THE VOLUME OF DISTRIBUTION OF SODIUM THIOSULFATE AS A MEASURE OF THE EXTRACELLULAR FLUID SPACE[1]

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The importance of total body fluid and electrolytes and their distribution in the body cell mass has been increasingly emphasized in the care of acutely and chronically ill patients. In order to determine distribution of water and electrolytes, a convenient and accurate technique for the estimation of the extracellular fluid space is essential.

Moore (1) has demonstrated that the chemical dissection of the patient (to determine total body composition) is feasible by the dilution of radioactive and stable isotope tracers used in conjunction with a means for measuring the extracellular fluid. While techniques such as deuterium dilution, tritium dilution, and antipyrine dilution for the *in vivo* measurement of total body water can be checked for absolute accuracy against desiccation and specific gravity measurements (2), no such criterion for the accuracy of extracellular fluid measurements is available. Evaluation of extracellular fluid techniques must depend upon their reproducibility and compatibility with microanatomical studies and estimations of the volume of distribution of normal extracellular constituents.

In addition to interstitial and plasma water, the extracellular compartment must be considered to include the cerebrospinal fluid, water in the gastrointestinal tract, in glandular lumina, and bone matrix water. No technique is at present available which has been proven to measure the absolute volume of the entire compartment. Indeed at certain points the exact boundaries of the "compartment" defy anatomical definition. In the absence of an absolute standard for extracellular fluid measurement, reasonable criteria for assessing a particular method would be that it must (*a*) include a significant fraction of the compartment, (*b*) vary in proportion to changes in the extracellular space, (*c*) be insensitive to changes in cell permeability, and (*d*) be reproducible and technically convenient.

Previous studies by Newman, Gilman, and others (3–5) employing sodium thiosulfate have demonstrated (*a*) that its equilibrium volume of distribution is in the range of the extracellular fluid volume, (*b*) that its rate of disappearance after equilibrium of distribution is proportional to its concentration, (*c*) that it diffuses rapidly, and (*d*) that it is metabolized slowly and at an exponential rate (4).

These properties suggested that the thiosulfate ion would prove to be a suitable and convenient measure of the extracellular fluid by a technique not requiring either urine collections or a long period of constant infusion such as is necessary with inulin.

The present study was undertaken to examine the volume of distribution of sodium thiosulfate observed after a brief single injection and the collection of several serial blood samples over a short interval of time.

METHODS

Seven adult mongrel dogs ranging from 8.5–14.4 kgm. in weight were used in the preliminary experiments. Normal healthy male adult humans, ages 18–30, were studied for the arterio-venous difference, reproducibility and erythrocyte penetration studies, and hospital patients as specifically described in the next section were studied in febrile and edema states.

Twenty-five to 30 ml. of sterile 10% commercial hydrated sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$)[4] were injected into the dogs in one to two minutes. For human studies, 12 gm. of sterile sodium thiosulfate were diluted

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in 120 ml. of sterile pyrogen free water, 20 ml. withdrawn for determination of density and titration, and the remaining material administered by infusion after careful weighing to 0.01 gm. On completion of the 10–12 minute infusion, the infusion set was washed through three times with saline. Studies on a fourth rinse reveal less than 0.01% of the dose remaining.

Four to 12 blood samples were withdrawn from an indwelling needle (Cournand) in the femoral artery and venous blood from the femoral vein in dogs and the antecubital veins in man.

Samples in the preliminary studies were analyzed according to the indirect iodometric macro-method of Newman (3). In later studies it was found that a microtechnique afforded equal accuracy and required much less blood, thus permitting more samples and better definition of the curve without excessive blood loss. This technique [5] was modified from that suggested by Newman.

Calculation of the thiosulfate space

Venous serum concentrations are plotted semilogarithmically against the mid-time of sample collection and the equilibrium concentration obtained by extrapolation to the time of commencement of infusion (zero time). The plotted values must demonstrate linearity within the limits of error of the experiment or extrapolation is invalid. The extrapolated concentration is divided into the dose injected to obtain the volume of distribution.[5]

RESULTS

1. *Accuracy of chemical technique*

Duplicate samples were reproducible within an average range of 4 parts per 1,000. Recovery from plasma by this method averaged 96.5% with a range of 93.3–100%. Recovery from water averaged 98% ranging from 95–100%.

[5] See Appendix II.

2. *Expected accuracy in absolute quantities*

Accuracy in absolute quantities was estimated by a "dummy" measurement of 7,052 ml. This was done to indicate whether significant changes in volume of distribution might be masked by the error of the technique. Calculated values in quintuplicate averaged 7,265.3 ± 58.7 ml. This is an error of 213 ml. or 3.0%.

