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Selenate as a Substitute for Sulfate in the Measurement of Extracellular Fluid Volume[1,5]

Solomon N. Albert, M.D., Chalom A. Albert, M.D., Erwin F. Hirsch, M.D.,[2] Ira N. Brecher,[3] M.D., Paul Numerof, Sc.D.[4]

Washington, D. C.

Extracellular fluid (E.C.F.) volume constitutes approximately 20 per cent of total body weight. Three-fourths of this volume of fluid (15% of body weight) is distributed in the interstitial space which is in constant equilibrium, across the capillary membrane, with the intravascular water content (five per cent of body weight) and the cellular membrane with the intracellular fluid space (1,2,34), (Fig. 1).

In recent years, great efforts have been made to measure extracellular fluid (E.C.F.) volume and to determine its significance in clinical medicine and the management of fluid therapy (23,31,42). E.C.F. volume is a reservoir of water and electrolytes. A major portion lies in the interstitial space between the cellular elements and the intravascular space. This latter volume of fluid serves to maintain tissue hydration and circulating blood volume. The transfer of nutrition and excretion of metabolites between the cellular elements of tissues and the blood stream takes effect through this buffer zone of the extracellular extravascular fluid space.

In recent years, various ions (3,4,5,6,7,8,9) and different molecular-size compounds (10,11,12,13,14,15) have been utilized to measure the E.C.F. volume by the dilution principle. Walser et al (16) introduced the sulfate ion labeled with ^{35}S as a tracer which rapidly equilibrates with a volume of fluid, designated as a sulfate water space, that seems to approximate the accessible portion of the E.C.F. volume.

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[1]Supported in part by Joseph L. and Ray L. Freund Foundation.

[2]Research Fellow in Surgery, Washington Hospital Center.

[3]Resident in Surgery, Washington Hospital Center.

[4]Manager Radiopharmaceutical Services, E. R. Squibb & Sons.

[5]From the Anesthesiology Research Laboratory and the Surgical Research Division—George Hyman Memorial Research Foundation, Washington Hospital Center.

Shires et al (17,18,19,20,21,22) measured the sulfate fluid space in hemorrhagic shock and demonstrated the importance of proper blood replacement and fluid therapy, in an attempt to replace the measured deficit in the functional E.C.F. volume. Walser et al (23) have demonstrated early accumulation of fluid in the E.C.F. space with radiosulfate in heart failure.

Sulfur-35 emits only weak beta particles. To determine the concentration of ^{35}S in blood, plasma must be separated, evaporated, and the residue counted in a gas flow counter.

An attempt was made to substitute ^{75}Se for ^{35}S. Selenium-75 decays mostly by electron capture with a half-life of 127 days (24). Gamma rays of at least ten energies are emitted in the decay, but three are of interest and they may be detected easily in a well-type scintillation counter. They are: 0.269 MeV (71%), 0.281 MeV (5%), and 0.405 MeV (14%).

Selenium seems to act biologically in a manner similar to sulfur. The existence of many biologically-active selenium analogues of sulfur compounds attests the closely related biochemical behavior of these two elements (25,26,27, 28,29). Galambos has demonstrated a parallel labelling of nondialyzable components of rabbit urine following ^{75}Se and sulfate (^{35}S) injection (30).

In this report data are presented to demonstrate that the portion of the E.C.F. measured as a selenate space closely approximates the established volume of fluid designated as a sulfate space (16). The technique is simplified and the use of ^{75}Se permits measurement on whole blood samples. Selenium-75 lends itself to triple tracer studies, especially—in this situation—to the quantitative measurement of iodinated albumin (^{125}I), ^{51}Cr-labelled cells, and sodium selenate (^{75}Se). The three tracers can be differentiated by pulse height analysis (32,33).

METHOD AND MATERIAL

The rate of equilibration, elimination, and volume of fluid measured expressed as selenate space was studied in healthy mongrel dogs.

