Simultaneous Measurement of Erythrocyte, Plasma and Extracellular Fluid Volumes with Radioactive Tracers

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The two components of blood were measured simultaneously with two gamma-emitting radioactive tracers—chromium-51-labeled cells for measuring erythrocyte volume and iodine-125-labeled albumin for measuring plasma volume. Extracellular fluid volume (ECF) was determined as a sulfate space utilizing sodium radiosulfate, a weak beta-emitting nuclide. A specially-designed anthracene cell attached to a photomultiplier scaler system was developed for radioanalysis of sulfur-35. Calculations for the three parameters were made according to the dilution principle.

Preferably, expected values for blood volume should be based on height and weight. Erythrocyte and plasma volumes were calculated on a 40 per cent whole body hematocrit ratio. When measured, ECF values varied considerably from expected values based on 20 per cent body weight. There was closer agreement between measured values and expected values determined as a function of plasma volume.

The distribution of isotonic crystalloid solution between the intravascular and interstitial compartments followed the normal distribution pattern of 1:3. Six per cent dextran 75 in normal saline solution was retained essentially in the intravascular compartment. Restricting fluid intake for 12–18 hours prior to surgery resulted in loss of fluid. This deficit in fluid volume may play an important role in transcapillary filling of the vascular bed in the event of blood loss.

IN MAN the extracellular fluid (ECF) volume constitutes approximately a third of the water content of the body, or 20 per cent of body weight. This fluid volume is distributed in two major compartments, interstitial (IF) and intravascular (IV). The volume of fluid in the two compartments are in equilibrium as a result of fluid and electrolyte exchange across the capillary membrane, with a distribution ratio of approximately 3:1 IF:IV in males and 3.5:1 in females.[1, 2, 3]

In recent years there has been great interest in estimating ECF volume in disease states, to determine the distribution of fluid between the IV and IF compartments and the relationship of ECF to maintenance of circulatory homeostasis.[4–8] The dilution method of measuring the two compartments of blood, plasma and erythrocytes is well established. Each compartment can be determined separately with an appropriate tracer.[9–12] Measuring ECF volume by the same technique presents problems in the choice of an appropriate tracer which will equilibrate in the ECF and remain within the confines of the ECF space for a reasonable period of time. The distribution of sulfate anions with the IF volume and their equilibration provide a measurement of a fluid volume closely approximating the actual ECF volume: the sulfate space.[8, 13, 14, 15] The method previously described,[12] utilizing three radioactive tracers to determine plasma, erythrocyte and ECF (sulfate) volumes, has been modified and simplified. (Iodine-125-labeled albumin (RIHSA) is used to measure plasma volume, chromium-51-labeled erythrocytes to measure erythrocyte volume, and sodium sulfate, ^{35}S, to measure ECF volume.) In this report the changes in technique are described. The rate of equilibration of sulfate anions in man, normal values for ECF volume as a sulfate space, and the distribution of fluids administered intravenously in the two compartments that constitute ECF volume are determined by this method.

Procedure

Plasma, erythrocyte, and extracellular fluid volumes of normal healthy volunteers and healthy patients admitted for minor surgical

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procedures, ingrown toenail, cysts, removal of scars, etc., were estimated. All volunteers and patients were informed of the procedure, and consent was obtained for administration of radioactive tracers.

Control studies were conducted while the subjects were at rest, after they had fasted for six to eight hours. Age, sex, weight, height and body build were noted. The subjects were asked to void before experimental measurements were made and again one hour later when the last blood sample had been drawn, and urinary output was measured.

^{125}I-labeled albumin, ^{51}Cr-labeled erythrocytes, and ^{35}S-labeled sodium sulfate were administered simultaneously intravenously. Four blood samples from each subject were drawn into 10-ml. heparinized syringes: a control sample (pre-mix) and three samples (post-mix) 15, 30, and 60 minutes, respectively, after administration of the tracers.

Analysis

The concentrations of ^{125}I and ^{51}Cr, gamma-emitting radioactive tracers, in the standard solution administered intravenously and in pre-mix, 15-minute and 30-minute post-mix samples were measured in a modified well-type scintillating counter (Omniwell),* attached to a dual-channel scaler analyzer. Each channel of the analyzer was pulse-heighted to detect one of the two tracers.