3. *Erythrocyte penetration*

Studies on erythrocytes incubated *in vitro* for 90 minutes at room temperature with oxygenation and gentle agitation indicated complete penetration of red cell water by sodium thiosulfate.

Studies *in vivo* were made by drawing blood 70 minutes after injection of thiosulfate, determining the hematocrit corrected for 3% trapped plasma, and splitting the sample, one-half of which was used for calculating plasma concentration. The other half was used for determining whole blood concentration, after hemolysis induced by alternate freezing and thawing. Table I summarizes the data obtained. Since at equilibrium of distribution, with complete penetration, the concentration of thiosulfate in both plasma water and red cell water will equal the concentration in whole blood water, the ratio of thiosulfate concentration in erythrocyte water to thiosulfate concentration in whole blood water was used as the index of penetration. In these experiments the ratios so obtained were 0.92, 0.42, and 0.62.

4. *Linearity of total clearance*

Preliminary studies were made on dogs employing an injection time of one to two minutes with

TABLE I

Penetration of thiosulfate into erythrocytes in vivo

Subject	Corr. hematocrit	Plasma $S_2O_3^=$	Whole blood $S_2O_3^=$	Plasma* water $S_2O_3^=$	Whole† blood water $S_2O_3^=$	Erythrocyte‡ water $S_2O_3^=$	EWT/WBWT§
		mgm./ml.	*mgm./ml.*	*mgm./ml.*	*mgm./ml.*	*mgm./ml.*	
D. H.	36	0.133	0.116	0.145	0.140	0.128	0.92
D. H.	39	0.142	0.100	0.154	0.121	0.051	0.42
J. R.	47.5	0.083	0.058	0.090	0.073	0.045	0.62

* Plasma water thiosulfate concentration = concentration of thiosulfate in plasma ÷ 0.92.

† Whole blood water thiosulfate concentration = concentration of thiosulfate in whole blood ÷ (0.92 [plasma volume] + 0.67 [red cell volume]).

‡ Erythrocyte water thiosulfate concentration = (thiosulfate content of 1 ml. whole blood − thiosulfate content in plasma fraction of 1 ml. whole blood) ÷ 0.67 × red cell volume per ml. of whole blood.

§ EWT/WBWT = ratio of the concentration of thiosulfate in erythrocyte water to the concentration in whole blood water.

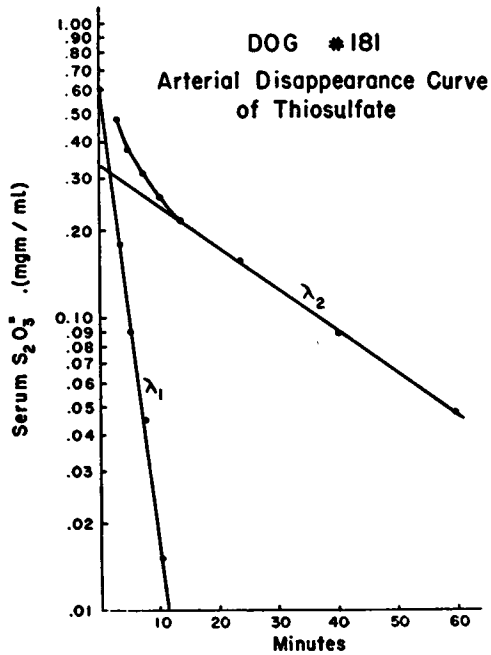

FIG. 1

Subject was a 13.0 kgm. dog. Twenty-five ml. 10% $Na_2S_2O_3 \cdot 5H_2O$ were injected into the femoral vein in one minute. Samples were withdrawn from the femoral artery through an indwelling (Cournand) needle and serum thiosulfate concentrations plotted against time. Curve analysis was by standard subtraction technique (11).

12 samples obtained from one to 70 minutes after the beginning of injection. In human subjects thiosulfate was administered by infusion lasting eight to 12 minutes and four to 12 arterial and/or venous samples were obtained. In 15 curves on seven dogs and 18 curves on 14 humans, linearity was demonstrated within the limits of error of the experiment. Typical examples of the resulting curves are presented in Figures 1 and 2.

TABLE II

Comparison of thiosulfate space determined simultaneously from arterial and venous blood levels

Subject	Arterial		Venous		Difference	
	Liters	% body weight	Liters	% body weight	Liters	% body weight
R. H. C.	10.88	17.5	10.00	16.1	−0.88	−1.4
R. H. C.	11.29	18.5	10.86	17.8	−0.45	−0.7
R. H.	10.50	13.3	10.33	13.0	−0.17	−0.3
J. R.	11.88	17.8	11.88	17.8	0.00	−0.0
T. D. B.	13.83	18.1	13.17	17.2	−0.66	−0.9
Mean	11.68	18.0	11.05	17.2	0.63	−0.8

5. *Effect of infusion time*

Infusion times in six reproducibility experiments are shown in Table IV. In general, infusions were set to run 10–12 minutes. Short infusions were avoided to minimize the increased renal loss with very high plasma concentration prior to equilibrium and because of the possibility of nausea and vomiting. The largest variation in infusion time was between 7.4 minutes on the first measurement and 12.6 minutes on the second measurement of one individual.

