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Simultaneous Red Cell Mass and Plasma Volume Determinations Using ^{51}Cr Tagged Red Cells and ^{125}I Labeled Albumin[1]

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Several techniques for the simultaneous determination of red cell mass and plasma volume have been developed and published (1-5). These techniques involve the use of ^{131}I labeled albumin and ^{51}Cr labeled red cells. It is difficult to measure quantitatively ^{131}I and ^{51}Cr simultaneously by energy spectrum analysis, because ^{131}I has its major photopeak at 364 kev and ^{51}Cr at 320 kev. Our laboratory was unable to resolve satisfactorily these two isotopes according to the method of Shires (5) and to make reproducible determinations of red cell mass and plasma volume.

With the availability of ^{125}I labeled albumin, our interest was renewed in the simultaneous determination technique. It was felt this isotope could be utilized satisfactorily, since ^{125}I has its major photopeak at 28 kev. A standard 2-inch Harshaw integral line NaI well crystal assembly was found to satisfactorily count radiations of this energy.

METHOD

Our technique consisted of tagging red cells with sodium radiochromate and injecting these cells into an antecubital vein followed by an injection of ^{125}I labeled albumin through the same needle. Since the ^{51}Cr tagged red cells and the ^{125}I labeled albumin were injected separately, dilutions of these materials could be made for use as standards in the study. However, the venous blood obtained from the patient contained both ^{125}I and ^{51}Cr. The following procedure was necessary to quantitatively evaluate the amounts of these two isotopes in our sample. The same sample was counted under two different spectrometer conditions, (1) an integral count of all radioactive emissions from the sample with a zero base line; (2) an integral count with a base line of 70 kev.

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Mathematically one may express Condition (1) which represents zero base line and Condition (2) which represents 70 kev base line in two simultaneous equations:

$$K_1 I + K_2 C = A \quad (1)$$

$$K_3 I + K_4 C = B \quad (2)$$

I = the count due to the total amount of iodine
C = the count due to the total amount of chromium
A = the observed count under Condition (1)
B = the observed count under Condition (2)
K_1, K_2, K_3, K_4 = the fractional portions of I and C which are represented in the counts A and B

Under Condition (1) (since zero base line was maintained) 100 per cent of the iodine and chromium in the sample were counted, *i.e.*, K_1 and K_2 factors are 1.0. The coefficients, K_3 and K_4 may be determined by counting samples of pure ^{125}I and pure ^{51}Cr under the different spectrometer Conditions (1) and (2) since

$$K_3 = \frac{\textit{Amount of } I \textit{ counted under Condition } 2}{\textit{Amount of } I \textit{ counted under Condition } 1}$$

$$K_4 = \frac{\textit{Amount of chromium under Condition } 2}{\textit{Amount of chromium under Condition } 1}$$

The K_3 and K_4 coefficients are fractions which are dependent only upon the settings of the spectrometer and not the amounts of the isotopes in the sample. The choice of a different base line value under Condition (2) will only change the value of the constants K_3 and K_4. Our choice of 70 kev was an attempt to minimize K_3 while maintaining a high value for K_4.

Dividing equation (2) by K_3 and subtracting to eliminate the factor I in the two equations yields a solution for C as

$$C = \frac{B - K_3 A}{K_4 - K_3}$$

and substitution of this solution into equation (1) yields a solution for the iodine.

$$I = \frac{K_4 A - B}{K_4 - K_3}$$

On our spectrometer, it was found that K_3 had a value of 0.005 and K_4 a value of 0.89, with less than 1 per cent variation. The fact that ^{125}I is still detected with a base line set at more than twice its peak spectrum probably only indicates coincidence counting.

Using 30 μc of chromium and 10 μc of ^{125}I, the value of $K_3 I$ was so small that assuming $K_3 = 0$ did not alter the final measured volume by more than 15 ml. This assumption simplifies the formulae to:

$$I = A - \frac{B}{K_4}$$

$$C = \frac{B}{K_4}$$

This approximation is valid, however, only if the ratio $\frac{K_3 I}{K_4 C}$ is very small.

Figure 1 shows an energy spectrum of ^{125}I and ^{51}Cr and the conditions under which the samples were counted to separate the two isotopes.