* Picker–Nuclear.

Hematocrit determinations of all blood samples were performed in duplicate by the micro-hematocrit technique, and the values corrected for trapped plasma.[16, 17] Plasma protein was measured by refractometry.

Sulfur-35, a weak beta-emitting radioactive tracer, was analyzed in clear solution, protein-free, by filling a specially-designed anthracene cell attached to a photomultiplier tube and scaler system.[18] The construction of the anthracene cell was modified and its efficiency greatly improved. Plasma removed from the three blood samples pre-mix, 30 and 60 minute post-mix, was treated with 50 per cent trichloroacetic acid (TCA), two parts plasma to one part 50 per cent TCA. The samples were centrifuged and the supernatant solution analyzed. A sample of the ^{35}S standard solution was prepared in 1:2000 dilution in banked plasma,† treated with 50 per cent TCA, two parts of the diluted standard solution to one part TCA, and prepared for analysis.

One-hour urinary output was measured volumetrically and a 1:50 dilution in distilled water was prepared. Two parts of the 1:50 dilution of urine were mixed with one part 50 per cent TCA and the mixture was analyzed in the anthracene cell to determine the amount of ^{35}S excreted during the one-hour period.

† Albumin was added to obtain a protein concentration of approximately 6.5 to 7 Gm./100 ml.

Calculations

$$\textit{Red cell volume} = \frac{[\text{Ct. } ^{51}\text{Cr std. injected} - \text{residue}] \times \text{Hct\%}}{\text{Net Ct. of equilibrated sample}} \quad (1)$$

$$\textit{Plasma volume} = \frac{[\text{Ct. } ^{125}\text{I std. injected} - \text{residue}] \times (1 - \text{Hct\%})}{\text{Net Ct. of } ^{125}\text{I in blood at zero time}} \quad (2)$$

$$\textit{Immediately-available ECF} = \frac{\text{Net Ct. } ^{35}\text{S std.} \times 2{,}000 \times \text{vol.} \times 0.91}{\text{Net zero time } ^{35}\text{S concentration in protein-free plasma}} \quad (3.1)$$

Net Ct. std. ^{35}S = Count of standard minus atmospheric background; 2,000 = Dilution prepared for analysis; Vol. = Volume of standard containing ^{35}S injected; 0.91 = Gibbs-Donnan factor; Zero time concentration = Value obtained by extrapolating the net concentration of ^{35}S in the 30- and 60-minute protein-free plasma samples.

$$\textit{Total ECF (60 min. ECF)} = \frac{(\text{Net Ct. } ^{35}\text{S std.} \times 2{,}000 \times \text{vol.}) - ^{35}\text{S excreted}}{\text{Net ct. 60 min. protein-free plasma sample}} \times 0.91 \quad (3.2)$$

$$^{35}S \textit{ Excreted in Urine} = \text{Net ct. urine sample} \times 50 \times \text{vol. urine output during 60 min.} \quad (3.3)$$

$$\textit{Interstitial fluid} = \text{ECF} - [\text{Plasma volume} \times (1 - \text{protein})] \quad (4)$$

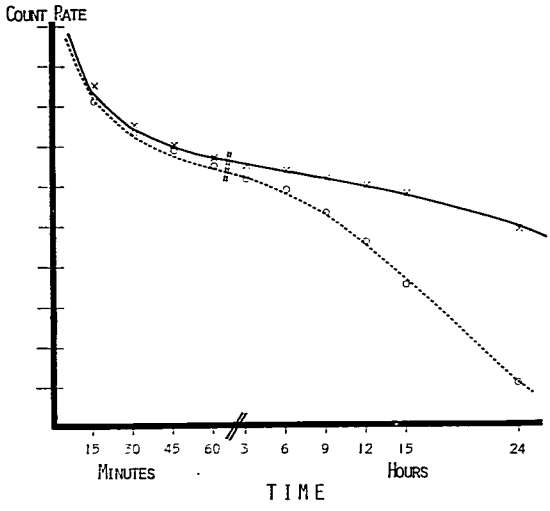

FIG. 1. Rate of decay of ^{35}S in plasma of dog and man. In dogs the rate is slow. In humans it varies with renal function and elimination proceeds at a more rapid rate. ——— = dog; - - - - - = man.

