MEASUREMENT OF BLOOD VOLUME USING RED CELLS LABELED WITH RADIOACTIVE CARBON MONOXIDE

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In the early 1940's, blood volume was measured by inhaling a gas mixture containing carbon monoxide (*1*). By measuring the concentration of carbon monoxide in the blood and by knowing the amount extracted from a closed-circuit breathing system, one can calculate the blood volume. A modification of this method, in which no blood samples are withdrawn, was described by Sjöstrand (*2*), but some of the assumptions on which his method is based have been questioned (*3*). It has been consistently demonstrated, however, that the dilution volume measured by inhaling carbon monoxide averages 12–16% more than the volume measured with cells labeled with radioactive phosphorus, chromium or iron (*4–7*).

Root and his co-workers (*8*) have shown that a similar difference is found with splenectomized dogs intravenously injected with red cells labeled *in vitro* with stable carbon monoxide. This work can be criticized, however, because the insensitivity of the chemical methods used means the amount of blood removed for labeling must range from 50 to 80 ml in dogs with blood volumes of between 200 and 350 ml. A second possible criticism is that a correction for the disappearance of the carbon monoxide label from the blood may not have been applied, although a disappearance rate of 36%/hr is quoted in the paper. Tobias *et al* (*9*), who used radioactive ^{11}C-carbon monoxide-labeled red cells to study the elimination of carbon monoxide from the body, have stressed the importance of this disappearance-rate measurement if reliable estimates of blood volume are to be made. If radioactive ^{11}C-carbon monoxide-labeled red cells are used to measure blood volume, the amount of labeled blood used is less than 5 ml in subjects with blood volumes of about 5,000 ml. The correction factor applied to account for the loss of ^{11}C activity from the blood can be determined easily.

The purpose of the present investigation was to obtain an accurate estimate of the clearance rate of ^{11}C-carbon monoxide from the blood and an accurate estimate of the difference in dilution space measured with ^{51}Cr-labeled red cells and ^{11}C-carbon monoxide-labeled red cells. As a further study of the possible usefulness of this method in practice, repeated measurements of blood volume using ^{11}C-carbon monoxide-labeled red cells were made in two volunteers.

METHODS

Production of ^{11}C-carbon monoxide. ^{11}C was produced by bombarding boron as boric oxide with 15-MeV deuterons from the Medical Research Council cyclotron (*10*). The nuclear reactions that occur are ^{10}B(d,n)^{11}C and ^{11}B(d,2n)^{11}C (*11,12*). Some ^{13}N is produced simultaneously by the ^{16}O(d,αn)^{13}N reaction. These reactions are the only ones that need to be considered for our purposes. The target vessel consisted essentially of a brass box containing a wedge that supports a thin layer of boric oxide (B_2O_3) on its serrated surface. The deuteron beam entered the box through a thin aluminum foil window. The radioactive products were swept out of the target vessel in a stream (50 ml/min) of carrier gas which consisted of 1% carbon monoxide in helium. The ^{11}C left the vessel only in the form of carbon monoxide or carbon dioxide, and the ^{13}N (about 16% of the total activity) left only as molecular nitrogen. The labeled carbon dioxide was reduced to monoxide by passing the gas over "active" carbon at 900°C; it then flowed through a soda-lime trap to remove any residual traces of carbon dioxide. The

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TABLE 1. DISAPPEARANCE RATE OF ^{11}C ACTIVITY FROM BLOOD

Patient	^{11}CO dilution space (ml)	Fractional loss rate (min^{-1})
AH	3,635 ± 120	0.00535 ± 0.00115
MF	3,776 ± 94	0.00530 ± 0.00095
EB	3,919 ± 157	0.00369 ± 0.00134
PC	1,917 ± 63	0.00433 ± 0.00122
AT	6,469 ± 115	0.00485 ± 0.00078
RE	4,638 ± 71	0.00304 ± 0.00046
JG	3,766 ± 127	0.00304 ± 0.00146
LC	3,045 ± 128	0.00564 ± 0.00158
MP	3,438 ± 113	0.00355 ± 0.00128
Weighted mean	—	0.00394 ± 0.00114
Average coefficient of variation (%)	3.03	—

composition of the final gas mixture used for labeling was checked with a gas chromatograph. Sterility was assured by Millipore filtration while the risk of possible pyrogen contamination was virtually eliminated by passing the gas through a small-bore silica tube at 300°C to denature any protein fragments. From this tube the gas flowed to waste through a small sterile vessel from which samples were withdrawn by piercing its soft rubber cap with a hypodermic needle.