LABORATORY PROCEDURE

With a method of determining the amount of ^{125}I and ^{51}Cr in a sample, the following laboratory procedure was developed.

1. Fifteen ml of blood is drawn from the patient, of which 10 ml are injected into a "safe tag" bottle[1] containing approximately 5 ml of special formula ACD solution. Five ml are injected into EDTA for use as a background sample.
2. Forty-five microcuries of sodium chromate ^{51}Cr are added to the 15 ml mixture of blood and ACD, giving a concentration of approximately 3 μc/ml.
3. After 15 minutes incubation at room temperature, 50 mgm of ascorbic

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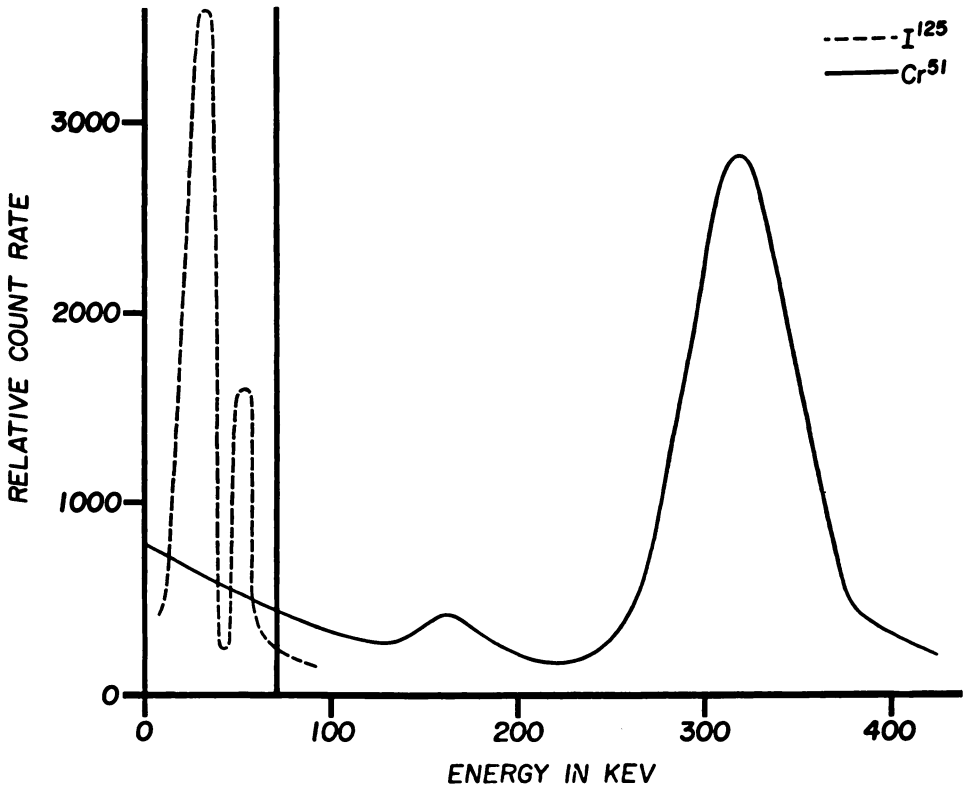

Fig. 1. Approximate energy spectra of ^{125}I and ^{51}Cr. Heavy line at 70 kev indicates position of base line under Condition (2) indicated in text.

acid are added to reduce the chromate ion to the chromic form and stop further tagging in the bottle.

4. Ten ml of the labeled red cell mixture are injected into a vein and, using the same needle, 5 ml of ^{125}I labeled albumin which had been diluted to a concentration of 2 μc/ml in normal sterile saline are injected.

5. Fifteen minutes postinjection, 7 ml of blood are withdrawn from the opposite arm and mixed with EDTA.

6. From the bottle of tagged blood, 1 ml of blood is withdrawn and diluted in 100 ml of normal saline. One ml of this mixture placed in a test tube, and labeled as Sample No. 3. A 3 ml sample of the tagged blood is centrifuged and 1 ml of plasma is removed and diluted in 100 ml of normal saline. Then, 1 ml of this mixture is placed in a test tube and labeled as Sample No. 4. A hematocrit is determined on the blood in the bottle and labeled Hematocrit No. 1.