Expected values for blood volume were determined according to the Hidalgo *et al.* equation, based on height and weight.[19, 20, 21] Expected plasma and erythrocyte volumes were calculated from the expected blood volume and a 40 per cent body hematocrit. The expected ECF (sulfate) volume was calculated as 20 per cent of body weight and also as a ratio of plasma volume.

Results

Figure 1 shows the rate of equilibration of radiosulfate anions in plasma water and elimination from the blood stream in ten human subjects and 20 anesthetized dogs. In man the concentration of radiosulfate anions in plasma water began to fall exponentially within 20 to 30 minutes. In anesthetized dogs equilibration was more rapid and the rate of elimination slower.

Table 1 shows the distribution of fluid between the interstitial and intravascular compartments in ten female subjects; table 2 shows the distribution in ten male subjects. From tables 1 and 2, it can be seen that the measured ECF is 4.44 times the plasma volume in women and 3.992 times the plasma volume in men.

Normal Values for ECF (Sulfate) Volume

Tables 3 and 4 show the ECF volumes in the control group, estimated from tables 1 and 2, compared with the expected ECF volumes.

Comparing the measured values for ECF (sulfate) with the expected ECF volumes (tables 3 and 4), it can be seen that when the expected values are calculated as 20 per cent of body weight, the standard error is higher, 0.5589 in males and 0.5949 in females, than when ECF (sulfate) is compared to expected ECF values calculated as a ratio of plasma volume, standard error 0.2004 in males and 0.2285 in females.

Distribution of Ringer's Solution and 6 Per Cent Dextran 75 in Saline in the ECF (Sulfate) Space

Four men and two women in the control series received 1,000 ml. of Ringer's solution. A second group, three men and two women, received 500 ml. of 6 per cent dextran 75 in

normal saline solution over a period of 30 minutes. Repeat studies were performed 30 minutes after termination of the infusion. Table 5 shows the resulting distribution of Ringer's solution in the ECF compartment. Ringer's solution tended to be distributed between the IF and IV compartments according to the pre-infusion ratio of IF and IV volumes.

Table 6 shows that 6 per cent dextran 75 in normal saline solution remained essentially in the intravascular compartment, with a small additional proportion of fluid drawn from the interstitial fluid compartment by transcapillary filling.

Effect on the ECF Volume of Fasting for 12–18 Hours

Five male and five female subjects received no food or water by mouth for 12–18 hours. Table 7 shows the resulting deficits in ECF volume, averaging 1.5 liters in both groups.

Discussion

When *in vivo* fluid volumes are measured indirectly by the dilution principle, the accuracy of the measurements depends to a great extent on uniform distribution of the tracer substance within the volume of fluid being measured. Another important consideration is that during the mixing period the tracer should remain within the confines of the space being measured. This is particularly important when fluid in more than one compartment is being measured and the spaces are separated by a semipermeable membrane. When measuring blood volume and ECF volume it is important to determine the appropriate time for blood sampling that would be representative of the

Table 1. Normal Distribution of Fluids in the ECF Compartments in Women

Age	Expected PV (ml.)	Measured PV (ml.)	Plasma Water PV × (1-Protein) (ml.)	IF (ECF—Plasma Water) (ml.)	Measured ECF/PV	(%) PV/IF
38	2,640	2,270	2,134	7,866	4.405	27.13
23	2,160	2,340	2,200	8,100	4.401	27.16
24	2,885	3,125	2,937	10,763	4.384	27.29
37	2,340	2,330	2,190	7,810	4.421	28.04
45	1,815	1,892	1,778	6,622	4.440	26.85
32	2,385	2,167	2,037	7,563	4.430	26.93
36	3,545	3,877	3,644	14,356	4.642	25.38
40	1,815	2,155	2,126	7,674	4.500	26.40
26	2,270	2,222	2,087	7,713	4.411	27.06
41	2,225	2,298	2,160	8,040	4.380	26.86
Average:					4.441	26.91

