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The elution of $^{99}Tc^{m}$ from red cells and its effect on red-cell volume measurement

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SYNOPSIS Technetium-labelled erythrocytes provide a satisfactory agent for the measurement of red-cell volume with the advantages that there is a low radiation dose and repeated measurements can be readily carried out. A method of labelling red cells using $^{99}Tc^{m}O_4^-$ with a small amount of stannous chloride has been evaluated in 20 patients, and compared with measurements of red-cell volume with ^{51}Cr-labelled erythrocytes carried out simultaneously in the same subjects.

No significant differences between the results by either method occurred if the period of observation was limited to 20 minutes after the injection of labelled cells. Thereafter, elution of Tc from the cells made the determination of red-cell volume with $^{99}Tc^{m}$ less reliable. Accordingly, the use of $^{99}Tc^{m}$ as the label for red-cell volume measurements will result in errors if mixing of the erythrocyte pool is delayed, unless an elution correction factor is applied.

Because of its short half-life and its radiation properties, the radionuclide $^{99}Tc^{m}$ appears to be particularly suitable as a red-cell label for measurements of the red-cell mass (Korubin, Maisey, and McIntyre, 1972) and the splenic red-cell volume, and for visualizing the spleen (Hegde, Williams, Lewis, Szur, Glass, and Pettit, 1973). Adding stannous chloride as a reducing agent permits satisfactory labelling efficiency (50-75%) as well as a good binding of the technetium to the erythrocyte (Eckelman, Richards, Hauser, and Atkins, 1971; Korubin, Maisey, and McIntyre, 1972). It has been shown that there is a negligible increase in the distribution space of the isotope, at least during the first 20 minutes after the injection of the labelled red cells.

However, if mixing of the labelled cells is delayed, as occurs in cardiac failure or shock (Baker, St. Ville, Suzuki, and Shoemaker, 1965) or in splenomegaly (Toghill, 1964), it is necessary to delay blood sampling for 30 to 60 minutes in order to avoid underestimating the red-cell volume.

The purpose of this study was to check the validity of Technetium-99^{m} as a red-cell label, with special reference to the elution of the isotope from the cells during the first hour following injection.

Patients

Red-cell volume was measured in 20 patients. Twelve of the patients suffered from polycythaemia rubra vera, one from myelosclerosis, two from aplastic anaemia, and five were being investigated because of the fortuitous discovery of a high packed cell volume. Only the patient with myelosclerosis had a massively enlarged spleen (F.A., see table). Although some of the other patients had varying degrees of splenomegaly, there did not appear to be any significant differences between the blood samples collected at 10 min and 60 min after the injection of ^{51}Cr-labelled red cells.

Methods

Twenty ml venous blood were collected into 3 ml of 'NIH A' acid citrate dextrose (ACD). The blood was centrifuged and the plasma removed. Half of the red cells were labelled with ^{51}Cr in accordance with the ICSH recommended methods (International Committee for Standardization in Hematology, 1973). Five hundred μCi of $^{99}Tc^{m}$ in the form of pertechnetate was added to the remainder and this suspension was allowed to stand for 15 min at room temperature. Two μg of a freshly prepared solution of stannous chloride in 9 g/l NaCl (passed through a 0·22 μm Millipore filter) was added per ml of red cells. After a further 5 min of standing, the red cells were washed three times in five volumes of 9 g/l NaCl.

The ^{51}Cr- and $^{99}Tc^{m}$-labelled red cells were mixed together thoroughly and suspended in saline so as

