EXPERIENCES WITH RADIOSULFATE IN THE ESTIMATION OF PHYSIOLOGIC EXTRACELLULAR WATER IN HEALTHY AND ABNORMAL MAN [1, 2]

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We have been interested in studying the changes in extracellular fluid volume in patients undergoing acute heat stress. Attempts to apply the inulin method to our needs were fruitless because of the prolonged time period required for equilibrium. Walser's radiosulfate method (1, 2) had appeal since it appeared suitable for repeated determinations within a short period of time.

Walser has suggested that within 15 to 20 minutes following a single intravenous injection, radiosulfate reached an apparent equilibrium between the vascular and interstitial spaces (1,2). He showed that the calculated volume of distribution of radiosulfate increased with time (1, 2) and that this increase could not be accounted for by urinary excretion (1, 2), fecal loss (3), or penetration of the muscle chloride space (4). He proposed that the 18-minute volume of distribution of radiosulfate could be used as an index of functioning extracellular fluid volume (1, 2).

The present report, dealing with the radiosulfate method, confirms and extends many of Walser's observations. In addition, it points out the desirability of calculating a "zero time" rather than an 18-minute volume of distribution, and proposes a modification of existing ways of calculating the "zero time" space.

METHODS

A. *Subjects:* Radiosulfate spaces were determined on 33 healthy individuals: eleven (Nos. 1–11) young, muscular soldiers; nine (Nos. 12–20) young, male medical students and physicians; five (Nos. 21–25) ambulatory, elderly males; and eight (Nos. 26–33) ambulatory, elderly females. Four patients with abnormalities of water metabolism were studied: two (Nos. 34, 35) were dehydrated and two (Nos. 36, 37) had ascites without peripheral edema.

In five subjects the radiosulfate space was redetermined at various intervals, ranging from 1 to 30 days, during which they continued their usual daily activities. Each of the eleven soldiers had a second determination following a three-week period of paratroop training.

B. *Procedure:* All subjects were studied in the morning after a twelve-hour fast. They were allowed water *ad libitum* during the preceding day and were given 200 ml. of water every two hours during the course of the test.

Approximately 100 microcuries of S^{35} as carrier-free sodium sulfate [3] were injected intravenously, from a calibrated syringe, over a period of one minute. An aliquot of the injected material was set aside as a standard. Timed blood samples were obtained from the arm opposite the site of the injection. Timed urine samples were obtained by indwelling catheters from subjects 21 through 35, and as voided samples in all others. Where catheters were used the bladder was washed with physiologic saline, followed by injection of air and manual expression. Ascitic fluid samples were obtained from indwelling polyethylene catheters with multiple openings.

In three subjects inulin space was simultaneously determined by the 5-hour constant infusion technique (4, 5). Inulin was determined in plasma and urine by Kendrick's modification (6) of the method of Harrison (7). The inulin space was calculated by dividing the inulin excreted in the urine (collected for 24 hours after completion of the infusion) by the plasma concentration at the end of the infusion. A timed blank correction was made on the urine sample. The space, thus calculated, was corrected for a plasma water content of 93 per cent.

C. *Method for the determination of radiosulfate:* S^{35} emits a low energy beta particle and has a physical half-life of 87.1 days. The chief problem concerned with its determination is the phenomenon of self-absorption.

As plasma is added to a fixed amount of S^{35}, the observed radioactivity decreases as the amount (or weight) of plasma increases. There is a rapid decrease in observed activity as dry weight increases from 0.1 to 12 mgm. Increments of weight about 12 mgm. produce a second slower rate of decrease in observed radioactivity.

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[2] The opinions expressed in this paper are those of the authors and do not necessarily represent the official views of any government agency.

[3] Supplied on allocation from the Atomic Energy Commission by Dr. Donald Tabern, Abbott Laboratories, North Chicago, Illinois.

This second slower rate of decrease may be expressed by the equation:

$$S = \frac{\log \frac{C_2}{C_1}}{W_2 - W_1}, \qquad (1)$$

where:

S = Slope (the exponential rate of decrease in observed activity per mgm. increase in sample weight)

C_2 = Observed counts per minute at Weight 2

C_1 = Observed counts per minute at Weight 1

W = Weight in mgm.

Knowing the slope (S), a sample of known weight and activity can be corrected to an activity of any arbitrary reference weight. Thus, samples of different weights may be made comparable. We selected 50-mgm. sample weight as an arbitrary reference point. In this situation, the equation for self-absorption correction becomes:

$$\log C_{50} = \log C_0 - S\,(W_0 - 50), \qquad (2)$$

where:

C_{50} = activity at 50-mgm. sample weight in counts per minute

C_0 = activity at the observed weight in counts per minute

S = exponential rate of decrease in observed activity with increasing weight

W_0 = observed weight in mgm.