6. *Arterio-venous difference*

Samples in all of the preliminary studies on dogs were drawn through an indwelling needle (Cournand) in the femoral artery. In transferring attention to man it was of obvious advantage to use venous rather than arterial blood. Two studies on dogs showed a marked lag in the fall of venous as compared with arterial concentration. Typical simultaneous arterio-venous curves in man are shown in Figure 3, and volumes of distribution

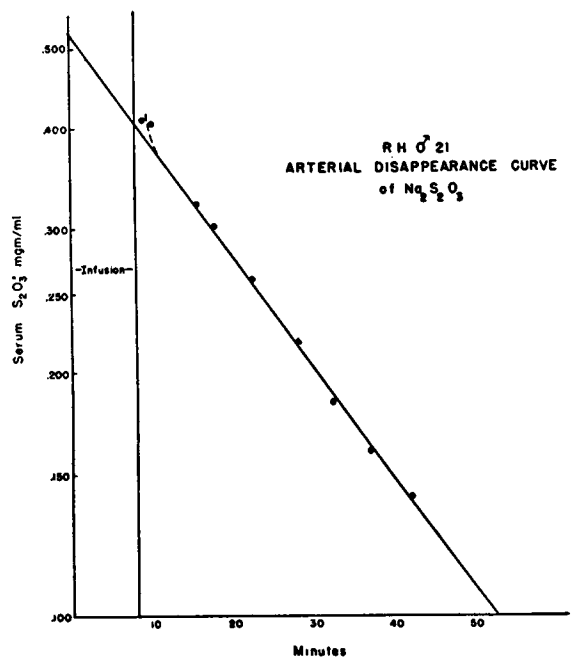

FIG. 2

Subject was a healthy male student, age 21. 102 ml. 10% $Na_2S_2O_3 \cdot 5H_2O$ were administered by infusion over 7.4 minutes. Samples were drawn from the femoral artery by an indwelling (Cournand) needle and serum thiosulfate concentrations plotted logarithmically against time.

TABLE III

Volume of distribution of sodium thiosulfate in dogs

Dog no.	Body weight	Thiosulfate space	
	kgm.	*liters*	*% body weight*
195	8.5	2.0	23.6
203	14.4	4.1	28.1
204	13.7	3.6	27.2
223	12.8	3.0	23.2
224	12.4	3.1	24.6
181	11.5	2.3	20.3
182	12.7	3.1	24.4
Mean	12.4	3.0	24.4

determined simultaneously from arterial and venous samples in four healthy young adult male humans are tabulated in Table II. The average volume of distribution was 0.63 liter (0.8% of body weight) smaller by venous concentration. The largest discrepancy in volume distribution was 0.9 liter or 1.4% of the body weight. At any one time the venous concentration of thiosulfate is higher than the arterial after distribution equilibrium has taken place (Figure 3). In addition, although not marked in every case, the venous concentration fell more slowly than the arterial. This discrepancy in rate of fall of thiosulfate concentration is less evident in the experiment given in Figure 3 than in some of the others studied.

7. *Volume of distribution and reproducibility*

The volumes of distribution of thiosulfate as determined in seven dogs under similar conditions are presented in Table III. The average value was 3 liters or 24.4% of body weight. Table IV shows the volumes of distribution in six healthy male adult humans studied on two occasions at various time intervals. As far as possible, subjects

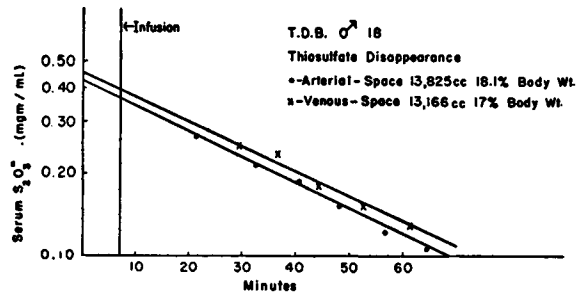

FIG. 3

Subject was a healthy male student, age 19. 101 ml. 10% $Na_2S_2O_3 \cdot 5H_2O$ were administered by infusion over 6.9 minutes. Arterial samples were drawn from the femoral artery using an indwelling (Cournand) needle. Venous samples were drawn from the antecubital veins and all serum thiosulfate concentrations plotted logarithmically against time.

in a "steady state" were chosen and no illness occurred between measurements. The average thiosulfate space was 12.2 liters or 16.6% of body weight and the average interval change of 0.42 liter or 0.38% of body weight is smaller than the expected general error of the measurement.

