TRIPLE-TRACER TECHNIQUE FOR MEASURING RED-BLOOD-CELL, PLASMA AND EXTRACELLULAR-FLUID VOLUME

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The measurement of body-fluid parameters is based on the dilution principle using appropriate tracers that are distributed and diluted in the various fluid compartments. The standard techniques for measuring the two components that constitute blood, red-cell and plasma volume use single tracers (*1-4*).

To simplify the technique for measuring blood volume further and to eliminate variables such as the red-cell distribution and F_{cell} ratio which may arise when a single tracer is used, we have developed a dual-tracer technique in which both red-cell and plasma volume are measured simultaneously on whole-blood samples (*5*). In this technique each component is measured by diluting an appropriate tracer—red-cell volume with ^{51}Cr-labeled red cells and plasma volume with ^{125}I-labeled albumin. The concentration of two tracers in whole blood is measured simultaneously in a dual-scaler analyzer (*6*).

The extracellular fluid compartment (ECF) is of particular interest because it comprises a relatively large volume of fluid (about 20% of body weight). The major portion of this fluid volume (15% of body weight) is found in the interstitial space where it acts as a buffer zone between the intravascular and intracellular space and represents a readily available pool of fluid.

Tracers used to measure ECF volume must meet three basic requirements: (1) The tracer should diffuse freely across the capillary membrane; (2) it should not penetrate the cellular membrane barrier; and (3) it should have a relatively slow rate of elimination from the system. Several tracers have been used for this purpose (*7-9*). Radiobromine ^{82}Br (*10, 11*), a gamma-emitting nuclide now being used, has several shortcomings: it has a short half-life (36 hr), it penetrates the cellular compartment to a limited extent and it is excreted through the gastrointestinal tract.

On the other hand, radiosulfate ^{35}S, a weak-beta-emitting nuclide (0.167 Mev), has a reasonably long half-life (87.2 days) and seems to meet most of the requirements for measuring ECF volume. The measurement of dilution volume expressed as sulfate space approximates the actual ECF volume. The only limitations for the widespread use of sulfate as a measure of ECF volume are the technical preparations of liquid samples for radioanalysis.

To remove the need of using weak-beta-emitting nuclides we introduced ^{75}Se, a multi-gamma-emitting nuclide with biologic properties closely resembling sulfur, in an earlier report (*12*). Values obtained for ECF volume measured as a selenate space closely approximated ECF volume measured with sulfate.

Further studies with selenate in humans substantiated observations reported by Nelp (*13*) and others (*14, 15*) that ^{75}Se tends to reappear protein-bound in plasma. In normal individuals the rate at which ^{75}Se reappears protein-bound in plasma is slow; for example, about 10% of the tracer is protein-bound in 40–60 min. At this rate the amount of protein-bound ^{75}Se does not significantly alter the ECF volume. Nevertheless, in several cases and in one particular patient, a young adult following a total colectomy, 90% of the ^{75}Se administered appeared to be protein-bound within 10 min. From experience gathered in pathological conditions, the rate at which ^{75}Se reappears protein-bound in plasma seems to be unpredictable. Attempts to block this protein-binding process of ^{75}Se by pretreating animals with "cold" selenium (10–20 μg/kg) (*16, 17*) or with sodium sulfate did not seem to alter the rate of reappearance of ^{75}Se protein-bound in plasma. We conclude at this time that ^{75}Se as selenate is a poor substitute for sulfate for measuring ECF volume.

In view of these findings we redirected our efforts to modify and simplify methods for radioanalysis of ^{35}S. To analyze plasma filtrate containing ^{35}S, we developed an anthracene cell placed in contact with a photomultiplier tube which is connected to a scaler (*18*). In addition, we developed a practical pro-