The slope (S) was determined by preparing a family of 19 curves covering a weight range of 30 to 80 mgm. and a range of activity from 300 to 40,000 counts per minute. For each curve, activity was held constant and weight varied by adding plasma without radioactivity to the planchet. The slopes of these curves were calculated and found to be quite constant, having a mean of -0.00633 (S.D. $\pm$ 0.00063). Substituting this value in equation (*2*), the correction equation becomes:

$$\log C_{50} = \log C_0 + .00633\,(W_0 - 50). \qquad (3)$$

Serum was separated by centrifuging at 3,000 rpm. Duplicate 500-lambda aliquots of serum were pipetted into clean, preweighed, disposable, nickel-plated planchets, dried under a 150-watt light bulb and weighed again. Counting in a windowless gas-flow counter went on until a minimum of 2,500 counts were recorded. Urine samples and the standard solution, after appropriate dilution, were treated in a similar fashion save that waste plasma (free of radioactivity) was added to bring them into the desired weight range. The results obtained according to equation (*3*) were corrected for physical decay. The mean difference between counts on duplicate samples was less than 3 per cent. (Care was taken to avoid uneven drying and particulate matter in the samples.)

CALCULATIONS

The mathematical principles for calculating the volumes of distribution of metabolized and nonmetabolized substances have been set forth (7–10). In essence, for substances that are excreted but not metabolized, the volume of distribution of the material, at any time following its administration is equal to the amount remaining in the body at that time divided by the plasma concentration at the same time.

$$VD_t = \frac{D - E_t}{P_t}, \qquad (4)$$

where:

VD = Volume of distribution in ml. at time t

D = administered dose

E_t = amount excreted by time t

P_t = plasma concentration in units per ml. at time t.

For materials that are not metabolized, or that do not enter other fluid compartments, the calculated volume remains constant. For substances that are metabolized or that penetrate other fluid spaces, equation (*4*) is inadequate. To compensate, equation (*4*) is calculated for "zero time," that theoretical moment when all the injected material is uniformly distributed in its assumed volume of distribution without being subject to excretion, metabolism or diffusion into other fluid compartments.

$$VD_0 = \frac{D}{P_0}, \qquad (5)$$

where:

VD_0 = Volume of distribution, in ml., at "zero time"

D = administered dose

P_0 = extrapolated "zero time" plasma concentration in units per ml.

Equation (*5*) assumes that any loss of material from the measured space occurs at a uniform rate. No material can instantaneously mix in the plasma and equilibrate between intra- and extravascular fluid. Since plasma concentration is initially higher than that of extravascular fluid, the rate of initial loss in the urine is greater than that prevailing when plasma and extravascular fluid have equilibrated. The material excreted during the equilibrium period never contributes to the post-equilibrium plasma concentrations or their "zero time" extrapolation. Failure to correct for this excessive equilibrium time excretion results in an overestimation of the volume of distribution when calculated by equation (*5*). The degree of overestimation is proportional to the equilibrium time excretion. This error may be minimized by one of two calculations. First, one may calculate the volume of distribution at various post-equilibrium times, using equation (*4*), and extrapolate the volumes of distribution to "zero time." Secondly, one may plot the amount of material remaining in the body (dose minus cumulative excretion) against time and extrapolate to "zero time." This may be regarded as the "effective" dose, that part of the administered dose that eventually becomes distributed in the given volume of distribution. This "effective" dose divided by the "zero time" plasma concentration yields a "zero time" volume of distribution corrected for

the excessive urinary loss before equilibrium has been reached.

$$S_0 = \frac{R_0}{P_0}, \qquad (6)$$

where:

S_0 = "zero time" volume of distribution in ml.

R_0 = "effective dose" ("zero time" extrapolation of the amount of material remaining in the body)

P_0 = "zero time" plasma concentration in units per ml. obtained by extrapolation.

The "zero time" volumes of distribution presented in this report were calculated by equation (6). These were then corrected for a plasma water content of 93 per cent and the Gibbs-Donnan phenomenon, assuming the factor to be 0.90. Since plasma volume was not determined, the Gibbs-Donnan factor was applied to the entire volume of distribution rather than the interstitial fluid volume. No correction was made for urinary lag time since urine flow rates were generally good.

RESULTS

A. *The disappearance of radiosulfur from the plasma*

Figure 1 shows, by means of an example, the disappearance of S^{35} from the plasma following a single intravenous injection of sodium radiosulfate. Two rates of disappearance are evident. The first is rapid but of brief duration. Within 15 to 20 minutes it is replaced by a second slower rate that persists for at least 8 to 9 hours. The second slower rate was calculated for each of our subjects. The results are presented in Table I. These rates are such that the plasma radioactivity decreases 50 per cent in 2 to 4 hours.

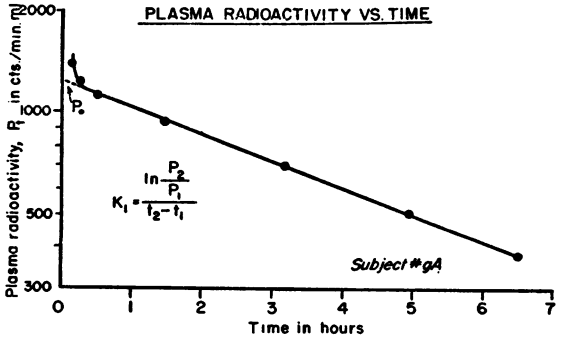

FIG. 1. THE DISAPPEARANCE OF RADIOSULFUR FROM THE PLASMA FOLLOWING A SINGLE INTRAVENOUS INJECTION

P_0 is the "zero time" extrapolation of the slower rate of disappearance.