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Patient	1 ^{51}Cr RCV	2 $^{99}Tc^m$ RCV (m = 10-20 min)	3 $\frac{^{99}Tc^m \text{ RCV}}{^{51}Cr \text{ RCV}} \times 100$	4 $^{99}Tc^m$ RCV (60 min)	5 $\frac{^{99}Tc^m \text{ RCV } 60 \text{ min}}{^{51}Cr \text{ RCV}} \times 100$	6 % Elution (60 min)	7 Corrected $^{99}Tc^m$ RCV 60 min	8 $\frac{\text{Corrected } ^{99}Tc^m \text{ RCV } 60 \text{ min}}{^{51}Cr \text{ RCV}} \times 100$
Mc.G.B.	2617	2697	103·5	2810	107·3	4	2490	95·1
A.A.	3520	3370	95·7	3737	106·1	14	3424	97·2
R.G.	2111	2110	99·9	2263	107·2	10	2086	98·8
F.A.	892	833	93·3	871	97·6	11	795	89·1
W.L.	3134	3100	98·9	3336	106·4	10	3093	98·6
W.T.	2017	1958	97	2101	104·1	13	1961	97·2
C.S.	1845	2111	114·4	2188	118·5	11	2019	109·4
W.E.	920	1025	111·4	1058	115	8	995	108·1
C.T.	3218	3139	97·5	3370	104·7	12	3108	96·5
D.W.	2678	3254	121·5	3533	131·9	16	3304	123·3
M.J.	1701	1787	105	1940	114	8	1787	105
G.R.	1495	1470	98·3	1635	109·3	11	1514	101·2
J.L.	777	754	97	747	96·1	4	781	100·5
R.J.	2808	2912	103·7	2990	106·7	5	2764	98·4
H.J.	749	728	97·1	744	99·3	12	787	105
B.C.	2629	2804	106·6	2889	109·3	6	2659	101·1
P.L.	2485	2484	99·9	2692	108·3	9	2495	100·4
W.J.	4697	3900	83	4259	90·6	11	3833	81·6
S.E.	2307	2218	96·1	2407	104·1	12	2289	98·2
B.I.	1945	1792	92·1	2065	106·1	17	1918	98·6
			m = 99·7% SD = 8·4		m = 106·9% SD = 8·7	m = 10·2		m = 98·8% SD = 8·2

Table *Comparison of red-cell volume measurements using ^{51}Cr and $^{99}Tc^m$*

to obtain a PCV of about 0·5. One ml of this suspension was retained as a standard. The amount of radioactivity in the supernatant of further aliquot portions of the suspension was determined at various times after the end of the tagging procedure.

Venous blood samples were obtained every 10 min for 60 min after the injection. The radioactivity was measured in a scintillation counter with a dual channel analyser (Packard model 3002). With this the counts due to each of the isotopes were easily distinguished, and, in the Tc counting conditions, the amount of scattered radiation due to ^{51}Cr from the small dose administered was minimal. The $^{99}Tc^m$-radioactivity attached to the red cells was calculated in each sample by subtracting the plasma activity (adjusted for PCV) from the activity of the whole blood. The value obtained for each time interval was used to calculate Tc red-cell volume. From the ^{51}Cr data the red-cell volume was calculated in the conventional way (International Committee for Standardization in Hematology, 1973). These results were compared with the Tc red-cell volume to enable the elution of technetium from the red cells to be calculated.

Results

Elution of technetium from the red cell in saline suspension *in vitro* was very slow and did not exceed 1% in one hour (fig 1).

Red-cell volume as measured with ^{51}Cr and $^{99}Tc^m$ did not differ significantly with the isotope used when calculated from the mean of the blood samples taken 10 and 20 min after the injection (table, columns 1-3). However, a significant increase (mean 7%) in the distribution space of technetium occurred when the 60-min sample was used (table, columns 4-5). This increase was due to elution of the label from the red cells (fig 2), the eluted technetium disappearing partially from the circulation. The presence of some free radioactivity in the blood at

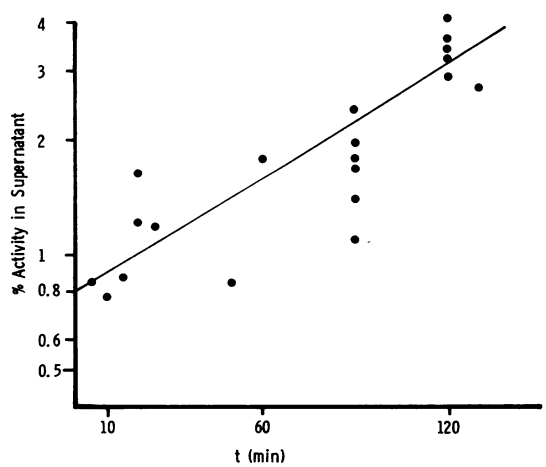

Fig 1 *Elution of $^{99}Tc^m$ from red cells* in vitro. *Note minimal elution during the first two hours.*