Table 2. Normal Distribution of Fluids in the ECF Comparments in Men

Age	Expected PV (ml.)	Measured PV (ml.)	Plasma Water PV × (1-Protein) (ml.)	IF (ECF—Plasma Water) (ml.)	Measured ECF/PV	(%) PV/IV
36	3,432	3,975	3,736	11,964	3.950	31.23
23	2,722	2,997	2,817	9,383	4.070	30.03
27	3,175	3,237	3,043	9,957	4.015	30.56
38	3,170	3,100	2,914	9,386	3.963	31.05
41	2,527	2,858	2,686	8,814	4.023	30.47
32	2,198	2,213	2,080	7,020	4.104	29.62
37	3,484	3,445	3,238	10,462	3.981	30.95
47	3,182	3,119	2,932	9,568	4.007	30.64
40	3,106	2,958	2,780	8,820	3.921	31.51
27	2,928	2,936	2,760	8,640	3.884	31.94
Average					3.992	30.80

TABLE 3. Measured Values of ECF Compared with Expected Values Obtained as a Fraction of Body Weight and as a Ratio to Expected Plasma Volume in Women

Age of Subject	Weight (kg.)	Height (inches)	Expected ECF calculated on basis of 20% body weight (liters)	Measured ECF (liters)	Expected ECF calculated on basis of 4.4 × expected plasma volume* (liters)
38	72.7	67	14.5	10.0	11.6
23	60.0	63	12.0	10.3	9.5
24	74.5	72	14.9	13.7	12.7
37	63.6	65	12.7	10.0	10.3
45	49.5	60	9.9	8.4	8.0
32	60.4	68	12.1	9.6	10.5
36	123.6	66	24.7	18.0	15.6
40	50.0	60	10.0	9.7	8.0
26	62.7	64	12.5	9.8	10.0
41	56.8	66	11.4	10.2	9.8
Average	67.4	65	13.5	11.0	10.6
Average difference			−2.5		+0.3
Average deviation			2.49		0.97
Standard error			0.5949		0.2285
Fluctuation			−6.7 to −0.3		+2.4 to −1.7

* Obtained from table 1, column 6.

TABLE 4. Measured Values of ECF Compared to Expected Values Obtained as a Fraction of Body Weight and as a Ratio to Expected Plasma Volume in Men

Age of Subject	Weight (kg.)	Height (inches)	Expected ECF calculated on body weight (liters)	Measured ECF (liters)	Expected ECF calculated on basis of 4.0 plasma volume* (liters)
36	95.4	69	19.1	15.7	13.7
23	65.0	67	13.0	12.2	10.9
27	94.5	64	18.9	13.0	12.7
38	77.7	72	15.5	12.3	12.7
41	65.0	62	13.0	11.5	10.1
32	44.5	67	8.9	9.1	8.8
37	92.3	72	18.5	13.7	13.9
47	77.3	72	15.5	12.5	12.7
40	77.7	70	15.5	11.6	12.4
27	68.2	68	13.6	11.4	11.7
Average	75.8	68	15.2	12.3	12.0
Average difference			−2.8		+0.34
Average deviation			2.89		0.72
Standard error			0.5589		0.2004
Fluctuation			−5.9 to +0.2		+2.0 to −0.2

* Obtained from table 2, column 6.

concentration of the tracer in the volume of fluid measured, and at the same time, to take into account the amount of tracer eliminated from the system during the mixing period.[22, 23] It becomes apparent that the disparately contracted ECF volume sometimes seen following hemorrhagic shock is an artifact of the single-sample technique.[24, 25, 26] Under normal conditions, when blood flow is not impaired, blood sampling over a 60-minute pe-

TABLE 5. Changes in ECF Following the Administration of 1,000 ml. of Ringer's Solution over a 30-minute Period