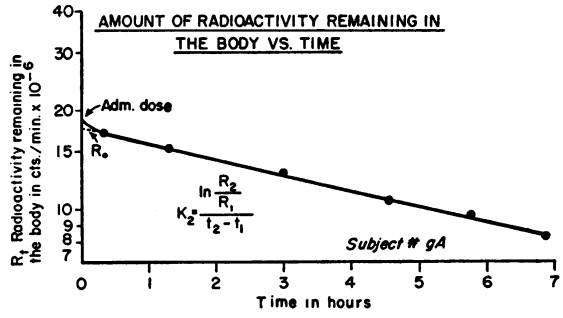

FIG. 2. THE AMOUNT OF RADIOSULFUR REMAINING IN THE BODY (DOSE-URINARY EXCRETION) FOLLOWING A SINGLE INTRAVENOUS INJECTION

R_0 is the "zero time" extrapolation of the slower rate of disappearance ("effective administered dose"). See text.

B. *The disappearance of radiosulfur from the body*

Intravenously injected S^{35} given as sodium sulfate is eliminated from the body chiefly by urinary excretion. Ninety-five per cent was recovered in the urine of Subject 14 within 5 days after administration. We have been able to account for only trace amounts in the sweat during a period of active sweating. Walser, Reid, and Seldin have found that fecal loss is negligible (3). Hence, for all practical purposes the amount of radioactivity remaining in the body may be expressed as the injected dose minus the urinary excretion.

Figure 2 shows the amount of S^{35} remaining in the body following a single intravenous injection. Two rates of disappearance are seen. The first is rapid and brief. It is replaced in 15 to 20 minutes by a second slower rate that lasts 8 to 9 hours.

The initial rapid rate of disappearance of S^{35} from the body is such that, on the average, 7.2 (S.D. $\pm$ 3.6) per cent of the administered dose is lost. This ranged from 1.0 to 19 per cent of the administered dose. Table I shows the data.

The second slow rate of disappearance of S^{35} from the body was calculated for each of our subjects (see Table I). This rate was such that 50 per cent of the injected dose was lost from the body in 4 to 8 hours. This was notably different from the rate of disappearance from the plasma (see above).

Renal clearance during the second slow phase of disappearance from the body was calculated for each subject. The mean clearance in ml. per

TABLE I

The rates of disappearance from the plasma and body, pre-equilibrium urinary excretion and zero time volume of distribution of radiosulfate in healthy individuals

Subject	Age *years*	Wt. *Kg.*	S.A. M^2	K_1* $\Delta ln/hr.$	K_2† $\Delta ln/hr.$	E. 3 hr.‡ % *adm. dose*	Vol. dist. *liters*	Vol. dist. *% body wt.*
Soldiers before paratroop training								
1A	22	73.5	1.89	−.170	−.108	2.3	16.4	22.3
2A	21	72.7	1.92	−.148	−.095	5.9	13.1	18.0
3A	19	63.6	1.78	−.139	−.100	5.1	11.9	18.8
4A	17	66.9	1.84	−.089	−.066	7.4	13.9	20.8
5A	17	71.0	1.84	−.145	−.131	5.1	14.4	20.3
6A	18	62.7	1.73	−.228	−.140	7.8	10.6	16.9
7A	18	74.7	1.96	−.174	−.116	7.5	14.7	19.7
8A	17	71.6	1.86	−.161	−.129	5.6	13.5	18.8
9A	19	74.6	1.92	−.181	−.110	4.2	12.2	16.3
10A	18	69.5	1.86	−.147	−.160	13.5	13.6	19.6
11A	19	70.9	1.85	−.140	−.132	9.5	13.6	19.0
Mean	18.6	70.2	1.86	−.157	−.117	6.7	13.4	19.1
Std. Dev.	±1.6	±4.1	±.07	±.034	±.040	±3.0	±1.5	±1.7
Soldiers after paratroop training								
1B	22	71.5	1.88	−.221	−.112	13.6	13.8	19.3
2B	21	71.4	1.90	−.175	−.179	11.1	14.2	19.9
3B	19	63.1	1.77	−.177	−.167	6.7	10.7	17.0
4B	17	66.6	1.84	−.206	−.251	7.7	10.8	16.2
5B	17	70.0	1.84	−.187	−.181	12.1	13.7	19.6
6B	18	64.2	1.74	−.222	−.084	3.4	10.3	20.2
7B	18	74.0	1.96	−.169	−.120	12.5	13.1	17.7
8B	17	69.7	1.84	−.209	−.186	10.6	12.1	17.1
9B	19	74.0	1.92	−.185	−.118	16.2	12.8	17.2
10B	18	68.4	1.85	−.169	−.096	6.6	14.3	20.8
11B	19	69.7	1.84	−.160	−.080	4.0	13.4	19.2
Mean	18.6	69.3	1.85	−.189	−.143	9.5	12.7	18.6
Std. Dev.	±1.6	±3.6	±0.06	±.022	±.054	±4.1	±1.5	±1.6
Young sedentary males								
12	31	82.5	1.97	−.167	−.131	15.3	11.6	14.1
13	28	61.0	1.74	−.183	−.189	5.8	9.6	15.8
14	30	78.5	1.99	−.174	−.139	6.6	13.0	16.5
15	23	70.6	1.86	−.151	−.111	12.8	15.5	21.9
16	22	67.1	1.75	−.209	−.158	19.2	10.6	15.8
17	23	79.6	1.96	−.168	−.175	13.0	13.5	17.0
18	30	67.7	1.87	−.141	−.213	2.5	11.4	16.8
19	25	78.8	2.01	−.166	−.144	10.1	13.5	17.2
20	25	58.6	1.69	−.222	−.183	4.0	9.0	15.4
Mean	26.3	71.6	±1.87	−.176	−.160	9.9	12.0	16.7
Std. Dev.	±3.5	±8.7	±0.12	±.026	±.032	±5.6	±2.1	±2.2
Elderly males								
21	63	77.7	1.83	−.168	−.106	4.5	12.5	16.1
22	61	62.3	1.71	−.132	−.125	7.2	10.8	17.3
23	61	70.9	1.76	−.150	−.142	8.6	10.8	15.2
24	63	60.9	1.73	−.171	−.065	1.0	10.0	16.4
25	65	95.5	2.14	−.191	−.100	1.0	9.0	9.4
Mean	62.6	73.5	1.83	−.162	−.108	4.5	10.6	14.9
Std. Dev.	±1.7	±14.1	±0.18	±.022	±.029	±3.5	±1.3	±3.2
Elderly females								
26	56	82.2	1.80	−.152	−.131	5.1	11.5	14.0
27	57	96.8	2.02	−.096	−.074	4.0	11.9	12.3
28	60	60.5	1.60	−.241	−.131	3.3	10.3	17.1
29	64	70.7	1.74	−.166	−.087	2.0	11.6	16.4
30	76	66.8	1.77	−.132	−.068	4.0	11.0	16.5
31	65	62.3	1.61	−.085	−.068	4.1	10.6	17.0
32	72	65.3	1.57	−.075	−.032	1.0	11.4	17.5
33	72	54.8	1.50	−.186	−.080	6.1	7.1	12.9
Mean	65.3	69.9	1.70	−.142	−.084	3.7	10.7	15.5
Std. Dev.	±7.8	±13.5	±0.17	±.018	±.011	±1.6	±1.5	±2.1