COUNTING TECHNIQUES

A scintillation detector with a two inch Tl-activated NaI crystal was modified by placing a hollow Pb shield in front of the crystal (Fig. 2) with two openings five inches apart that would lodge 6ml plastic syringes.[2] Counting and settings to discriminate three isotopes, ^{51}Cr, ^{75}Se and ^{125}I were performed by changing the MeV range on a Picker Spectroscaler III. Sodium selenate (^{75}Se) was prepared, by E. R. Squibb & Sons, with a specific activity of 7,000 mC per gram of selenium. Six-ml aliquots of standard prepared by drawing the solution into the plastic syringe to the 6 ml mark was counted in the distal opening B for three minutes. Venous blood, drawn to the 6 ml mark in plastic syringes were counted in the front opening for three minutes. The three isotopes could be adequately differentiated by simply changing the gain level. Blood samples were, therefore, counted on three different MeV ranges: 2, 1, and 0.25. The pulse height window was calibrated by adjusting the high voltage using a ^{137}Cs source.

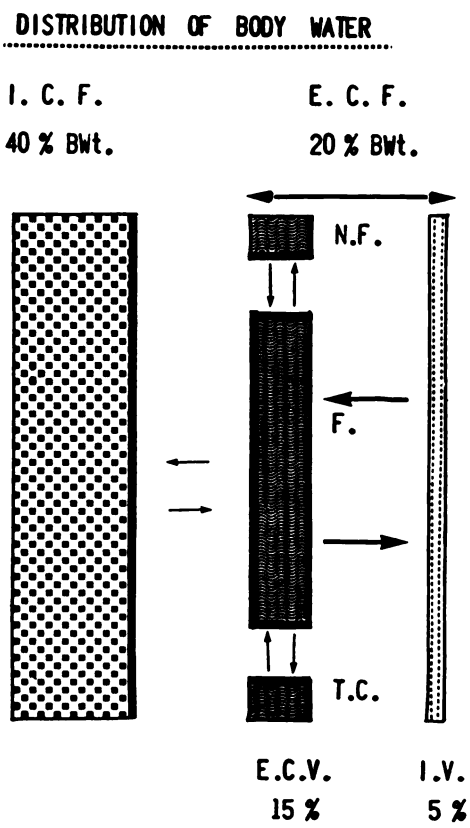

Fig. 1. Body water constitutes approximately 60 per cent of body weight. This is subdivided into the following components: I.C.F., intracellular fluid, 40 per cent of body weight, separated from the E.C.F. space by a slow transfer membrane; E.C.F. extracellular fluid, 20 per cent of body weight, is subdivided into two compartments: 1) I.V., intravascular fluid, five per cent of body weight, separated from the functional E.C.V., extracellular-extravascular space, by a rapid transfer membrane, 2) E.C.V., extracellular-extravascular fluid, 15 per cent of body weight, comprises (a) F, a large functional fluid volume, (b) N.F., nonfunctional volume, i.e. E.C.V. fluid found in connective tissue, cartilage, tendons and bones and (c) T.C., transcellular fluid, found in cerebrospinal fluid, vitreous of eyes and gastrointestinal mucosa.

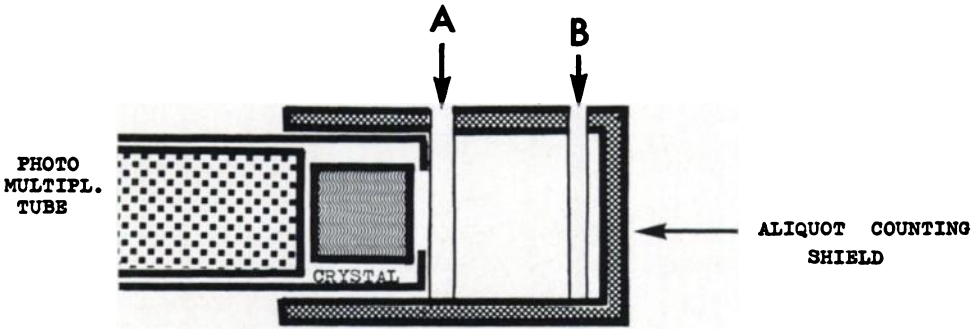

Fig. 2. Modified scintillation counter for measuring samples in syringes. A. Front position for measuring dilution samples. B. Back position for measuring standard.

Although the energies of ^{75}Se and ^{51}Cr are close, we were able, by utilizing Table I, to differentiate and calculate the concentration of each tracer separately. Since the concentration of ^{75}Se is much higher, it was found more practical in *in vitro* experiments to count the ^{75}Se standard separately and count the ^{51}Cr and ^{125}I combined. Table I presents the proportions of spillover of each isotope for the three different MeV ranges obtained in this particular counting system. Window setting was maintained at 16% and the lower level range at 0.80.