8. *Volumes of distribution in febrile patients*

Sensitivity of the thiosulfate ion to changes in cell permeability was tested in three hospital patients with febrile illnesses. Volumes of distribution are presented in Table V. An effort was made to choose patients free from any chronic underlying disease or complication which might affect fluid or electrolyte distribution or kinetics. All were adequately hydrated at the time of measurement but were not edematous. Volumes of distribution in these patients were 17.9, 18.4, and 15.9% of body weight.

TABLE IV

Volume of distribution and reproducibility of thiosulfate space in adult male humans

Subject	Age	First measurement							Second measurement							Difference		
		Date	Infusion time	Body weight	Surface area	Thiosulfate space			Date	Infusion time	Body weight	Surface area	Thiosulfate space					
	yrs.		*min.*	*kgm.*	M^2	*liters*	*% b. wt.*	L/M^2		*min.*	*kgm.*	M^2	*liters*	*% b. wt.*	L/M^2	*liters*	*% b. wt.*	L/M^2
R. H. C.	30	12/8/50	13.6	62.2	1.68	10.00	16.1	5.95	1/11/51	16.5	61.1	1.67	10.68	17.8	6.50	+0.68	+1.7	+0.55
J. R.	18	1/16/51	11.0	66.8	1.86	11.88	17.8	6.39	2/9/51	10.8	67.6	1.87	11.78	17.4	6.30	−0.10	−0.4	−0.09
T. D. B.	19	1/19/51	6.9	76.2	1.99	13.17	17.2	6.62	2/12/51	11.3	75.0	1.97	11.93	15.9	6.06	−1.24	−1.3	−0.56
G. C.	18	1/23/51	8.4	82.6	2.06	13.74	16.6	6.67	2/5/51	11.6	82.9	2.07	13.74	16.5	6.64	0.00	−0.1	−0.03
F. R. C.	21	1/22/51	12.4	76.7	2.00	12.48	16.3	6.24	2/8/51	11.1	75.2	1.98	11.79	15.7	5.95	−0.69	−0.6	−0.29
R. H.	21	1/26/51	12.6	81.2	2.04	13.39	16.5	6.56	3/6/51	7.4	80.6	2.03	12.05	14.9	5.94	−1.3	−1.6	−0.65
Mean			10.8			12.44	16.75	6.41		11.4			12.02	16.37	6.23	−0.42	−0.38	−0.18

TABLE V

Volume of thiosulfate distribution in febrile patients

Patient	Sex	Age	Diagnosis	Temp. range	Body weight	S_2O_3 space	
		yrs.		*° F.*	*kgm.*	*liters*	*% body wt.*
M. R.	F	18	Infectious mononucleosis	103	54.8	10.0	17.9
E. C.	M	68	Bronchopneumonia	101.8–103.5	63.2	11.5	18.4
S. K.	M	27	Post-lithotomy fever	101.4–102.4	72.0	11.5	15.9

9. Edema

As a first step in evaluating the relative accuracy of thiosulfate dilution as a measure of the extracellular fluid volume, patients with obvious edema were studied. Table VI lists the determinations carried out in five such patients. In these subjects the thiosulfate volume of dilution ranged from 14.6 to 20.7 liters, corresponding to 21.5–28.7% of the body weight. All of these values are considerably greater than those found in any of the normals. In two of the five subjects the volumes obtained were close to twice that obtained in the normal. In these instances the thiosulfate space has changed in the same direction and is of reasonable magnitude based on the clinical appearance of the subjects. It is of interest that none of these edematous patients showed thiosulfate spaces in the "excessive" range (over 35% of body weight) often found in observing the radiosodium space or thiocyanate space in sick patients. Similarly it is reassuring that they all manifested increases of an absolute magnitude comparable to the volume of clinical edema (diuresis).

DISCUSSION

I. Characteristics of thiosulfate as an "extracellular" ion

Sodium thiosulfate was first investigated as an antichemical-warfare agent and was found non-toxic even in very large doses, the only untoward effects observed being nausea and vomiting. These appeared when high concentrations were injected rapidly and were considered due to hypertonicity. It was also observed that the ion appeared in the urine at a rate which suggested that its excretion was a function of glomerular filtration. Thereafter it was extensively studied as a renal clearance test and found to have a clearance ratio to inulin and creatinine of 1.0 (both in normals and in renal and cardiac disease states [3–5]). Gilman, Philips, and Koelle (4) suggested and Brun (5) unequivocally asserted that the thiosulfate ion was confined to the extracellular space and not absorbed by the renal tubules. The former (4) calculated volumes of distribution using the amount recovered in the urine while Schwartz, using a constant infusion technique, estimated the space in dogs and man (6).