* K_1—Exponential rate of disappearance of radiosulfate from the plasma.
† K_2—Exponential rate of disappearance of radiosulfate from the body.
‡ Urinary excretion during the Equilibrium period expressed as E. 3 hr.-percentage of the administered dose.

min. per 1.73 m² was found to be 36.2 for 31 determinations on 22 young men, 25.8 for elderly men, and 21.7 for elderly women. The values for young men are comparable to those previously reported (2, 11, 12). The lower values in elderly people are probably related to the decrease in glomerular filtration rate that occurs with increasing age (13).

C. *The volume of distribution of radiosulfur as a function of time*

Figure 3 shows the volume of distribution of radiosulfate at various post-equilibrium times (calculated by equation 4). The calculated volume increases exponentially with time. The rate of increase is the difference between the rate of disappearance of S^{35} from the plasma and from the body:

$$VD_t = S_0 e^{(k_2 - k_1)}, \qquad (7)$$

where:

VD_t = volume of distribution in ml., at time t
S_0 = "zero time" volume of distribution in ml. (obtained by extrapolation or equation 6)
k_2 = exponential rate of disappearance of radiosulfur from the body
k_1 = exponential rate of disappearance of radiosulfur from the plasma
t = time.

Most of our subjects had volumes of distribution that increased with time. Six (No. 10A, 2B, 4B, 13, 17, 18), however, had volumes that decreased with time. Our data do not give an explanation for these decreasing volumes. A loss of water from the plasma or a return of radioactive sulfate to the plasma from some pool of high specific activity could result in such a phenomena.

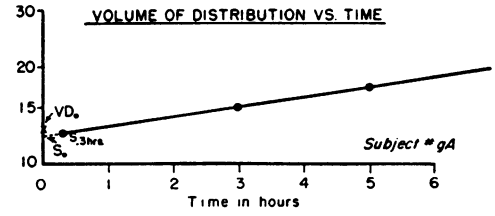

FIG. 3. THE CHANGE OF VOLUME OF DISTRIBUTION OF RADIOSULFUR, WITH TIME, FOLLOWING A SINGLE INTRAVENOUS INJECTION

S_0 is the extrapolated "zero time" volume, $S_{.3\,hrs.}$ the equilibrium time volume and VD_0 the "zero time" volume of distribution calculated as the dose divided by the "zero time" plasma concentration.

D. *The zero time volume of distribution of radiosulfate in healthy individuals*

Data are presented in Table I. The mean "zero time" radiosulfate space was found to be 19.1 (S.D. ± 1.7) per cent of body weight in muscular young soldiers (1A to 11A) before 3 weeks of paratroop training and 18.7 (S.D. ± 1.6) per cent of body weight after training (1B to 11B). The mean "zero time" radiosulfate space was 16.7 (S.O. ± 2.2) per cent of body weight in sedentary young males, 14.9 (S.D. ± 3.2) per cent of body weight in elderly males, and 15.5 (S.D. ± 2.1) per cent of body weight in elderly females.