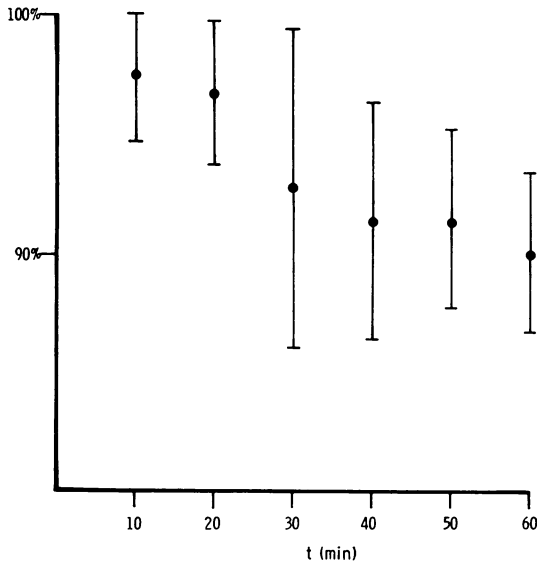

Fig 2 *Elution of $^{99}Tc^{m}$ from red cells* in vivo. *Note increasing elution with time. The vertical bars represent 2 SD.*

60 min explains the 7% increase in red cell volume at that time: this corresponds to an elution of 10% of the injected radioactivity which would have been found had all unbound technetium disappeared immediately from the circulation (table I, column 6).

As shown in the table (columns 7-8), it is possible to apply to the data a correction factor of 10% for the elution at 60 min, and thus to calculate the blood volume from the formula:

$$\frac{\text{cpm of standard} \times \text{dilution of standard} \times \text{vol injected} \times 0{\cdot}9 \times \text{PCV}}{\text{cpm of postinjection sample at 60 min} - (\text{cpm of plasma at 60 min} \times (1 - \text{PCV}))}$$

Discussion

At the present time, red-cell volume is most commonly measured by labelling the red cells with ^{51}Cr. It would be considerably advantageous to have a satisfactory radioactive red-cell label which has a short half-life and could thus be used when repeated estimations of red-cell volume are required. This would make for greater accuracy and a lower radiation dose. Red-cell survival studies are usually carried out with cells labelled with ^{51}Cr or DFP. In cases when a steady state does not exist, ie, when red cell production and destruction are not equal, errors may be introduced in whichever way the survival data are analysed. Here again red-cell volume estimations at suitable intervals may prove of considerable value.

This study confirms that the red-cell labelling technique using $^{99}Tc^{m}O^{-}_{4}$ and stannous chloride provides a reliable red-cell label which has stability *in vitro* and a satisfactory binding to the red cells. There is a difference in elution of $^{99}Tc^{m}$ *in vitro* and *in vivo*. The possible explanations for this are either the presence of a fraction of technetium which is more loosely bound and which elutes more rapidly when subjected to the environmental conditions of the circulation, or alternatively, rapid removal from circulation after reinjection of a small percentage of labelled but non-viable cells.

Measurements can be made of the red-cell mass using venous samples collected at 10 and 20 min, with an accuracy which does not differ significantly from that of the standard ^{51}Cr procedure. However, towards 60 min after the injection, a significant elution occurs from the red cells. Thus, in the case of slow mixing, as in splenomegaly, cardiac failure, or shock, where a sampling delay of 30 min or longer is necessary, the value obtained for the red-cell volume measurement should be corrected for elution in order to avoid overestimation.

References

Baker, R. J., St. Ville, J. M., Suzuki, F., and Shoemaker, W. C. (1965). Evaluation of red cell equilibration in hemorrhagic shock. *Arch. Surg.*, **90**, 538-544.

Eckelman, W., Richards, P., Hauser, W., and Atkins, H. (1971). Technetium labeled red blood cells. *J. nucl. Med.*, **12**, 22-24.

Hegde, U. M., Williams, E. D., Lewis, S. M., Szur, L., Glass, H. I., and Pettit, J. E. (1973). Measurement of splenic red cell volume and visualization of the spleen with $^{99}Tc^{m}$. *J. nucl. Med.*, **14**, 769-771.

International Committee for Standardization in Hematology (1973). Standard techniques for the measurement of red-cell and plasma volume. *Brit. J. Haemat.*, **25**, 801-814.

Korubin, V., Maisey, M. N., and McIntyre, P. A. (1972). Evaluation of technetium-labeled red cells for determination of red cell volume in man. *J. nucl. Med.*, **13**, 760-762.

Toghill, P. J. (1964). Red-cell pooling in enlarged spleens. *Brit. J. Haemat.*, **10**, 347-357.