TABLE I

Gain Level	^{51}Cr	^{75}Se	^{125}I
2	100%	73%	0
1	29.7%	100%	<.1%
0.25	6.5%	5.9%	100%
[3]Front to Back Ratio	55	63	73

[2]Monoject 6ml plastic syringes (506-S), Roehr Products Co.

[3]Count rate relationship when samples were counted in front position (A) to the count rate obtained on the same sample in back position (B) (Figure 2).

According to Table I we have three equations for three unknowns. The concentration of each tracer can be calculated by substituting values according to the count rate obtained on each MeV range.

Since our initial studies were geared to establish the feasibility and the validity of a selenate space to equate the sulfate space, only two tracers were used, sodium selenate (^{75}Se) and iodinated albumin (^{125}I). Measurement of the standard and whole blood samples were performed on MeV ranges of 1 and 0.25 as per Table I. The spillover of ^{125}I on MeV range of one was ignored and considered zero.

CALCULATIONS

The results presented in Tables II and III, in columns A and B were calculated by two different methods.

Extended Method of Calculation—(Column A in Tables II and III)

Calculations are based on ^{75}Se count rate extrapolated to zero time taking two points of reference: the 20 and 40 minute samples count rate (Figure 3).

A.1 Plasma water = Plasma vol. measured with ^{125}I albumin[1] × (1–Protein[2])

$$\text{A.2 Count } ^{75}\text{Se in plasma water} = \frac{\text{Extrapolated } ^{75}\text{Se Count in Blood}}{(1 - \text{Hct}) \times (1 - \text{Protein})}$$

[1]Plasma volume calculated from the ^{125}I standard injected and the dilution count corrected for the ^{75}Se spillover and the dilution count further corrected to zero time (44,45).

[2]Protein content of plasma measured by refractometer, Bausch & Lomb.

A.3 Total ^{75}Se counts in plasma water =
Count rate ^{75}Se in plasma water (A2) × plasma water (A1)

A.4 Count rate ^{75}Se in extracellular extravascular fluid space =
^{75}Se count rate in plasma water × 1.115 (Gibbs-Donnan factor)

A.5 Amount of ^{75}Se injected in extracellular extravascular space =
^{75}Se standard injected − ^{75}Se count in plasma water (A3)

A.6 Extracellular extravascular fluid volume =
$$\frac{\text{Total } ^{75}Se \text{ in extravascular space (A5)}}{\text{Corrected } ^{75}Se \text{ count in extravascular space (A4)}}$$

A.7 E.C.F. Volume (Selenate space) =
Extravascular fluid volume (A6) + Plasma water (A1)

Simplified Method of Calculation—(Column B in Tables II and III)

The selenate fluid volume was established by equation B1 (16,23) based on the count rate obtained on a 20 minute blood sample corrected for:

a. an average five per cent loss during the 20 minute equilibration period
b. the Gibbs-Donnan factor 0.90
c. the correction factor for water content of plasma 0.93.

B.1 $$Vd = \frac{\text{Counts injected} \times 0.95}{\text{Counts in plasma} \times (0.9 \times 0.93)} = 0.8 \frac{\text{Counts injected}}{\text{Counts in plasma}}$$

RESULTS

Eleven mongrel dogs weighing between 9.1 and 22 kgs were kept fasting for 12 hours after being received from the dog pound. They received 12 μC of sodium selenate (^{75}Se) and 1.5 μC of iodinated albumin (^{125}I) per 10 kgs in a 6 ml. volume.

RATE OF EQUILIBRATION

Venous blood samples were drawn at 5, 10, 20, 30, 40, 60 and 90 minutes intervals from an indwelling catheter; blood samples were also drawn at the time the animals were sacrificed. The rate of equilibration is presented in Figure 4, an average of 10 animals studied. This curve closely approximates the curve obtained with sodium selenate (^{75}Se) as presented by Walser *et al* (16) and Ryan (31). In some instances, there was a slight rise in count rate in the following 24 hour period. The rate of elimination of ^{75}Se in urine, presented in Figure 4, fluctuated between 0.5 — 7 per cent of the total dose injected; this varied between individual animals and with the state of hydration.