The difference between the values obtained before and after paratroop training was not significant. The muscular soldiers had significantly larger radiosulfate spaces than the sedentary young males ($P < .01$). The differences between sedentary young and elderly males and between elderly men and women were not significant.

TABLE II

The reproducibility of the zero time radiosulfate space in healthy individuals

Subject	Interval between determinations	First determination		Second determination		Difference	
		liters	*% body wt.*	*liters*	*% body wt.*	*liters*	*% body wt.*
	days						
12	1	11.6	14.1	12.7	15.4	+1.1	+1.3
13	7	9.6	15.8	9.2	15.8	−0.4	−0.0
15	1	15.5	21.9	15.1	21.4	−0.4	−0.5
19	1	13.5	17.2	13.3	17.0	−0.2	−0.2
26	30	12.2	14.8	11.5	14.0	−0.7	−0.8
Mean		12.48	16.76	12.36	16.72	−0.12	−0.04

TABLE III

Simultaneous radiosulfate and inulin spaces in healthy individuals

Subject	Radiosulfate space in liters	Inulin space in liters	Ratio: Radiosulfate / Inulin
22	10.8	10.5	1.03
23	10.8	10.9	0.99
28	10.3	11.1	0.93

E. *The reproducibility of the zero time radiosulfate space*

Five individuals (Nos. 12, 13, 15, 19, 26) had second determinations at various intervals ranging from 1 to 30 days following their initial test. Data are presented in Table II. The mean difference between the two determinations was − 0.12 liter with a range of + 1.1 to − 0.7 liters. The mean difference expressed as percentage of body weight was − 0.04 with a range of + 1.3 to − 0.8.

F. *Comparison of the zero time radiosulfate space with inulin space*

Table III shows the values obtained when we simultaneously determined "zero time" radiosulfate and 5-hour constant-infusion inulin spaces on three subjects. The mean radiosulfate-inulin space ratio was 0.98.

G. *The volume of distribution of radiosulfate in patients with abnormal water metabolism*

Table IV presents data on 4 patients. Subject 34 was a 60-year old female admitted in diabetic acidosis. Clinically, she was moderately dehydrated. Her "zero time" radiosulfate space was 8.2 liters or 10.5 per cent of body weight. Subject 35 was a 65-year old man who had suffered a cerebro-vascular accident approximately 48 hours before being found and brought to the hospital. He was severely dehydrated, oliguric and had hyperchloremia and hypernatremia. His radiosulfate space was 4.9 liters or 7.2 per cent of body weight.

Subject 36 was a 42-year old man with rheumatic mitral and tricuspid stenosis and insufficiency. He had 7,600 ml. of ascitic fluid obtained by paracentesis, but no peripheral edema. His radiosulfate space[4] was 16.3 liters or 26.2 per cent of body weight. Subtracting his ascitic fluid volume from his radiosulfate space gives a value of 8.7 liters or 16.8 per cent of his weight after paracentesis, a value well within the normal range. Subject 37 was a 60-year old man with Laennec's cirrhosis and ascites (3,600 ml.) but no peripheral edema. His radiosulfate space was 16.7 liters or 22.8 per cent of his weight. After subtraction of

TABLE IV

The zero time radiosulfate space in abnormal individuals

Subject	Clinical diagnosis	Abnormality of water metabolism	V.D. *liters*	V.D. *% body wt.*
34	Diabetes mellitus in acidosis	Moderate dehydration	8.2	10.5
35	C.V.A. with unconsciousness	Severe dehydration	4.9	7.2
36	Rheumatic heart disease	Ascites (7,600 ml.) without peripheral edema	16.3	26.2
37	Laennec's cirrhosis	Ascites (3,200 ml.) without peripheral edema	16.7	22.8

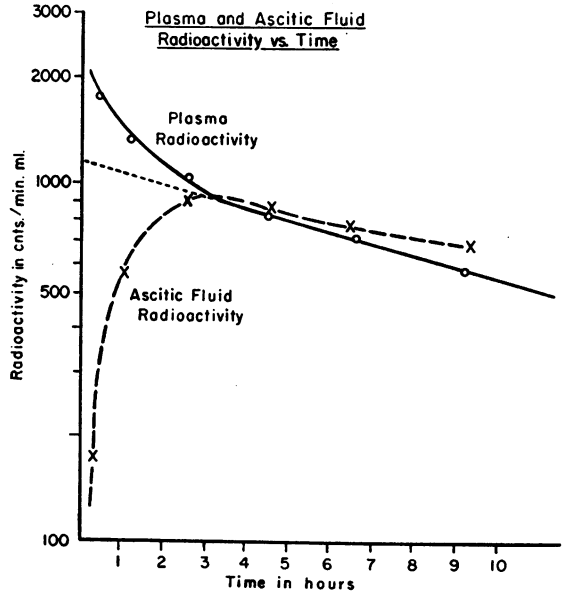

FIG. 4. THE CHANGE IN PLASMA AND ASCITIC FLUID RADIOACTIVITY, WITH TIME, FOLLOWING A SINGLE INTRAVENOUS INJECTION OF RADIOSULFUR

[4] For the subjects with ascites the volume of distribution was calculated at the time of equilibrium between plasma and ascitic fluid using the calculation described by Deane (14).

his ascitic fluid volume from his radiosulfate space he had a value of 13.1 liters or 18.7 per cent of his "dry" weight.