7. One ml of the postmix blood sample from the patient is put into a test tube and labeled Sample No. 5. A 3 ml sample of blood from the patient is centrifuged and 1 ml of plasma is put into a test tube and labeled Hematocrit No. 2.

8. Five ml of the albumin solution are injected into 1000 ml of normal saline and well mixed. One ml of this mixture is put into a tube and labeled Sample No. 2.

9. One ml of background blood is put into a tube and labeled Sample No. 7. Three ml of this blood are centrifuged and 1 ml of plasma is put into a tube labeled Sample No. 8.

Counting all samples under Conditions (1) and (2) gives data from the following samples:

1. Room Background
2. RISA Standard
3. Whole Blood ^{51}Cr Standard
4. Plasma ^{51}Cr Standard
5. Whole Blood Sample
6. Plasma Sample
7. Whole Blood Background Sample
8. Plasma Background Sample

It was decided to indicate counts by means of a coding system such that Sample No. 1, counted under Condition A and B would be Sample No. 1A or Sample No. 1B. For example, 2A would be the RISA Standard counted under the Condition of zero base line and 3B would be the whole blood ^{51}Cr Standard counted under 70 kev base line Condition.

DERIVATION OF FORMULAE

In general, the principle of measuring a volume by dilution techniques is that the volume is equal to the total amount of material injected, divided by the concentration of a sample drawn after complete mixing (6).

$$V = \frac{N}{n}$$

N = Total injected material

n = Concentration of sample

In order to measure red cell volume it is necessary to determine the total amount of ^{51}Cr tagged red cells which was injected and the concentration of tagged red cells in the postmix sample. Since whole blood is counted, this count must be corrected to indicate the activity due to 1 ml of red cells.

$$\text{Therefore, } N_c = \frac{\textit{Activity in 1 ml whole blood} - \textit{Activity of 1 ml plasma } (1 - H_1)}{H_1}$$

where N_c = Activity of 1 ml red cells
H_1 = Fractional portion of the whole blood standard which is red cells.

Now $N = N_c \times M \times H_1$ where M is the number of ml of whole blood injected and $M \times H_1$ is the number of ml of red cells injected. N indicates the total amount of Cr which is tagged to red cells and injected. The Cr activity in the sample which is withdrawn after mixing is:

$$n = \frac{\textit{Activity 1 ml whole blood} - \textit{Activity 1 ml plasma } (1 - H_2)}{H_2}$$

where H_2 is the fraction of the whole blood sample which is red cells. Division by H_2 corrects the count to 1 ml of red cells. Since the standard is diluted, it must be multiplied by a dilution correction factor DF. Hence, the formula for red cell volume using our notation is:

$$RBCV = \frac{3A - [4A\ (1 - H_1)]}{5B - [6B\ (1 - H_2)]} \times H_2 \times M \times DF \times K_4$$

where H_1 = Hematocrit of bottled blood
H_2 = Hematocrit of patient
M = ml injected (10)
DF = Dilution factor (100)

In the derivation of the formula for the plasma volume the same basic formula $V = \frac{N}{n}$ is used. It is possible to directly measure 1 ml of plasma so that hematocrit corrections are eliminated. N is equal to the product of the activity of the standard, the number of ml injected, and the dilution factor. The quantity n is the difference between the total activity in the plasma and the Cr activity in the plasma. The formula for plasma volume, using our notation, is:

$$PV = \frac{2A}{6A - \frac{6B}{K_4}} \times M \times DF$$

where M = ml injected (5)

DF = Dilution factor (200)

Although in the formulae the numbers indicated are gross counts, it is necessary to correct the numbers to net counts per minute per milliliter before using them. The corrections for the activity in the background blood are made by subtracting