The exponential decay curve is presented in Figure 3. The curve is extrapolated to zero time taking the 20 and 40 minute count rate.

Table II presents the results obtained as E.C.F. selenate space percent body weight. Column A calculated according to the extended method of calculation (A1 thru A7) and Column B calculated according to equation (B1).

TABLE II

24 Hours After Receiving From Pound Fasting 12 Hours

		A		*B*	
Dog #	*Wt. in Kgs.*	*E.C.F. % Wt.*	*E.C.F. Volume*	*E.C.F. Volume*	*E.C.F. % Wt.*
1	18.20	18.5	3375	3901	21.4
2	15.90	19.3	3070	4045	25.4
3	19.90	18.3	3650	4200	21.0
4	9.10	18.3	1640	1872	20.6
5	15.90	18.0	2866	3107	19.5
6	15.30	18.8	2883	3326	21.7
7	18.20	19.3	3521	4087	22.4
8	22.00	18.6	4112	4547	20.6
9	17.30	18.0	3116	3806	22.0
10	16.25	18.8	3069	3464	21.3
11	16.00	19.0	3090	3198	20.0
Mean.		18.6			21.5
S.D.	±0.44				±1.49
Fluct.	−.63 cc to +.67				−1.9 to +3.95

TABLE III

Repeat Measurement After 3 Days
Hydration & Feeding
6 Hours Fasting

		A		*B*	
Dog #	*Wt. in kg.*	*E.C.F. % Body Wt.*	*E.C.F. Volume*	*E.C.F. Volume*	*E.C.F. % Body Wt.*
1	20.00	22.9	4586	4866	25.8
2	16.30	22.2	3627	4232	25.9
3	12.00	22.1	4435	4779	23.9
4	9.10	19.6	1780	1790	19.7
5	16.20	20.3	3299	3299	20.3
6	15.40	20.4	3150	3256	21.0
Mean		21.2			22.7
S.D.	±1.2				±2.55

Table III presents the results obtained on six dogs, one through six, on repeat determinations following three days of feeding, hydration and fasting for three hours prior to performing the measurement.

Table IV presents individual studies to compare changes in measured ^{75}Selenate volume changes as they occur with loss of fluid by starvation, evaporation and fluid replacement.

DISTRIBUTION OF ^{75}SELENATE IN TISSUES

Table V presents the amount of ^{75}Se in tissues. Each figure is the average of three specimens taken on three dogs sacrificed at 3, 6, 24, 48, and 96 hours. Calculations were reduced to a common denominator.

The percentage of dose retained is expressed per 100 gms of whole tissue on the basis of 12 μC of ^{75}Se/10 kg body weight.

DISCUSSION

Extracellular fluid volume is an ill-defined component of total body water which comprises several fractions, a static extracellular fluid volume, transcellular water and a functional extracellular fluid volume. Each fraction constitutes an entity in itself which accommodates variable proportions of the different molecules or ions utilized as tracers (1). The estimated volume measured varies with the rate of equilibration, permeability of the capillary membrane, size of the functional capillary bed, blood flow, metabolism of the tracer and the rate of flow of the tracer across the cellular membrane. Accurate measurement of the extracellular fluid volume entails many variables (34). Of practical value is the