Figure 4 shows plasma and ascitic fluid radioactivity following a single intravenous injection of S^{35} in Subject 37. A transient equilibrium between plasma and ascitic fluid concentrations occurred 3.4 hours following injection. In Subject 36, who had a larger amount of ascitic fluid, equilibrium occurred at 5.1 hours. These equilibrium times are comparable to those reported by Madison, Teng, Seldin, Reid, and MacDonald (15).

DISCUSSION

A. *The zero time radiosulfate space*

In theory, the extracellular fluids consist of plasma, lymph, interstitial fluid, cerebrospinal fluid, aqueous humor, vitreous humor, synovial, pleural and pericardial fluids, glandular secretions and urine. These anatomic entities, however, do not behave as a single physiologic unit. The physiologic extracellular water consists of the plasma and those fluid compartments with which it freely exchanges ions and small molecules. It differs from the anatomical extracellular fluid by excluding secretions and possibly the ocular humors and cerebrospinal fluid. The relationship of ground substance and its bound water to either the anatomic or physiologic extracellular water is not clear. Neither the anatomic nor the physiologic extracellular fluid can be measured by direct means. They must be estimated from the dilution of an administered chemical. As yet, no chemical has been found which is confined to the physiologic extracellular water. However, since ions and small molecules generally diffuse rapidly through the physiologic extracellular water and slowly into other fluid compartments an estimation of extracellular water can be made. The problems dealing with estimations of extracellular fluid volume, *in toto,* and in various tissues have recently been reviewed and discussed by Manery (16).

The relationship of the radiosulfate space to an anatomically defined extracellular space is obscure. There are not sufficient data to define accurately the distribution of radiosulfate. Very little radiosulfate has been found in gastric juice (2) or sweat. Sulfate does not readily penetrate brain tissue or cerebrospinal fluid from the blood (17). It has been found to equilibrate with peritoneal fluid and therefore it should equilibrate with pleural and pericardial fluid. It has been shown that the radiosulfate space of muscle is approximately 20 per cent smaller than the muscle chloride space and that it remains constant between 20 and 250 minutes following its administration (4). This is probably due to the higher concentration of chloride in muscle connective tissue and the slowness or lack of penetration of sulfate into connective tissue water. Data are lacking on the distribution of radiosulfate in lymph, interstitial fluid, synovial fluid, and the ocular humors. Radiosulfate does equilibrate with edema fluid water in about 11 hours (15). From the available data it appears that radiosulfate space is not a measure of an anatomical extracellular space, but is an index of physiologic extracellular water, *i.e.,* the plasma and those fluid compartments with which it rapidly exchanges ions and small molecules.

From the data of Sheatz and Wilde on the transcapillary migration rate of radiosulfate (17), and the initial rapid rate of disappearance of radiosulfate from the plasma noted by Walser, Seldin, and Grollman (1, 2), it appears that radiosulfate has a rapid diffusion into an extravascular water compartment that we presume is the physiologic extracellular water. Since radiosulfate is not bound to plasma proteins,[5] it seems reasonable to assume that the concentration of radiosulfate in plasma is representative of that in the physiologic extracellular space when the Gibbs-Donnan phenomenon is taken into account. However, since the calculated radiosulfate space increases with time, it is apparent that the isotope is not confined to the physiologic space. It must be metabolized or penetrate other body fluid compartments. The extent and rate of loss of radiosulfate, in this fashion, will be discussed below. Suffice it to say here, that the loss occurs at a relatively slow rate and that the error introduced by such a loss can be minimized by calculation of a "zero time" space.

Whether one calculates a "zero time" or an 18-minute volume of distribution, as Walser did, it cannot be assumed, as has been proposed (2), that four per cent of the injected material is excreted in the urine during the equilibrium period. Our

[5] Plasma radiosulfur can be completely dialyzed and is unprecipitable with trichloracetic acid and phosphotungstic acid.

data show that the urinary excretion during the assumed equilibrium time of 18 minutes is quite variable and ranges from 1 to 19 per cent of the injected material.

The relationship of the radiosulfate space to other indices of extracellular fluid volume is not precisely established. The "zero time" volumes of distribution of radiosulfate, seen in our healthy individuals, are comparable to those previously reported (1, 2, 18), and are generally smaller than the volumes of distribution of chloride (19, 20), bromide (20), and thiocyanate (19, 21). The discrepancies might be due to the penetration of the latter substances into connective tissue water (22), into cell water (23–25), sequestration in the gastrointestinal tract (26), or, in the case of thiocyanate, binding to protein (27). Recently, Berson and Yalow (28) have shown that calculation of the volume of distribution of radiosodium and radiobromide from their early rates of disappearance from the plasma yields values that amount to approximately 18 per cent of body weight, even though their ultimate volumes of distribution are much greater. These values are comparable to those found for radiosulfate and suggest that the discrepancy between chloride and radiosulfate spaces might be eliminated by calculation of chloride space from its early rate of disappearance from plasma.