TABLE VI

Volume of thiosulfate distribution in edematous patients

Patient	Sex	Age	Diagnosis	Body weight	Thiosulfate space	
		yrs.		*kgm.*	*liters*	*% b. wt.*
M. L.	F	37	Lymphedema, etiology unknown	93.2	20.0	21.5
K. R. M.	M	42	Malignant hypertension	68.7	19.8	28.7
L. H.	F	25	Nephrotic syndrome	59.8	14.6	24.5
F. M.	M	74	Congestive heart failure	67.2	16.3	24.3
J. J.	M	70	Chronic nephritis, uremia	96.6	20.7	21.9

Contrasted with inulin which has a large molecular weight of 5,100 and a slow diffusion coefficient of 0.20/cm.²/day, thiosulfate has a molecular weight of only 135 and diffuses rapidly with a diffusion coefficient of 0.68/cm.²/day (7). In this respect it lies between inulin and the halides and light metals whose atomic weights are under 100 and which diffuse very rapidly at a rate of approximately 1.4/cm.²/day (7, 8).

Binding to plasma proteins similar to that which occurs with the thiocyanate ion has been investigated by Kowalski and Rutstein (9). Their *in vitro* studies indicate that no protein binding of thiosulfate takes place.

From the data presented in Table I it is apparent that significant penetration of thiosulfate into erythrocyte water occurs after 70 minutes. If penetration does not occur instantaneously, the extrapolation method used will correct for loss into erythrocyte water.

It is well known that thiosulfate crosses some cell membranes since it cannot be recovered com-

pletely from the urine. In a large series of dogs, Gilman, Philips and Koelle (4) were able to rerover 70–80% in the urine by the time the plasma concentration had fallen below 1.0 mgm.%. In dogs with ligated ureters he observed that the slope of disappearance was linear at 0.05% to 0.10% per minute and represented about 7% of the renal clearance. In this laboratory, 65% of the dose administered to a normal healthy adult male human was recovered by the time the plasma concentration was 1.0 mgm.% and only negligible amounts thereafter. This suggests that recovery in man may be slightly less than in the dog where extremely rapid elimination through the kidneys compared with the volume of distribution reduces the time during which significant metabolism may occur. Gilman and his associates (4) concluded that thiosulfate degradation occurs primarily during and immediately following injection and that after equilibration only slow destruction occurs. One observation offered in support of this postulation was the expanding volume of distribution from approximately 35% of the body weight at 30 minutes to 80% of the body weight at 120 minutes, noted in four dogs and calculated on the basis of the retained dose (the difference between the injected dose and the amount excreted in the urine). These data indicate that metabolism of thiosulfate during the post-equilibrium period must account for the expanding volume of distribution noted. In fact, if one plots their data on semi-logarithmic coordinates and extrapolates to zero time, the thiosulfate space so obtained is approximately 25% of the body weight, which agrees well with the values obtained in our series (24.4% of the body weight, *cf.* Table III) and with those obtained by these authors (4) in the same animals using the Newman formula (10). One is therefore forced to conclude that destruction of thiosulfate is uniform throughout the post-injection period. The discrepancy that remains unexplained is that between the rate of degradation of thiosulfate based on the slow fall in plasma thiosulfate concentration in the anuric dog and the rate of degradation based on the expanding volume of distribution during the post-equilibrium period with intact renal function.

II. The mathematical model

The volume of distribution of a hypothetical substance which could be completely and equally mixed throughout this volume instantly, could be expressed by:

$$V = \frac{A}{P} \qquad (1)$$

V = the volume of distribution,
A = the total substance introduced,
P = the concentration after mixing.

The volume of distribution of any substance which is very rapidly and freely diffusible throughout its volume and which disappears by any route at a rate proportional to its concentration may be determined by plotting its concentration semi-logarithmically against time and extrapolating to its theoretical equilibrium concentration at zero time for substitution in equation (*1*).

$$V = \frac{A}{P \text{ at } t_0}. \qquad (2)$$

That is, the volume of distribution is equal to the amount of material dissipated into the space divided by the concentration which would have occurred had it been instantly distributed throughout the volume at zero-time. The mathematical justification and experimental basis for equation (*2*) is implicit in the derivations of Newman, Bordley, and Winternitz (10) which relates the volume of distribution of a non-metabolized substance to the renal clearance and the time decrement of the natural logarithm of the plasma concentration and in the Schwartz (6) modification which takes into account clearance by all routes.