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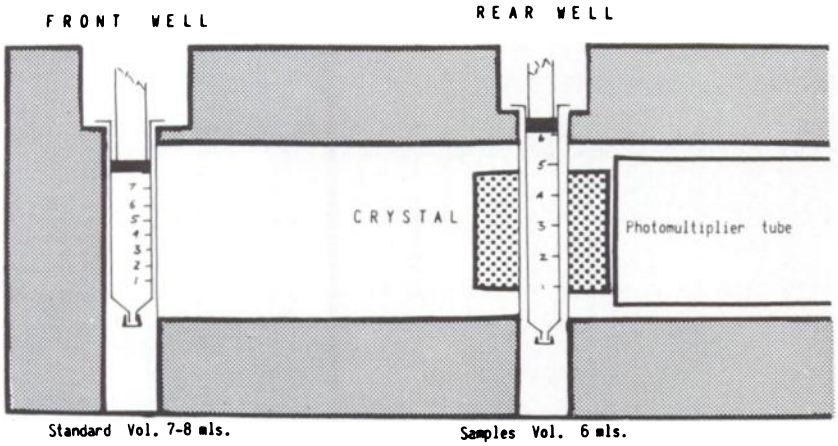

FIG. 1. Modified scintillation well counter used for measuring ^{51}Cr and ^{125}I.

cedure for measuring red-cell, plasma and ECF volume simultaneously using the three radioactive tracers ^{51}Cr, ^{125}I and ^{35}S as sulfate, and we present the results in this report.

PROCEDURE

Red-cell and plasma volume are measured simultaneously using two tracers: ^{51}Cr-labeled red blood cells for red-cell volume and ^{125}I-labeled albumin for plasma volume. Both tracers are mixed together in a single plastic syringe and administered into an accessible vein. Immediately after administration, a premeasured volume of standard containing a known quantity of ^{35}S (sulfate) is injected. The administered activity of the ^{51}Cr-labeled red cells is approximately 0.3 μc/kg body weight; that of ^{125}I-labeled protein is about 0.03 μc/kg body weight and that of ^{35}S-labeled sodium sulfate is 1.0 μc/kg body weight. Blood samples are drawn at 10, 20, 40, 60 or 80-min intervals in heparinized syringes. Radioanalysis is followed in two separate counting systems: a scintillation-detector system for the gamma-emitting ^{51}Cr and ^{125}I and the anthracene-cell system for the weak-beta-emitting ^{35}S.

Counting ^{51}Cr and ^{125}I. ^{51}Cr and ^{125}I are analyzed simultaneously in a dual-scaler analyzer and a modified scintillation counter (Picker-Nuclear Twinscaler II) (Fig. 1). The counter has two positions for counting liquid samples in plastic syringes. The front position, facing the 2-in. NaI(Tl) crystal, is for measuring the standard. In this position the total volume of standard to be administered (7–8 ml) is exposed to the crystal. The rear position, with an opening through the crystal, is used for counting pre- and post-mix blood samples. The samples are measured accurately to the 6-ml mark in plastic syringes. The dual-scaler analyzer is standardized so that scaler A with analyzer A is set for counting ^{51}Cr and scaler B with analyzer B is set to measure ^{125}I*. We obtain less than 4% spillover of ^{51}Cr counting rate on the ^{125}I level. Since both tracers are diluted by approximately the same amount, the error introduced by this low rate of spillover is negligible. We estimate that an error of 40 ml can be expected if the dilution volumes of ^{51}Cr cells and ^{125}I albumin differ by 1,000 ml.

Measuring ^{125}I together with ^{51}Cr presents certain problems of coincidence, summation effect and apparent spectral changes when geometry is changed and samples are moved from the front to the rear position. To overcome these difficulties, it is important to establish beforehand a geometric ratio value between the front and rear well position for the two tracers when they are used together. To do this we perform accurate *in vitro* volumetric measurements using approximately the same proportion of ^{125}I and ^{51}Cr that is normally injected to measure blood volume *in vivo*. The 7–8-ml standard containing both ^{51}Cr and ^{125}I is drawn into a 10-ml plastic syringe which is placed in the front position of the counter and analyzed with the dual-scaler analyzer. The standard is then mixed with an accurately measured 4,000-ml volume of water. A 6-ml dilution sample is drawn into a 6-ml plastic syringe that fits the rear well, and the concentration of the tracers is established after dilution. The front-to-rear ratio (F/R) is calculated separately for each tracer from the counting-rate values obtained on each channel of the scaler (scaler A set for ^{51}Cr and scaler B set for ^{125}I) according to the following equation:

$$\text{Ratio (F/R)} = \frac{4{,}000 \times (\text{Ct. of dilution sample} - \text{Bkgd.})}{\text{Count of standard} - \text{Residue in syringe}}$$

The final ratio for each tracer is established on an

* ^{51}Cr settings: gain 1, lower level 300, window 40; ^{125}I settings: gain 0.25, lower level 126, window 20.

average of 5–10 *in vitro* experiments. Although the F/R ratio for each tracer is stable, it should be checked once a week.