Age of Subject	Ht. (in.)	Wt. (kg.)	Plasma Water (Control) (ml.)	ECF (Control) (liters)	Plasma Water Experimental* (ml.)	ECF (Experimental*) (liters)	Per Cent Increase ECF	Per Cent Increase Plasma Water	Per Cent Increase IF	Ratio of IF to Plasma Water
Men										
28	69	75.0	3,084	11.8	3,317	12.7	+7.62	+7.55	+7.65	2.86
23	67	65.0	2,817	12.2	3,025	13.0	+6.56	+7.38	+6.31	2.85
40	70	77.7	2,780	11.6	2,990	12.5	+7.76	+7.55	+7.82	3.28
47	72	77.3	2,932	12.5	3,123	13.3	+6.40	+6.51	+6.30	3.19
Average	69.5	73.75	2,903	12.02	3,114	12.87	+7.08	+7.25	+7.03	3.045
Women										
41	66	56.8	2,160	10.2	2,340	11.0	+7.84	+8.33	+7.71	3.44
26	64	62.7	2,087	9.8	2,287	10.7	+9.18	+9.58	+7.14	3.50
Average	65	59.75	2,123	10.0	2,313	10.85	+8.51	+8.95	+7.42	3.470

* 30 minutes following administration of 1,000 ml. Ringer's solution.

TABLE 6. Changes in ECF Following the Administration of 500 ml. of Dextran (75) over a 30-minute Period

Age of Subject	Ht. (in.)	Wt. (kg.)	Plasma Water (Control) (ml.)	ECF (Control) (liters)	Plasma Water (Experimental*) (ml.)	ECF (Experimental*) (liters)	Per Cent Increase ECF	Per Cent Increase Plasma Water	Per Cent Increase IF
Men									
30	68	74.1	2,853	12.5	3,353	12.9	+3.20	+17.52	−1.04
27	68	68.2	2,760	11.4	3,310	11.8	+3.51	+19.93	−1.70
35	66	60.9	2,445	10.5	2,968	10.9	+3.81	+21.39	−1.45
Average	67.3	67.7	2,686	11.46	3,210	11.87	+3.50	+19.61	−1.40
Women									
45	60	49.5	1,778	8.4	2,308	8.8	+4.75	+29.81	−1.96
32	58	60.4	2,037	9.6	2,557	10.1	+5.21	+20.37	−0.26
Average	64.0	54.95	1,907	9.0	2,432	9.45	+4.98	+25.09	−1.11

* 30 minutes following administration of 500 ml. dextran (75).

TABLE 7. Effect on ECF of Restricting Fluid and Food Intake for 12 to 18 Hours

Age of Subject	Weight (kg.)	Height (inches)	Expected* ECF (liters)	Measured ECF (liters)		Loss (liters)
				On Admission	After 12–18 hours NPO	
Men						
37	92.3	72	13.9	13.7	12.1	−1.6
36	95.4	69	13.7	15.7	13.3	−2.4
38	77.7	72	12.7	12.3	11.0	−1.3
41	65.0	62	10.1	11.5	10.1	−1.4
32	44.5	67	8.8	9.1	8.3	−0.8
Average Fluctuation	75.0	68	11.8	12.5	11.0	−1.5 {−0.8 {−2.4
Women						
38	60.0	63	9.5	10.3	8.7	−1.6
23	72.7	67	11.6	10.0	8.9	−1.1
37	63.6	65	10.3	10.0	8.9	−1.1
24	74.5	72	12.7	13.7	11.9	−1.8
40	50.0	60	8.0	9.7	8.0	−1.7
Average Fluctuation	64.2	65	10.4	10.7	9.3	−1.5 {−1.1 {−1.8

* Calculated according to expected plasma volume and body surface. Men: 4.0 × expected plasma volume. Women: 4.4 × expected plasma volume. Average loss for both men and women = 1.48 liters; S.E. 0.1421 liters.

riod is adequate; but with derangement of blood flow, blood pooling, or when shunting mechanisms are in effect, blood sampling should be extended for 120–180 minutes.[27, 28]

Extracellular fluid is compartmented. Expressing the volume of fluid measured as immediately available ECF is based on the extrapolated zero-time concentration of tracer in plasma water from the 30- and 60-minute samples. The total ECF volume is determined by the concentration of tracer in plasma water obtained from the 60-minute sample, and in some instances the 120-minute sample, corrected for the amount of tracer excreted in the urine. The difference between the value at zero time and the final concentration indicates the amount of fluid sequestered. Under normal conditions, the measured volumes obtained at zero time and at 60 minutes are of equal magnitude. In three patients we were able to determine the amount of ascitic fluid by taking into account the differences in ECF volumes obtained at the zero-time concentrations of tracer in plasma and at 60-minute concentrations in plasma water.