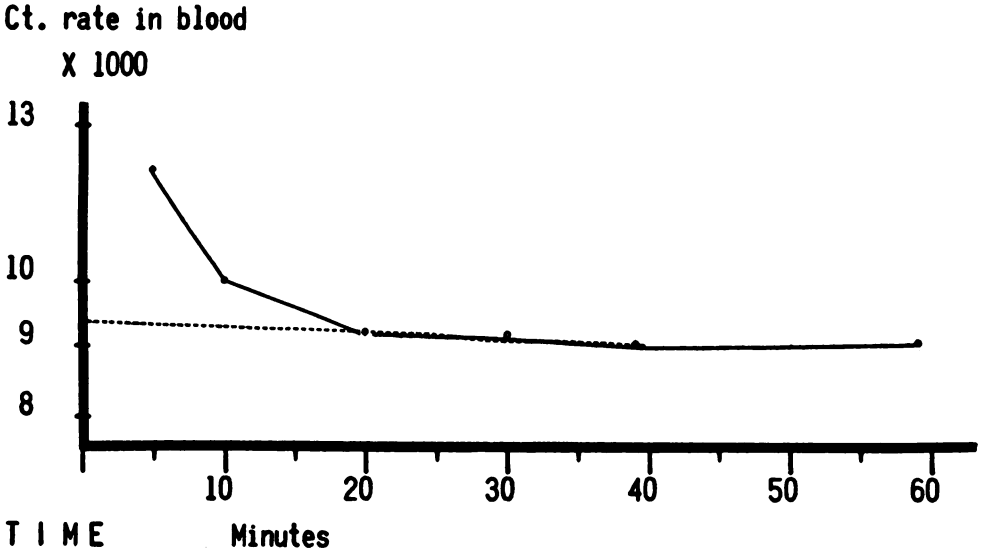

Fig. 3. Decay curve of ^{75}Se in venous blood. Curve extrapolated to zero time from the count rate obtained on the 20 and 40 minute blood samples to determine the adjusted concentration of ^{75}Se.

measurement of the functional available fluid volume that acts as a buffer zone, as a reservoir of fluid and electrolytes to maintain cellular hydration and blood volume.

This functional extracellular fluid volume should be correctly described as the fluid volume determined by dilution of the specific tracer utilized. In this report, we are referring essentially to a sulfate space which seems to reflect the portion of extracellular fluid volume, a functional volume, which equilibrates rapidly with the intravascular fluid volume. Shires *et al.* have demonstrated that this reservoir of fluid is altered in acute conditions and other investigators have shown changes in distribution and retention of fluid in chronic pathological conditions (11,23).

Undoubtedly, exogenous sulfate labelled with ^{35}S is metabolized and eventually appears in various enzymes, amino acids and proteins. Selenium resembles closely, both in chemical and physical properties, ^{35}S as characterized