The primary error involved in applying equation (*2*) is the renal loss incurred at concentrations greater than that accounted for by extrapolation during the period of equilibration (Figure 1). For a rapidly diffusible substance equilibrium time is short enough to make this error insignificant. Employment of such a substance eliminates the necessity for constant infusion or urine collections. It permits the use of a substance lost by extrarenal routes (including metabolism) provided that cell penetration is slow as compared with the rate of distribution in the interstitial fluid. The studies reported in this paper indicate that sodium thiosulfate approaches the requirements for the use of this expression.

Examination of Figure 1 reveals that equilibrium occurs in 10–12 minutes in that the concentration falls logarithmically from this time on and that the

curve may be analyzed into "fast" and "slow" rates, the former probably representing transfer across the capillaries while the latter represents total clearance by all routes. The curve may be described by the general formula (11):

$$C_s = C_1e^{-\lambda_1 t} + C_2e^{-\lambda_2 t}, \qquad (3)$$

where C_s is the concentration at any time t in serum.

C_1 is the concentration obtained by extrapolation of the rapid component of the curve to time zero.

C_2 is the concentration obtained by extrapolation of the slow or equilibrium component of the curve to time zero.

λ_1 is the slope of the rapid component of the curve.

λ_2 is the slope of the equilibrium or slow component of the curve.

Since λ_1 is much faster than λ_2, the requirement of rapid distribution as compared to its total clearance is met. Further evidence indicating rapid equilibration as compared to total clearance is the close agreement for thiosulfate volumes of dilution given by simultaneous arterial-venous sampling. If there were a sizeable gradient across the capillary, one would expect significant arterial-venous differences.

Study was focussed on the equilibrium disappearance rate. The linearity of total clearance as observed by other investigators (3, 4, 6) was confirmed.

The single injection technique described in this paper eliminates the elaborate constant infusion equipment, the long equilibration period, and the addition of a large volume of fluid added to the extracellular compartment. Like the Schwartz technique (6), it does not require urine collections or bladder washouts and obviates the possible errors due to changing urine blank and renal delay time.

Its sole theoretical disadvantage is the possible error due to loss (by all routes) while the concentration of thiosulfate in the plasma is in excess of the extrapolated equilibrium value.

Validity of the single injection technique is dependent on three requirements: (*1*) The extracellular space must remain constant throughout the

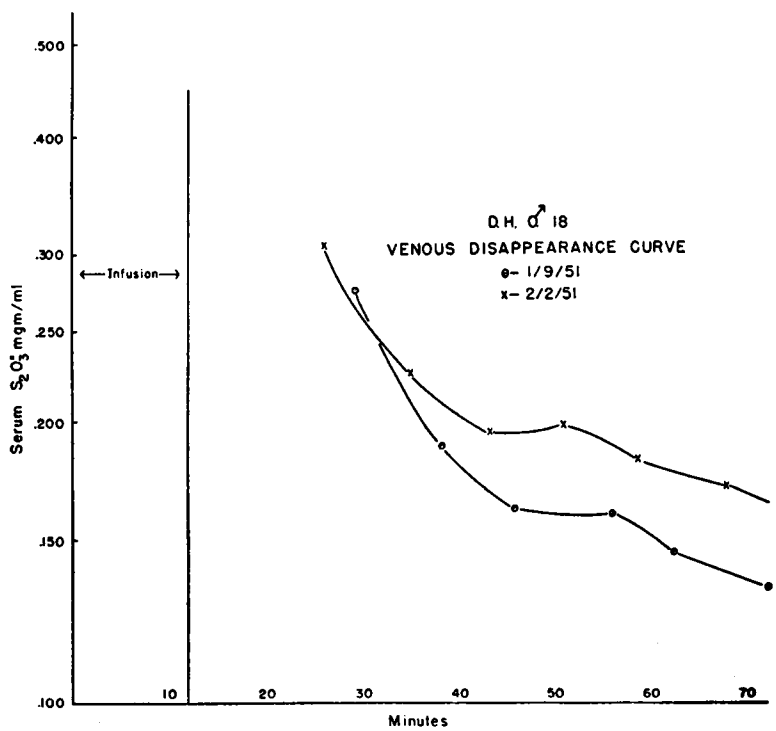

FIG. 4

Subject was a healthy male student, age 18. No history of renal or other disease. 102 ml. 10% $Na_2S_2O_3 \cdot 5H_2O$ were administered by infusion over 11.3 minutes. Samples were drawn from the antecubital veins and serum thiosulfate plotted logarithmically against time.

experiment. The small amount of fluid added by the technique described and the short time required for the measurement tend to insure this. (*2*) There must be uniform and rapid distribution of the substance throughout the space. This appears to occur in about 10 minutes for the thiosulfate ion. (*3*) The substance must be removed from its volume of distribution by all routes at an exponential rate. This has been shown to occur.