The values we have obtained for "zero time" radiosulfate space are comparable to those reported for the 5-hour constant-infusion inulin space (5, 29, 30), constant-infusion sucrose space (14, 19, 31), and "zero time" thiosulfate space (32). They are slightly smaller than reported mannitol spaces (33).[6] Walser found a ratio of 0.95 between simultaneously determined radiosulfate and inulin spaces. On three subjects we also found good correspondence between inulin and radiosulfate spaces. This apparent agreement of these several methods may be coincidental. Becker and Heinemann (18) found that the inulin-radiosulfate ratio approached unity, but that the coefficient of correlation between the two was only 0.3. Swan, Madisso, and Pitts (34) recently determined simultaneous "zero time" mannitol and inulin, sucrose, raffinose, thiosulfate and radiosulfate spaces in nephrectomized dogs. Using mannitol as a reference they found ratios of 0.74 for inulin, 0.87 for raffinose, 0.91 for sucrose, 0.99 for thiosulfate and 1.0 for radiosulfate. This may be due to a relatively slow penetration of the larger inulin, raffinose and sucrose molecules into some relatively inaccessible fraction of extracellular water. The data of Cotlove (35) and Nichols, Nichols, Weil, and Wallace (36) suggest that this may be some connective tissue phase.

[6] These mannitol spaces were not corrected for the excessive urinary loss of mannitol during the equilibrium period and therefore are too large.

B. *The slowly exchanging sulfate pool*

Since injected S^{35} leaves the plasma at a faster rate then it appears in the urine it is apparent that it is being metabolized or entering fluid compartments other than its "zero time" volume of distribution. We have attempted to shed some light on this slowly exchanging pool using the mathematical approach presented in the Appendix. This approach has been applied to the data obtained on the eleven young soldiers and the results are given in Table V and illustrated in Figure 5.

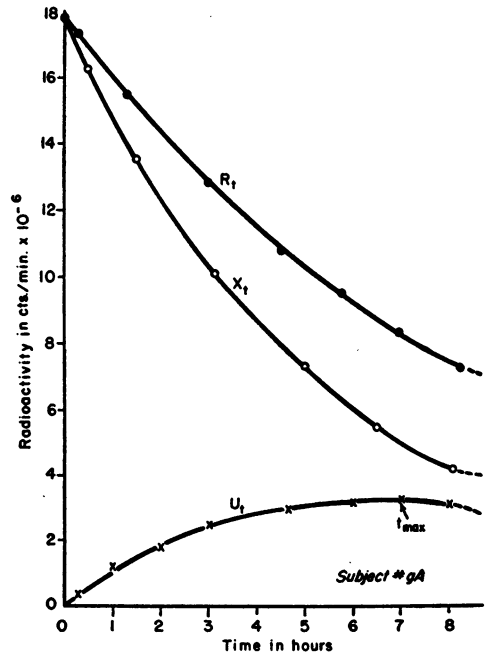

FIG. 5. THE EXTRA-URINARY LOSS OF RADIOSULFUR (U_t) FOLLOWING A SINGLE INTRAVENOUS INJECTION

R_t represents the disappearance of radiosulfur from the body; X_t the disappearance of radiosulfur from the physiologic extracellular space and t_{max} the time at which the extra-urinary loss of radiosulfur is maximum.

TABLE V

The extra-urinary loss of radiosulfate in eleven soldiers before and after paratroop training

Subject	Before				After			
	t_{max}*	Ut_{max}†	VU‡ *liters*	VU‡ *% body wt.*	t_{max}	Ut_{max}	VU *liters*	VU *% body wt.*
1	7.3	16.0	9.0	12.2	6.3	22.9	13.6	19.0
2	8.3	14.5	8.5	11.7	—	—	—	—
3	8.5	11.9	4.6	7.2	5.8	2.2	0.7	1.0
4	12.7	10.4	4.7	7.0	—	—	—	—
5	7.3	2.7	0.8	1.1	5.5	1.1	0.3	0.4
6	5.6	17.1	7.2	11.5	7.1	33.4	22.0	34.3
7	7.0	14.3	7.6	10.2	7.0	11.8	5.5	7.5
8	6.9	8.5	3.5	4.9	5.1	4.1	1.5	2.2
9	7.0	18.0	7.9	10.6	6.7	14.2	7.2	9.8
10	—	—	—	—	7.8	19.7	10.9	15.9
11	7.4	2.2	0.9	1.3	8.7	24.6	13.4	19.3
Mean	7.8	11.6	5.5	7.8	6.7	14.9	8.3	12.6
Std. Dev.	±1.90	±5.63	±3.04	±4.20	±1.14	±11.19	±7.29	±11.13

* t_{max} = The time of maximum extra-urinary loss of radiosulfate in hours.
† Ut_{max} = The maximum extra-urinary loss of radiosulfate expressed as per cent of the administered dose.
‡ VU = The volume that the extra-urinary loss would occupy if it were contained in a fluid compartment in simple diffusion equilibrium with the "extra-cellular space" (see text).

The maximum amount of radiosulfate entering the slowly exchanging pool was found to be approximately 10 to 15 per cent of the injected dose. This maximum was reached 6 to 8 hours after injection. Considerable variation was noted. The maximum amount of radiosulfate entering the slowly exchanging pool during the first twenty minutes after injection was 1.3 (S.D. ± 1.2) per cent of the injected dose. It must be stressed that this is an over-estimation since during the first twenty minutes after the injection the concentration of radiosulfate is greater in the plasma than in the interstitial fluid.