Counting ^{35}S. The weak beta emitter ^{35}S must be measured in a separate detector system. Plasma filtrate containing ^{35}S is prepared as follows. Plasma is separated from red blood cells by centrifugation and the plasma proteins are precipitated with 20% trichloracetic acid (TCA) in a 1:1 ratio. The mixture is shaken and centrifuged. The supernatant fluid, designated as plasma protein-free filtrate, is removed and checked for free ^{51}Cr and ^{125}I by placing the filtrate in the scintillation-counting system. Plasma-filtrate samples are then introduced into the specially designed anthracene cell which is placed in contact with a photomultiplier tube connected to a scaler. The counting rate of ^{35}S in the sample is determined, and the concentration of ^{35}S in the plasma filtrate is obtained from the concentration curve established by plotting known concentrations of ^{35}S as functions of counting rate (*18*).

CALCULATIONS

The equations we used to determine red-cell, plasma and ECF volume are:

$$\text{Red cell volume (Scaler A cts.)} = \frac{(\text{Ct. of standard} - \text{Residue}) \times F/R \times \text{Hct\%}}{\text{Net count of sample}}$$

$$\text{Plasma volume (Scaler B cts.)} = \frac{(\text{Ct. of standard} - \text{Residue}) \times F/R \times 1 - \text{Hct\%}}{\text{Zero-time extrapolated net count of 2 samples*}}$$

$$\text{ECF volume} = \frac{\text{Standard injected } (\mu c) \times 0.87}{\text{Concentration of filtrate } (\mu c/\text{liter}) \times 2}$$

The concentration of ^{35}S in plasma is obtained by multiplying the concentration of ^{35}S in the filtrate by 2 since the filtrate is a 1:1 dilution and is corrected for one-half the plasma-protein content and the Gibbs-Donnan effect. The product of these two correction factors is 0.87. No corrections are made for the rate of elimination of ^{35}S because this is taken into account when the concentration of ^{35}S in the samples is extrapolated to zero time on a semilog scale when the dilution curve reaches equilibration.

PREPARING THE STANDARD

To prepare the standard we made a suspension of washed red blood cells labeled with ^{51}Cr in 5–6-ml normal saline to which 1–2 ml of ^{125}I albumin is added. We then place the total volume of the injectable standard (7–8 ml) in a 10-ml plastic syringe for radioanalysis.

After a sterile stock solution containing 10 μc of ^{35}S per ml is prepared, multiples of 1 ml are drawn accurately into calibrated glass syringes for injection. Because ^{35}S has a long half-life (87.2 days), the standard is considered to contain 10 μc/ml for the 2–3 weeks the solution is available for use. Three dilutions of 2, 5 and either 7 or 8 μc/liter are prepared from the stock sterile standard in 100-ml glass stoppered volumetric flasks for calibrating the anthracene cell. These test dilutions are used to standardize the cell while the injectable sterile stock solution is available.

The cell is calibrated from the counting rate obtained on each of the test dilutions. The standardization curve for the cell is obtained by plotting the net counting rate as a function of concentration in μc/liter. The concentration of ^{35}S in μc/liter in plasma filtrates is obtained from the standardization curve and the counting rate of the samples analyzed. Figure 2 outlines the steps involved in determining red-cell, plasma and extracellular-fluid volume.