It is customary to express volume measurements as a ratio of body weight. This presumes that the distribution of the various elements that constitute body weight is within normal limits. In clinical conditions where there is loss of weight or accumulation of adipose tissue, body weight alone is not a sound basis for calculating expected normal values. From the results presented in this report, the mean values of the measured ECF (sulfate) volumes in the controls, "group of normal individuals," seemed to deviate significantly from expected values determined as a percentage of body weight (20 per cent). There seems to be closer correlation between measured values and expected values for ECF (sulfate) when the expected values are calculated as a ratio of the expected plasma volume which is established on two parameters, height and weight.

Six per cent dextran 75 in normal saline, 315 milliosmols/kg. (normal range of plasma osmolality: 282–298 milliosmols/kg.), when administered intravenously, tended to remain in the intravascular compartment. With this

infusion, we noted an added expansion of the intravascular volume by transcapillary filling as a result of the elevated osmotic pressure of the solution.

The distribution of isotonic crystalloid infusions, Ringer's solution, 285 milliosmols/kg., between the intravascular and interstitial compartments followed closely the normal ratio of distribution of fluids to these two compartments, IF:IV 3:1 in males and 3.5:1 in females. These observations agree with some reports,[4] but are at variance with results reported by McNeill *et al.*[29] A possible explanation for the differences is transcapillary filling resulting from the large amount of blood removed from dogs during sampling.[30, 31, 32] From the observations presented, it would seem that four unit volumes of isotonic crystalloid infusions are necessary to expand the intravascular volume by one unit volume. We tend to agree that intravenous administration of isotonic crystalloid infusions should be limited to the actual fluid needs of the patient [6, 33, 34] and that plasma expanders should be used to maintain intravascular volume and adequate venous return to the right side of the heart. Overhydration and expansion of the IF compartment will interfere with cellular metabolism. Gases being transferred, nutritive material, and metabolic end products being eliminated, have to cross the IF space. The greater the distance between the source of blood supply and the cellular elements, the slower the process of diffusion.[35, 36]

The normal response to loss of intravascular volume or disparity between volume and vascular tone results in transcapillary filling and hemodilution. This response is rapid and effective in order to maintain venous return. Often transcapillary filling is delayed or even absent; this varies with the amount and availability of fluid in the interstitial compartment.[37, 38, 39] Restricting food and fluid intake for 12 to 18 hours depletes fluid reserves in the body by 1.5 liters. Such a deficit in the elderly chronically-ill patient may be detrimental, depriving the patient of fluid reserves which could be mobilized in the event of blood loss or depressed vasomotor tone.

Summary and Conclusions

A modified technique for measuring plasma, erythrocyte and ECF (sulfate) volumes utilizing three radioactive tracers is presented. The technique is simple and adaptable to clinical practice. Expected values for blood volume should be based, preferably, upon a ratio of body surface rather than body weight, and ECF volume upon a ratio to the expected plasma volume. The distributions of isotonic crystalloid solutions and 6 per cent dextran 75 in normal saline between the intravascular and interstitial space, are described. Isotonic crystalloid solutions tend to equilibrate in a 1:3 ratio between the intravascular and interstitial compartments. The implications of overhydration are discussed.

Restricting fluid intake for 12–18 hours prior to surgery results in a loss of fluid which might be important in maintenance of circulatory homeostasis in the elderly and chronically-ill patient.

Addendum

Since this report was submitted, the beta-counting anthracene cell has been modified to improve its efficiency. Anthracene has been replaced by europium-activated calcium fluoride crystals.*

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