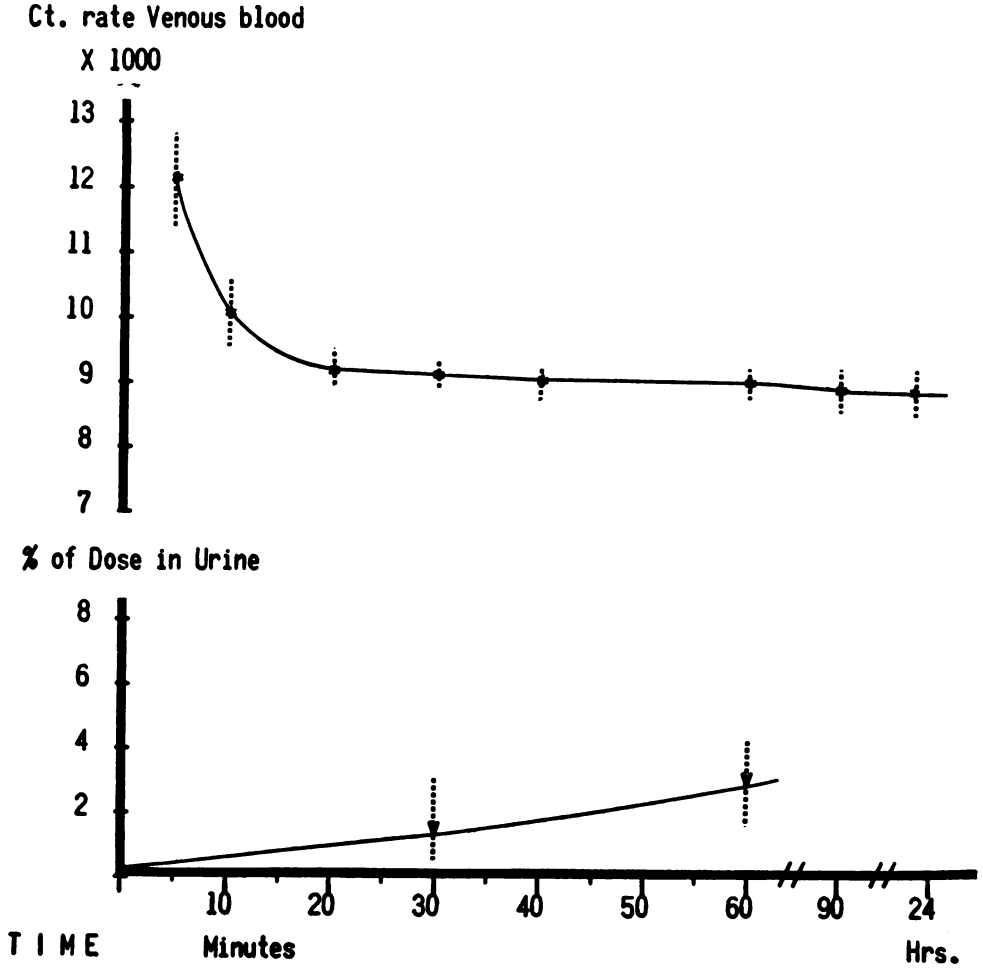

Fig. 4. Rate of equilibration of ^{75}Se in blood and the accumulated urine excretion.

TABLE IV

Repeat Studies			Special Studies Cases							
			A			B				
Dog #	*Wt./Kgs*	*Remarks*	*E.C.F. % Body Wt.*	*E.C.F. Volume*	*Plasma Volume*	*E.C.F. Volume*	*E.C.F. % Body Wt.*	*Hct.*[1] *%*	*Prot.*[2] *Gm. %*	*mOs./L*[3]
12	15.0	Repeat Study	19.3	2891	707	3255	21.5	33.5	5.9	305
		on Anesth. Dog	19.3	2937	700	3105	20.7	34.0	5.7	305
13	20.0	Hydrated Dog.	22.2	4435	1047	4779	23.9	27.5	5.2	303
		Received 100cc Ringer's Sol.	22.9	4586	948	4866	24.3	27.5	5.0	304
		Rec. 500cc Ringer's Sol.	25.9	5198	1017	5223	26.1	27.0	4.8	296
14	16.3	Repeat Studies Evaporated Control	19.0	3090	812	3198	19.6	36.0	5.3	305
	15.6	2 Hrs Abd. Cont. Exposed	16.8	2620	809	2620	16.1	37.5	5.8	314
	16.2	600cc Saline Inf.	17.5	2840	792	3005	18.5	35.0	5.6	299
15	9.1	Repeat Studies Hydration	18.3	1640	443	1872	20.6	40.0	5.5	300
		250cc Ringer's Sol.	19.6	1790	400	1790	19.9	44.0	5.9	303
16	15.9		18.0	2866	592	3107	19.5	48.0	6.8	325
		500cc Ringer's Sol.	20.0	3221	615	3196	20.0	46.0	6.6	315

TABLE IV—*Continued*

Dog #	Wt./Kgs	Repeat Studies Remarks	Special Studies Cases A E.C.F. % Body Wt.	A E.C.F. Volume	Plasma Volume	B E.C.F. Volume	B E.C.F. % Body Wt.	Hct.[1] %	Prot.[2] Gm. %	mOs./L[3]
17		Repeat Studies After Exposure 6 Hours.								
	11.36		16.3	1837	485	2118	18.7	44.4	6.2	320
		100cc Ringer's Sol.	16.3	1842	499	2174	19.2	43.6	6.2	321
		500cc Ringer's Sol.	20.0	2276	594	2530	22.6	42.0	6.0	310
18	16.3	Control Hydrated	22.2	3627	865	4232	25.7	35.2	5.3	308
	15.3	48 Hrs. Fasting	18.8	2883	820	3326	21.6	37.0	6.0	315
	16.3	4 Days later feeding & hydration	20.0	3299	693	3502	21.5	39.0	5.5	320

[1]Microhematocrit method corrected for trapped plasma.
[2]Refractometer method—Bausch & Lomb.
[3]Freeze Osmometer—Advance Inst., Inc.

by the inorganic and organic compounds they form. Although Se follows a metabolic pathway distinct from that of S, analogous compounds containing Se and S respectively, may under prescribed conditions follow identical metabolic routes (25,26,27,28,29,30).

The specific activity of the sodium selenate (^{75}Se) used in this study was 7,000 mC/g of selenium, or 7μC/μg. Doses for each determination were calculated on the basis of 1.2μC/kg, or 0.17 μg of selenium. The suggested dose in humans for a single volume determination will be approximately 1 μC/kg, containing 0.143 μg of selenium. This amounts to about 10 μg of selenium for an adult weighing 70 kg. This amount of selenium is small when compared to 1 μg/kg, or 70 μg, of selenium administered for scanning the pancreas. Total-body absorbed radiation dose resulting from three doses of sodium selenate (^{75}Se) for measuring the selenate fluid volume would amount to less than half the absorbed radiation from a single administration of selenomethionine (^{75}Se).