The slopes of decrement (in two studies on one subject) in which total clearance rate appears to have been variable are shown in Figure 4. Obviously this curve does not permit extrapolation to a zero-time value and it is anticipated that when such individuals are encountered clinically they will be at once recognized by the characteristics of the curve and no attempt made to estimate the volume of dilution by this method.

III. Evaluation of data and comparison with other techniques

Comparison of simultaneous arterial and venous rates of disappearance shows a consistent lag in the rate of fall of venous values, even though the difference in volume of distribution at zero-time obtained by extrapolation is insignificant, as described above. Brun, Hilden, and Raaschou working with diodrast in dogs (12) point out that the A-V difference is due to the presence in the right ventricle of cleared blood from the renal veins. The much greater difference in dogs is explained by the fact that the ratio of the renal clearance to the volume of distribution in dogs is 70/3 compared with 130/12 in man or almost 2.5 times as great in dogs. Hence a far larger part of the volume of distribution is being cleared in dogs per unit time.

The volumes of distribution of thiosulfate in dogs and man have been determined in this small series to be 24.4% and 16.6% of body weight, respectively. The latter figures were reproducible within 2% of the body weight after a month or more in a steady state. The lower volumes obtained by Schwartz in dogs (17% of the body weight) may be due to a greater fat content in his animals. Gilman, Philips, and Koelle (4) obtained volumes of 22% of the body weight in dogs, agreeing with our values and Schwartz obtained values of 15.7% and 19.5% in two human subjects, agreeing well with our series (Table IV). The volumes are close to those determined with inulin by Gaudino and other investigators (13, 14) of 21–23% for the dog and 13.5–17.5% for man. Turning to the other extracellular dilution indices, chloride, bromide, and sodium are excreted slowly but are known to enter cells to varying degrees and to be concentrated in certain cellular areas (14–18). The sulfate ion is endogenously produced at varying and unpredictable rates and is therefore unsatisfactory (19, 20).

Thiocyanate has been extensively studied as a measure of the extracellular compartment (21, 22). It diffuses rapidly and is slowly excreted permitting long equilibration (22). Its volume of distribution (22–27% of body weight) is similar to that for bromide and sodium which are known to have considerable intracellular fractions and is considerably larger than the inulin space where the large molecule is presumed to be limited to extracellular areas. Protein binding of thiocyanate has been demonstrated by Scheinberg and Kowalski (23). It is known to penetrate the red cell and to be concentrated in saliva and other gastrointestinal secretions (24).

Overman (25) has shown that in febrile states the permeability of the cell membrane to thiocyanate is altered and that its volume of distribution approaches the total body water. Three studies (reported herein), with thiosulfate in febrile patients, resulted in volumes of distribution in the normal range; hence it is probable that no gross changes in cell permeability to thiosulfate occur as a result of fever.

As part of the preliminary evaluation of this technique, five patients with obvious edema were studied. The finding of an increased volume of dilution in all of these patients supports the concepts that by this method an accurate estimate of the extracellular fluid volume can be made.

The final evaluation of this method will depend on the results obtained after wide application by many workers. Further studies on the rates of penetration of thiosulfate into fluid compartments and repeat studies in patients with known losses and/or gains of extracellular fluid are essential.

SUMMARY

A technique for measurement of the extracellular fluid space employing a single brief injection of sodium thiosulfate and collection of several small

blood samples over a short period is presented. This method obviates constant infusion and urine collection. Evidence is presented that sodium thiosulfate is a suitable substance for the relative measurement of the volume of extracellular water.

While there was a consistent lag in the fall of venous concentration behind arterial concentration, no significant difference in the volume of distribution obtained by extrapolation was demonstrated.

The volumes of distribution of sodium thiosulfate in seven dogs and six humans averaged 3 liters or 24.4% of body weight and 12.2 liters or 16.6% of body weight, respectively.

Thiosulfate space in patients with fevers of 102–103.5° F. were in the normal range and in patients with edema were well above the normal range. The volume of distribution of thiosulfate in edematous patients was compatible with known increase in weight and clinical estimates of edema fluid.

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SINGLE INJECTION TECHNIQUE FOR MEASUREMENT OF THE VOLUME OF DISTRIBUTION OF THIOSULFATE

APPENDIX I

Preparation—(under sterile conditions): Twelve 1.0 gm. ampules of $Na_2S_2O_3 \cdot 5H_2O$* are dissolved in a sterile beaker with 120 ml. sterile pyrogen-free water and thoroughly mixed. Twenty ml. are withdrawn for the determination of the density of the solution and titration of the concentration of thiosulfate. The remainder is used to fill a calibrated 100 ml. syringe.

Administration: An infusion is started with saline or 5% glucose using an open-top set. When the chamber of the infusion set is empty, the thiosulfate solution is quickly introduced from the calibrated syringe.