Labeling procedures. To label the red cells with ^{11}C-carbon monoxide, 5 ml of venous blood were withdrawn into a 50-ml syringe containing 1.5 ml of acid citrate dextrose anticoagulant (ACD). Forty-five milliliters of ^{11}C-carbon monoxide plus helium carrier gas mixture were drawn into the syringe which was then rotated at 10 rpm for 10 min. The excess gas was expelled. Because of the high radioactivity per milliliter of blood achieved by this method (75 μCi/ml), there was adequate time to prepare standards and doses. The amount of ^{11}C-carbon monoxide contained in the plasma was less than 0.01%. Therefore it was not necessary to wash the plasma from the red cells after labeling.

To label the red cells with ^{11}C-carbon monoxide and ^{51}Cr, 10 ml of venous blood were withdrawn into a 50-ml syringe containing 1.5 ml of ACD. Less than 100 μCi of ^{51}Cr (as sodium chromate) was added to this syringe under sterile conditions. Forty milliliters of the ^{11}C-carbon monoxide gas mixture (containing 500 μCi of activity) was drawn into the syringe. The syringe was rotated for 10 min at 10 rpm. The excess gas was expelled, and the red cells were washed three times with isotonic saline (four centrifugations at 3,000 rpm) for 5 min each time. The amount of ^{51}Cr activity contained in the washing was checked. Virtually no activity (less than 0.01%) remained in the supernatant after the washing procedure. The washed labeled red cells were resuspended in saline up to the original volume of 10 ml.

RADIATION DOSIMETRY

^{11}C has a physical half-life of 20.34 min and emits positrons with a maximum energy of 0.97 MeV which yield 0.51-MeV annihilation photons. The k-factor (specific gamma-ray emission) is 5.8 R/hr/mCi at 1 cm. Assuming a body weight of 65 kg and a height of 160 cm, the average geometrical factor (*13*) is 126 and the geometrical factor at the center of the body is 180. Instantaneous mixing and uniform distribution within the body is assumed. The effective half-time clearance from the blood is 18 min since the biological half-life is 161 min (see below). The average beta dose is 0.0059 mrads/μCi and the gamma dose is 0.0049 mrads/μCi, giving a total dose of 0.011 mrads/μCi. An injected dose of 30 μCi gives a whole-body dose of 0.33 mrads. The total-body dose from an injection of 30 μCi of ^{51}Cr is 7.3 mrads.

RESULTS

Clearance of ^{11}C-carbon monoxide from the blood. One hundred microcuries of ^{11}C-carbon monoxide-labeled autologous red cells was administered to nine normal female patients, and five samples were taken at approximately 10-min intervals from 8 min up to 50 min after injection. The blood clearance rate was estimated by fitting a single exponential function to the data using the computer program devised by Marquardt (*14*). This program also yields estimates of the errors in the calculated parameters. The results are shown in Table 1. The average fractional loss rate is 0.0039 ± 0.0011 min^{-1} which corresponds to a clearance half-time of 176 min; i.e., the rate of disappearance from the blood is 25%/hr. This is identical with the value of 0.0039 previously reported by Pace *et al* (*15*) in five women following inhalation of stable carbon monoxide. Although these authors report a difference in carbon monoxide clearance rate between men and women, our own results on 11 male athletes (*16*) do not confirm this difference when a small quantity of ^{11}C-carbon monoxide-labeled cells is used.

Comparison of blood volume measured with ^{11}C-carbon monoxide- and ^{51}Cr-labeled red cells. Approximately 2 ml of the labeled red cells were taken up into each of two 5-ml syringes. Three syringes were necessary if two standards were to be used for an occasional check. Each syringe contained 30 μCi

of ^{11}C-carbon monoxide and 20 μCi of ^{51}Cr. One of the syringes was used to make up a standard by injecting the contents directly into a plastic vial containing 0.2 ml of saturated saponin solution and 0.5 ml of liquid paraffin. We used paraffin originally as a control to investigate whether any loss of ^{11}C-carbon monoxide occurred when the red cells were hemolyzed with saponin. No detectable loss occurred, but because the paraffin prevented the blood from being trapped near the stopper of the plastic vial during mixing, we continued to use it. The volume of the standard was made up to 5 ml with water. The contents of the remaining 5-ml syringe were injected intravenously into the patient and washed in by drawing back the venous blood four times. The residual activity in the syringe was less than 0.01% of the injected dose. The total time between removing the patient's blood and reinjecting the labeled blood was always less than 1 hr.

