it.

There is no particular difficulty in labelling red cells with Cr–51, it has been successfully attempted in animals as small as the mouse (18) and the hamster (19). Counting techniques are now standard and should not limit the accuracy of the experiment. The measurements most obviously prone to error are the volume of blood injected and the haematocrit of the blood withdrawn for sampling. The former difficulty is readily overcome by the weighed-syringe method and good injection technique, and the latter by the use of the capillary-tube haematocrit, which gives

exceedingly consistent results, although it may contain a systematic error; some users claim that it does not (10) but there is evidence that very high accelerations (>6000g) are required to remove all of the trapped plasma (21).

The reliability of the withdrawal technique is shown by the similarity of the serial measurements obtained on a single animal (Table I); this also shows that elution of the label is insignificant in the first few hours after injection. The high consistency of the results in one set of results (females; Table III) indicates that the overall technique

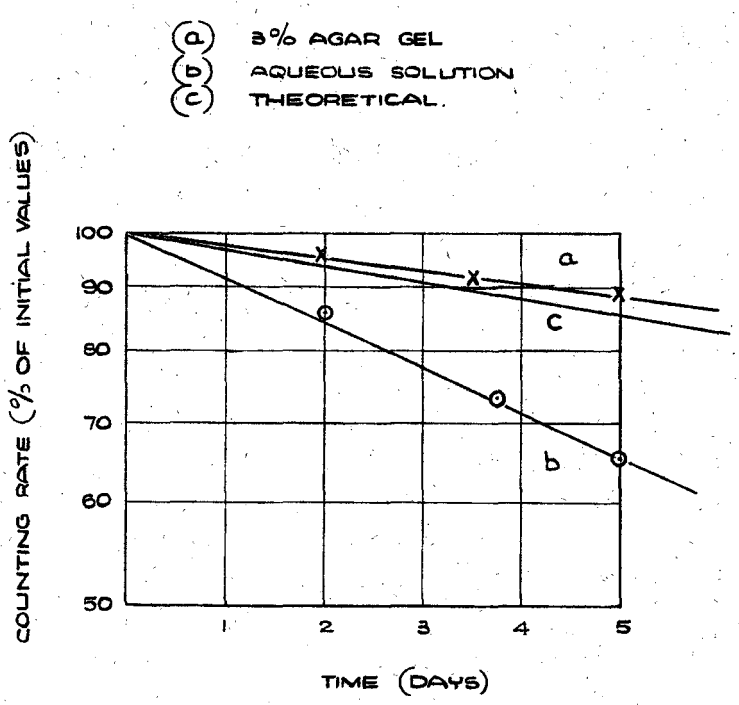

Figure 3. *Comparison of decay of standards*

is also reliable, and suggests that the larger variation seen in other sets (Table II and males Table III), and by other observers (5) is of genuine biological origin. Two obvious causes of such variation are the splenic emptying (11, 12) and the stomach size in ruminants.

Many methods other than chromium labelling may be used to measure red cell volume, but all are either more complicated or less reliable. In vitro labelling of cells with inorganic P–32 phosphate is complicated by the rapid elution of the label (5, 9), while labelling with P–32 as DFP is very expensive for routine use (13). In vivo labelling of cells with Fe–59 is lengthy (14), while carbon monoxide labelling by the respiratory route requires very careful control (15).

Chromium labelling measures the red cell volume directly, not the blood volume. There is excellent evidence that the venous haematocrit is greater than that of the total circulation, both in man and animals (13, 16); the difference anmouts to about 10% in those species tested. Theoretically, therefore, one should adjust the apparent blood volume (calculated as under methods) by this factor. This has not usually been attempted because the factor is uncertain, so that most measures of blood volume (including those reported in this paper), are probably too low by about 10%.

Chromium is an unsatisfactory label for the measurement of red-cell life-span, because it elutes too rapidly. This has been shown in sheep (20; of Fig. 1) and although there continue to be attempts to measure life-span in various species with chromium (17–19) it is obvious from the shortness and variability of the results reported by these authors, that the measure is virtually useless in many animals.

The rapid loss of activity shown by a dilute "standard" solution of sodium chromate noted here and elsewhere (4), is of practical interest. It can be evaded completely by the formation of a standard gel, which indicates that it is caused by precipitation of the chromate, or its absorption on to the walls of the vessel.

Summary

1. A method of measurement of red cell volume in sheep and rabbits, using Cr–51, is described.
2. Its accuracy is calculated and its limitations discussed.

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