With the thought that the slowly exchanging pool might represent a fluid compartment, we attempted to calculate its volume using the principles set forth by Best (37) in his work with fluid analogues. The calculated volumes varied from 0.3 to 22.0 liters (see Table V). They were not reproducible. They do not conform to any known fluid compartment. We can only conclude that the extra-urinary loss of radiosulfur is not due to simple diffusion from "physiologic extracellular water" to some other fluid compartment.

We do not know what the slowly exchanging sulfate pool represents. It may be the net result of several simultaneously occurring phenomena. Everett and Simmons (38) report that in the rat, S^{35} is excreted in the bile and upper gastrointestinal tract and subsequently is reabsorbed in the large gut. It is incorporated into taurocholic acid (39). Dziewiatkowski, Benesch, and Benesch (40, 41) report that S^{35} is concentrated in cartilage (as chondroitin sulfate) and bone marrow. Radiosulfate enters into the formation of ethereal sulfate (42, 43). Ling feels that some sulfate enters muscle cells (44).

SUMMARY AND CONCLUSIONS

1. A method for determining radiosulfate in plasma and urine has been presented.

2. The disappearance of radiosulfate from the plasma and the body was discussed. A method of calculating a "zero time" radiosulfate space corrected for excessive equilibrium time excretion was derived. This calculated space has some advantages over the equilibrium time space and the "zero time" space uncorrected for urinary excretion.

3. Corrected "zero time" radiosulfate spaces were determined on 11 soldiers, 9 sedentary young males, 5 elderly males, and 8 elderly females. They were found to average 19.1, 16.7, 14.9, and 15.5 per cent of body weight, respectively. This "zero time" space was found to be reproducible in the steady state and to vary in the clinically indicated degree and direction in patients with abnormal water metabolism. Approximately 5 hours were required for radiosulfur to equilibrate between plasma and ascitic fluid.

4. The distribution of radiosulfate and the relationship of radiosulfate space to other indices of extracellular fluid volume have been discussed. It is concluded that the "zero time" radiosulfate space is an index of physiologic extracellular water.

5. A method of calculating the slowly exchanging sulfate pool has been presented. The significance of this pool has been discussed.

APPENDIX

Injected S^{35} leaves the plasma at a faster rate than it appears in the urine. Assuming that the "zero time" radiosulfate space remains constant, the extra-urinary loss of S^{35} during the post-equilibrium period can be calculated. This is the amount remaining in the body (dose minus excretion) minus that remaining in the "zero time" space. The amount remaining in the "zero time" space is the product of its volume and its concentration:

$$X_t = cS_0P_0e^{K_1t}, \tag{8}$$

where:

X_t = radioactivity remaining in the "zero time" radiosulfate space at time t in counts per minute

c = constant correcting for plasma water content and Gibbs-Donnan effect (assumed to be 1.194)

S_0 = "zero time" radiosulfate space in ml.

P_0 = "zero time" plasma concentration in counts per minute per ml.

K_1 = exponential rate of disappearance of radiosulfate from the plasma

t = time in hours.

The S^{35} remaining in the body after the equilibrium period is:

$$R_t = R_0e^{K_2t}, \tag{9}$$

where:

R_t = amount of radiosulfate remaining in the body at time t in counts per minute

R_0 = amount of radiosulfate remaining in the body at "zero time" (obtained by extrapolation) in counts per minute

K_2 = exponential rate of disappearance of radiosulfate from the body

t = time in hours.

The amount of S^{35} being metabolized or entering fluid compartments other than its original volume of distribution is the difference between equations (*9*) and (*8*). This simplifies to:

$$U_t = R_0(e^{K_2t} - e^{K_1t}), \tag{10}$$

where:

U_t = the amount of radiosulfate, in counts per minute, lost by extra-urinary sources by time t.

It is apparent that the extra-urinary loss of S^{35} reaches a maximum and thereafter decreases (see Figure 5). The time at which this maximum is reached can be calculated from equation (*10*):

$$t_{max} = \frac{\ln \frac{k_2}{k_1}}{k_2 - k_1}, \tag{11}$$

where:

t_{max} = time of maximum extra-urinary loss of radiosulfate in hours

k_1, k_2 = the exponential rates of loss of radiosulfate from the plasma and the body, respectively.

Knowing the time at which the extra-urinary loss is maximum the maximum amount may be calculated.

$$Ut_{max} = R_0e^{t_{max}}(k_2 - k_1). \tag{12}$$

If one assumes that the extra-urinary loss of S^{35} is due to simple diffusion into a second fluid compartment, then it is reasonable to assume that there is no net exchange between the two compartments when the radioactivity in the second compartment reaches a maximum. At this time the concentrations in the two compartments would be equal and would be determinable since the concentration in one can be measured. Thus, knowing the total amount of S^{35} (by equation *12*) and the concentration of S^{35} in the second compartment, its volume can be calculated:

$$VU = \frac{Ut_{max}}{cPt_{max}}, \tag{13}$$

where:

VU = the volume of the "second radiosulfate space" in ml.

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