Venous blood samples were taken into plastic syringes containing three drops of heparin solution (5,000 units/ml) from a vein in the opposite arm approximately 8, 10 and 12 min after injection. These were immediately put into plastic counting vials containing 0.5 ml of saturated saponin solution and 0.5 ml of paraffin. The exact amount placed in each vial was not measured because the amount does not affect the ratio of the dilution volumes measured by the two isotopes and is only important if actual volumes are of interest. The samples and standard were then mixed on a rotary mixer for 10 min and counted after letting them stand for 5 min to allow the frothing caused by the saponin to subside. The ^{11}C activity relative to the standard was then calculated. Allowance was made for the decay of ^{11}C activity during the counting time. Using the value of the clearance rate obtained previously, the value of the dilution measured by each sample of ^{11}C-carbon monoxide was corrected to zero time from a knowledge of the exact time at which the sample was taken. The average value of the three samples corrected in this manner was calculated. Twenty-four hours later the ^{51}Cr content of the sample was measured. The difference between the dilution factors measured with ^{11}C-carbon monoxide and ^{51}Cr was expressed as a percentage of the factor measured with ^{51}Cr.

The results of measurements on 21 normal female patients is shown in Table 2. It can be seen that the average value of the dilution space measured with ^{11}C-carbon monoxide is 6.2% larger than that measured with ^{51}Cr, but the variation in this difference—although large—is within useful limits.

Measured accuracy of repeated blood volumes using ^{11}C-carbon monoxide. The relative activities of two syringes containing approximately 2 ml of labeled blood were measured by supporting each syringe over a well counter in a fixed mechanical jig. The contents of one syringe were reinjected into the patient by washing four times with venous blood and the contents of the other syringe were injected into a plastic counting vial containing 0.2 ml of saponin solution. The volume was made up to nearly 5 ml by washing the activity remaining in the syringe into the counting vial and making the final volume up to a mark on the vial. Blood samples were taken at 8, 10 and 12 min. Exactly 5 ml of each sample were pipetted directly into a counting vial containing 0.2 ml of saponin solution. The standard and samples were mixed for 10 min and counted after allowing them to stand for 5 min. The total time between taking the blood and reinjecting it into the patient in this procedure was less than 30 min. The same procedure was repeated for the second and third measurements with intervals of about 90 min between measurements. The activity remaining in the blood from the previous measurement was assessed and was found in all cases to be negligible compared with activity due to the later injection. The values obtained were corrected to zero time using the value for clearance rate determined when the measurement was carried out for the third time and five instead of three samples were taken. The results are shown in Table 3. The measured coefficient of variation is 3.88% in one case and 1.48% in the other.

TABLE 2. COMPARISON OF BLOOD VOLUME MEASURED WITH ^{11}CO- AND ^{51}Cr-LABELED CELLS

Patient	$\left(\frac{^{11}CO \text{ dilution factor}}{^{51}Cr \text{ dilution factor}} - 1\right) \times 100$
AB	+ 4.40
EG	+ 9.41
LC	+15.24
GJ	+13.00
JW	+ 0.00
MK	+ 1.95
RD	+ 4.43
FG	+13.79
JP	+ 5.03
BT	+ 2.62
PB	+ 4.89
EH	+ 3.38
JC	+ 6.43
PC	+ 0.00
AP	+ 8.14
DJ	+ 3.50
JB	+17.26
MC	+ 3.04
MP	+ 3.74
RP	+ 3.35
AMcH	+ 6.81
Average	6.21
Standard deviation	± 4.89

TABLE 3. REPEATED ESTIMATION OF ^{11}CO BLOOD VOLUME IN TWO PATIENTS

	^{11}CO blood volume (ml)	
	Patient 1	Patient 2
1st estimate (3 samples)	3,701	3,043
2nd estimate (3 samples)	3,501	3,153
3rd estimate (5 samples)	3,386	3,131
Weighted mean	3,503	3,113
Standard deviation	± 136	± 46
Coefficient of variation (%)	3.88	1.48

DISCUSSION

The results in Table 1 indicate that if samples are taken approximately 10 min after injection and if no correction is made for the clearance of ^{11}C-carbon monoxide from the blood, the estimated carbon monoxide dilution space will be approximately 4% too large. Unless extreme accuracy is required, it appears to be unnecessary to carry out blood-clearance estimations on individual patients. Instead it is sufficient to apply the average correction. This has been confirmed by subsequent studies (*16*).