Figure 3 shows the rate at which ^{35}S equilibrates in plasma and compares this (Curve A) with the concentration of the tracer in plasma filtrate (Curve B). There is an initial fall in concentration in the first 20–30 min. The curve does not flatten at the 20-min time interval but seems to decay gradually at a slow rate. The variation in decay rate is due to the rate of equilibration of the tracer across

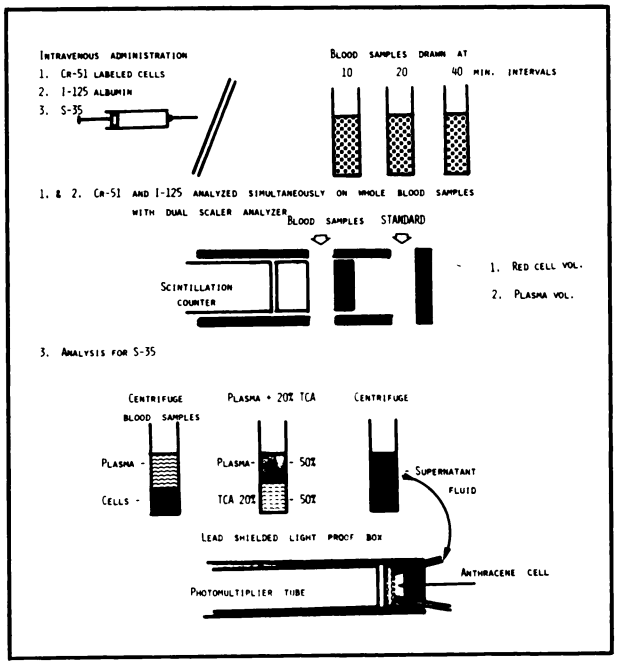

FIG. 2. Various steps involved in determining red-cell, plasma and extracellular fluid volume using triple-tracer method.

* 10 and 20-min sample counts extrapolated to zero time.

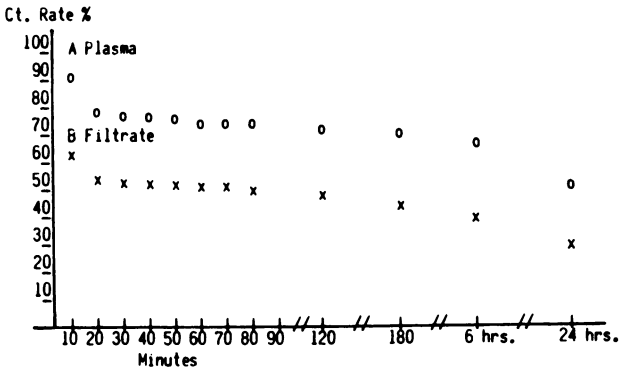

FIG. 3. Dilution curves comparing the rate at which ^{35}S equilibrates in plasma and plasma filtrates over a 24-hr period.

the capillary membrane with the interstitial-fluid volume and the rate of elimination of the sulfate anion by the kidneys. To obtain equilibrated values, we often use the 40–60 or 40–80-min sample concentration in our extrapolation to zero-time concentration instead of the 20–40-min concentration.

In Curve B the concentration of ^{35}S in plasma filtrate is protein-free in 1:1 dilution with 20% TCA. The two curves for plasma and plasma filtrate run parallel for 24 hr. There seems to be no protein binding of sulfur. The proportionally higher counting rate encountered in the filtrate is due to the light-quenching produced by plasma discoloration.

Table 1 shows duplicate measurements of red-cell, plasma and ECF volume in five adult males while Table 2 lists duplicate measurements in dogs. The average values closely approximate the figures obtained by Walser *et al* (*7*), and the average values for ECF in humans is similar to the figures obtained by Savoie *et al* (*19*).

DISCUSSION

To understand better the dynamics of shifts in fluid and electrolytes between the intravascular and extravascular compartment, one must measure the available ECF volume. The value of measuring extracellular fluid volume can only be established if a practical and relatively rapid technique is available for routine use in conjunction with blood-volume measurements. We believe that the triple-tracer technique described in this report is a reliable and accurate measure of the two readily accessible fluid compartments, ECF and intravascular-fluid volume.

The distribution of ^{35}S as sulfate seems to be a representative measure of the ECF volume. The only drawback is the tedious and time-consuming procedure for radioanalysis of weak-beta-emitting radioisotopes. The complexities of the procedure are considerably simplified by using the anthracene-cell counter we have developed.