A stop-watch is started when the thiosulfate solution reaches the needle and this is considered zero-time for calculations.

Flow should be regulated, before addition of the measured thiosulfate, to approximate seven to 10 ml. per minute so that the infusion will last ten to 15 minutes. Upon completion of the infusion, the time should be noted and the set washed through with 20–30 ml. of normal saline or 5% dextrose solution three times.

Samples: Blood samples should be 1–2 ml. in volume. The blank titration is considerable (about 0.04 mgm./ml.). A blank should be drawn at the time the needle is inserted for the infusion. Venous samples must be drawn without stasis and midtimes recorded. Between samples the arm should be kept under a blanket. A minimum of four samples evenly spaced over 20–70 minutes after the start of infusion should be drawn.

APPENDIX II

Chemical Method

I. Reagents

1% Na_2WO_4
$\frac{N}{15}$ H_2SO_4
0.001 N (exact) KIO_3
0.0005 N $Na_2S_2O_3$
2 N HCl
10% KI
1% soluble starch.

Comment: The KIO_3 must be made up precisely and is stable. The $Na_2S_2O_3$ tends to deteriorate and must be checked at the beginning and end of each run. Deterioration is due to sulfur-metabolizing bacteria and may be slowed by using sterile distilled water and adding 0.1 gm. Na_2CO_3 per liter. KI should be prepared freshly with each set of determinations and kept in a brown or opaque container.

The starch solution is prepared by boiling. It is then centrifuged and decanted, and can be stored on ice for a few days.

II. Procedure

A. Protein-free filtrates

Samples are centrifuged as soon as possible and clear serum must be obtained.

To 2 ml. $\frac{N}{15}$ H_2SO_4 in 16 ml. H_2O, add 0.2 ml. serum slowly with agitation.
Then add 2.0 ml. 1.0% Na_2WO_4 slowly with agitation.
Permit to stand 10 minutes, centrifuge, decant, and save clear supernatant fluid.

B. Infused solution

The weight of the infused thiosulfate solution contained in a calibrated 10 ml. pipette is determined on a chemical balance and the density calculated.

Quadruplicate 0.10 ml. aliquots are then weighed to 0.1 mgm. and diluted in liter volumetric flasks with distilled water and 8 ml. aliquots of both dilutions titrated as below.

* Sulfactol, Winthrop-Stearns, Inc., N. Y. C.

*C. Titration**

To 5 ml. 0.001 N KIO_3, add 8 ml. protein-free filtrate and 1 ml. 2 N HCl.

Let stand at least five minutes and not more than 10 minutes.

Then add

1 ml. 10% KI and titrate immediately with 0.0005 N $Na_2S_2O_3$ using 2 to 3 drops 1% starch indicator.

Comment: Duplicate 8 ml. aliquots of the diluted infused solutions, serum blank, serum samples, and a reagent blank are titrated. Starch should not be added until the yellow iodine color has almost disappeared.

III. Calculations

$$A = W_I \times C_I, \qquad (1)$$

where: A = mgm. of $S_2O_3^=$ administered.

W_I = volume in ml. delivered by the calibrated 100 ml. syringe

C_I = concentration in mgm./ml. of the infused thiosulfate solution.

$$C_I = (t_s - t_u)\left(\frac{0.158}{8}\right)\left(\frac{5}{t_s}\right)\left(\frac{D.F.}{8}\right) \qquad (2)\dagger$$

$$C_p = (t_B - t_u)\left(\frac{0.158}{8}\right)\left(\frac{5}{t_s}\right)\left(\frac{100}{8}\right), \qquad (3)\dagger$$

where: C_p = concentration in mgm./ml. of thiosulfate in serum.

t_s = ml. of 0.0005 N $Na_2S_2O_3$ required to titrate 5 ml. of 0.001 N KIO_3 (reagent blank).

t_u = ml. of 0.0005 N $Na_2S_2O_3$ required to titrate unknowns other than serum blank.

t_B = ml. of 0.0005 N $Na_2S_2O_3$ required to titrate serum blank.

D.F. = dilution factor, obtained by dividing 1000 by the weight of the aliquot and this divided by the density of the infused solution.

$$V = \frac{A}{C_{Po}}, \qquad (4)$$

where: V = volume of distribution for thiosulfate

C_{Po} = concentration in mgm./ml. of thiosulfate in serum at zero-time. This is obtained by plotting on semi-log coordinates the concentrations of thiosulfate in the serum samples against the mid-point of the time at which they were drawn and extrapolating to zero-time.

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* All glassware must be extremely clean and oxidizing agents must be avoided.

† For derivation of formulas 2 and 3 see Newman, Gilman, and Philips (3).

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