Despite the fact that the ^{11}C-carbon monoxide was administered labeled to red cells, the volume measured is greater than that measured with ^{51}Cr-labeled cells (Table 2). Roughton and Root (*17*) have suggested that the rate of transfer of carbon monoxide from red cells to myoglobin is far faster than is generally supposed, and they have calculated a reaction half-time of 10 sec. The relative amounts of myoglobin to hemoglobin in the body have been estimated to be 15% (*18*), 6.2% (*19*) and 4.4% (*20*). The proportion of carbon monoxide combined with the myoglobin in man has been estimated at about 5% of that combined with hemoglobin (*21*).

The result obtained by us (Table 2) appears to suggest that with ^{11}C-carbon monoxide-labeled red cells the total hemoglobin and myoglobin space in the body is being measured, and the difference of 6.2% between ^{11}C-carbon monoxide- and ^{51}Cr-measured volumes is close to the expected difference of approximately 5%.

It is not easy to account for the larger difference of 12–16% noted by other workers (*4–7*) although part of this may be due to the failure of some previous workers in some cases to correct for the disappearance of carbon monoxide from the blood.

If it is considered necessary to correct the volume measured with ^{11}C-carbon monoxide by the 6.2% factor to obtain a red cell equivalent volume, then, assuming that the error in estimating the dilution space is 3.03% (see below), the error in the blood-volume estimate will increase to ±5.2%. It should be pointed out that the ^{11}C-carbon monoxide dilution spaces measured in this comparison test were all calculated using the average fractional loss-rate correction determined previously. In many clinical situations it is change of blood volume that is important within individual patients, and it is therefore probably not always necessary to apply the 6.2% correction factor with its associated error.

The average coefficient of variation in the method determined by repeated measurements on two patients is 2.68%. This can be compared with an error of ±3.8% claimed for the modified closed-circuit breathing method of Sjöstrand (*22*). The computer program used to fit the five-sample data provides an independent estimate of the accuracy of the method by estimating the error in the intercept. The average coefficient of variation in the measured dilution volume estimated by this method in nine patients is 3.0% (Table 1).

In this investigation whole-blood samples were counted and the hematocrits of the samples were not used to obtain red-cell volume or plasma volume allowing for the difference between venous and whole-body hematocrit. The estimate of hematocrit might itself be expected to be subject to a ±2% error.

In estimating the $^{51}Cr/^{11}C$-carbon monoxide ratio, several simplifying and time-saving procedures were introduced. Labeling was carried out by gentle rotation of the syringe for 5–10 min instead of the more usual 40–60 min. No hemolysis was measurable after labeling by this technique. No washing is necessary when ^{11}C only is used for labeling the red cells and the blood is reinjected into the patient within 30 min after the sample is taken. This insures that the blood is outside the body for only a short time and reduces the likelihood of the red cells becoming damaged. By using counting equipment with a short resolution time (0.7 μsec) and by delaying the time at which counting is carried out, the entire standard can be counted directly. This eliminates the need to make up a separate standard in a dilute form and to count an aliquot of this new standard. It also avoids the additional errors involved in the volumetric manipulations associated with a secondary standard.

CONCLUSIONS

If a cyclotron is available on site, ^{11}C-carbon monoxide-labeled red cells offer a convenient and accurate way of measuring the carbon monoxide dilution space in the body. If a correction is applied, the red cell equivalent volume can be estimated—but this correction introduces an additional error. Using the average clearance rate calculated on a group of nine

normal female patients instead of measuring individual clearance rates does not appear to add significantly to the error. It is probably desirable to measure individual clearances if the greatest possible accuracy is required. The blood volume can therefore be estimated by taking two samples at known times after injection, having allowed approximately 10 min for adequate mixing to take place. The accuracy of the method determined by repeated estimates in two patients is $\pm 2.7\%$, which compares well with the computer-derived estimate of $\pm 3.0\%$ based on measurements on nine patients. For multiple studies and in situations in which it is especially desirable to use minimal radiation doses (such as in pregnancy and in measurements on children), the ^{11}C-carbon monoxide method is of particular value.

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