Whenever the dilution principle is used for measurements, it is important to follow the rate of equilibration of the tracer with the volume of fluid being measured by taking multiple blood samples (*20*). For practical purposes, 10, 20, 40, 60 or 80-min sampling is adequate. In hypotensive conditions and in shock, however, the rate of equilibration is slow, and samples must be drawn over a longer period of time. On the other hand, the rate of transfer of the tracer across the capillary membrane depends on the available area of the capillary bed and the state of the capillary membrane. We observed that equilibration of ^{35}S with the interstitial-fluid volume is frequently delayed in apprehensive patients and in those with a marked element of vasoconstriction. In studying ECF one can infer that it is important in each case to obtain and analyze multiple samples to establish an equilibration curve.

At this time it is worth noting that although blood-volume measurements may be within normal limits, the interstitial-fluid volume may be contracted. Under these conditions, the rate of hemodilution is slow following blood loss and is related directly to the amount of the available interstitial-fluid volume.

TABLE 1. HUMAN STUDIES

Weight (kg)	Red cell volume		Plasma volume		Total blood volume		Corr. Hct. (%)		ECF (ml)		ECF (ml %)	
	C	R	C	R	C	R	C	R	C	R	C	R
110	2,935	2,842	4,572	4,594	7,507	7,436	43.0	42.1	19,818	19,593	18.0	17.8
98	2,396	2,257	4,198	4,028	6,594	6,285	41.3	39.9	18,253	18,634	18.6	19.0
86	2,258	2,207	3,425	3,789	5,683	5,996	42.6	40.4	18,071	17,626	21.0	20.5
71	1,907	1,841	3,346	3,132	5,253	4,973	40.8	41.7	13,629	13,426	19.2	18.9
58	1,500	1,510	2,489	2,438	3,989	3,948	41.3	41.3	11,357	11,146	19.6	19.2
	Average: 25.6 ml/kg		Average: 42.6 ml/kg		Average: 68.2 ml/kg						Average: 19.18%	

C = Control study, first determination; R = Repeat study, second determination.

TABLE 2. STUDIES IN DOGS

Dog No.	Weight (kg)	Red cell volume		Plasma volume		Total blood volume		Corr. Hct. (%)		ECF (ml)		ECF* (ml %)		ECF† (ml %)	
		C	R	C	R	C	R	C	R	C	R	C	R	C	R
1	16.2	478	426	705	665	1,183	1,091	43.5	43.0	3,213	3,188	19.8	19.7	21.2	20.4
2	14.0	415	370	621	587	1,036	957	43.5	42.6	2,859	2,726	20.4	19.5	19.5	18.5
3	17.7	512	472	808	788	1,320	1,260	42.6	41.3	3,487	3,535	19.7	20.0	20.5	21.2
4	12.2	377	322	538	502	915	824	43.9	42.6	2,547	2,461	20.9	20.2	21.0	22.0
5	14.2	427	381	640	594	1,067	975	43.5	43.0	2,600	2,550	18.3	18.0	18.6	19.0
6	11.7	355	303	535	489	890	792	44.4	42.1	2,311	2,355	19.8	20.1	21.0	20.6
7	13.6	385	345	621	563	1,006	908	41.7	40.4	2,450	2,475	18.0	18.2	19.5	21.0
8	15.0	426	381	649	619	1,075	1,000	43.0	40.4	2,752	2,683	18.3	17.9	18.0	19.5
9	10.5	301	256	501	461	802	717	41.3	39.5	2,140	2,061	20.4	19.6	20.1	21.0
10	9.2	272	237	426	420	698	657	43.5	41.7	1,751	1,709	19.0	18.6	21.0	21.4
		Average: 29.4 ml/kg		Average: 45.0 ml/kg		Average: 74.4 ml/kg						Average: 19.5%			

C = Control study, first determination; R = Repeat study, second determination.
* Calculated according to formula reported in this report—extrapolated values.
† Calculated on the 20-min sample according to the formula suggested by Walser et al. (21).

SUMMARY

A practical method for measuring red-cell, plasma and extracellular-fluid volume using three tracers, ^{51}Cr, ^{125}I and ^{35}S as sulfate, is described. An anthracene cell has been designed which greatly simplifies measuring ^{35}S in liquid samples. Measurements obtained in humans and dogs are presented.

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