TABLE I

RESULTS IN 10 NORMAL SUBJECTS WITH TWO-HOUR INTERVAL BETWEEN DETERMINATIONS

				First Determination				*Second Determination*			
Subject	*Sex*	*Estimated Blood Vol*	*Venous Hematocrit*	*Red Cell Mass*	*Plasma Volume*	*Blood Volume*	*Isotope Hematocrit*	*Red Cell Mass*	*Plasma Volume*	*Blood Volume*	*Isotope Hematocrit*
JB	F	4049	45	1382	2367	3749	37	1534	2259	3793	40
PH	F	3545	45	1238	2321	3559	35	1489	2198	3687	40
DH	M	4729	44	1789	2834	4623	39	1554	2383	3937	39
RW	M	5265	39	1848	2768	4616	40	1896	2858	4754	40
LS	F	4517	42	1634	2812	4446	37	1923	3133	5036	38
JF	M	5846	47	2373	2437	4810	49	2650	2756	5406	49
RH	M	4780	44	1672	2575	4247	39	1314	2761	4075	32
CR	M	5400	47	2613	3336	5949	44	2011	3538	5549	36
GE	M	5225	45	1926	2903	4829	40	1800	2838	4638	39
JL	M	4865	44	1707	2563	4217	40	1786	2513	4299	42
Mean		4822	44.2	1818	2692	4505	40.0	1795	2727	4517	39.5
SD				416	305			370	406		

samples 7 and 8 from 5 and 6, respectively, prior to the time 5 and 6 are used in the formula. This correction is necessary only if the patient has had prior radioisotope studies. The values of K_3 and K_4 can be calculated from the ratio of the counts, $K_3 = \frac{2B}{2A}$ and $K_4 = \frac{3B}{3A}$. This procedure eliminates keeping separate pure samples of ^{125}I and ^{51}Cr to compute the values of these two constants.

RESULTS

In evaluating the reproducibility of this technique, ten normal subjects were chosen and the measurement was performed on them twice with a two-hour interval. No attempt was made to restrict the hydration, food intake or exercise of these subjects since it was desired to evaluate the errors in this method and apply them to the estimation of errors in patients who are seen in a random manner and are not at a base line condition. The results of these studies are shown in Table I. As can be seen in the red cell volume determinations, the first determination average was 1818 ml with a standard of 416. The second determination was 1795 with a standard deviation of 370. A statistical analysis of the differences between the two determinations showed that they were not different at the 0.05 significance level.

In the plasma volumes, the first determination was 2692 with a standard deviation of 305; and the second determination, 2727 with a standard deviation of 406. Again, these determinations are not statistically different indicating that the method is reproducible.

A group of patients with no known cardiovascular abnormalities was also studied by a single determination of this method in order to evaluate our results in comparison to other authors. A summary of the data from this group appears in Table II. In evaluating these patients, the ratio of the isotope computed hematocrit to the peripheral hematocrit was examined. The ratio average was .894 with a standard deviation of 0.08. This value agrees with the finds of other authors (2, 7). Total measured blood volume was compared to blood volume estimated on the basis of height cubed, and weight, as reported by Nadler *et al* (7). The mean of the estimated blood volumes was 4,415 with a standard deviation of 546. The mean of the measured blood volumes in this group of patients was 4,166 with a standard deviation of 756. The differences between these two

TABLE II

SUMMARY OF RESULTS IN 41 PATIENTS WITH NO KNOWN CARDIOVASCULAR ABNORMALITIES

	Isotope Hematocrit / *Peripheral Hematocrit*	*Estimated Blood Volume*	*Measured Blood Volume*	*Per Cent Tagging of ^{51}Cr*
Mean	.894	4415	4166	92.4
Standard Deviation	.08	546	756	3.3
Number of Subjects	41	37	41	41

values of estimated and measured blood volume are not statistically significant at the 0.05 level.

Since the red cells were not washed to remove excess ^{51}Cr prior to injection, the per cent tagging of red cells was investigated. A per cent tagging mean of 92 with a standard deviation of 3.3 per cent was found. Approximately 8 per cent of the chromium is unbound after 15 minutes of tagging, and suitable corrections must be made when counting whole blood in order to obtain the amount of chromium bound to red cells.

SUMMARY

The simultaneous measurement of red cell mass and plasma volume is the best way to study both blood compartments under the same physiological conditions. ^{131}I and ^{51}Cr are difficult tracers to use because of their overlapping energy spectra.

We have successfully used ^{125}I labeled albumin and ^{51}Cr labeled red cells as tracers. A method for this measurement is presented along with results in 10 normal subjects and 41 patients.

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