The toxicity of selenium is well documented (35,36,37,38,39). It seems that selenium acts by substitution of sulfur in enzyme systems containing a sulfhydryl group (25). In the mouse, 800 μg/kg selenium produced ataxia immediately after injection, some collapsed for approximately one minute and all appeared normal within 10 minutes. None died during the 10-day observation period. In dogs, a single intravenous injection of an aqueous solution of selenomethionine of 12.5 to 100 μg/kg of selenium showed no gross reactions (25).

TABLE V

Average Weight of Animals: 15 Kgs.
Dose: 12μC ^{75}Se/10 Kgs
Per Cent of Dose in 100 Gms of Tissue

Organs	*Av. Wt. Gms.*	*3 Hrs.*	*6 Hrs.*	*24 Hrs.*	*48 Hrs.*	*96 Hrs.*
Lungs	120	1.7×10^{-3}	1.42×10^{-3}	2.28×10^{-3}	5.00×10^{-4}	2.00×10^{-4}
Heart	85	0.9×10^{-3}	5.1×10^{-4}	1.00×10^{-3}	8.5×10^{-4}	6.43×10^{-4}
Liver	550	3.6×10^{-3}	2.2×10^{-3}	2.65×10^{-3}	2.1×10^{-3}	1.02×10^{-3}
Spleen	40	1.27×10^{-3}	3.4×10^{-3}	2.3×10^{-3}	1.3×10^{-3}	1.29×10^{-3}
Pancreas	40	1.95×10^{-3}	2.1×10^{-3}	1.3×10^{-3}	8.2×10^{-4}	6.6×10^{-4}
Adrenals	3.2	5.7×10^{-4}	6.8×10^{-4}	6.4×10^{-4}	5.3×10^{-5}	0.9×10^{-5}
Kidneys	127	8.1×10^{-3}	4.35×10^{-3}	9.15×10^{-3}	8.6×10^{-3}	7.3×10^{-3}
Small Bowel	43	5.6×10^{-3}	6.2×10^{-3}	2.17×10^{-3}	1.9×10^{-3}	1.3×10^{-3}
Large Bowel	108	———	2.78×10^{-3}	1.98×10^{-3}	6.00×10^{-4}	3.2×10^{-5}
Thyroid	1.3	1.3×10^{-3}	9.2×10^{-4}	9.6×10^{-4}	7.6×10^{-4}	5.2×10^{-4}
Brain	80	———	5.68×10^{-5}	2.8×10^{-3}	3.62×10^{-5}	1.72×10^{-5}
C.S.F.	32	———	1.81×10^{-2}	3.10×10^{-4}	———	———
Vitreous	1.8	———	2.00×10^{-4}	———	———	7.4×10^{-6}
Bile	8	2.8×10^{-4}	4.72×10^{-4}	6.00×10^{-4}	3.2×10^{-5}	———
Muscle		3.9×10^{-4}	2.93×10^{-4}	2.00×10^{-4}	1.96×10^{-4}	1.82×10^{-4}
Urine Excretion			0.5 – 3%			
Small Bowel Content			1.69×10^{-2}			
Total Urine and Feces Excreted				4 – 5%	15.3	21.8
% Dose remaining in body			97.5	95.0	83.1	74.8
Biological Half Life : 7.2 days.						

From the data presented in this report, sodium selenate seems to be excreted in bile and is apparently reabsorbed from small bowel (40). The slight rise in concentration of ^{75}Se in blood samples, at times encountered in the first 24 hour period, is probably due to the reappearance of ^{75}Se labeled compounds in blood by reabsorption and metabolism. Within 6-24 hours, ^{75}Se reappears in blood attached to the red cells and to protein fractions of plasma. This has also been shown to take effect when the selenium is administered as selenomethionine (41). From Table IV one can note that a portion of the ^{75}Se is found in transcellular fluid, C.S.F. and vitreous of the eye within 3-12 hours, which seems to be transient and disappears after 24 hours as noted in Table V.

Urine excretion and elimination of the ^{75}Se when administered as selenate is variable and seems to approximate the rate of excretion seen with radiosulfate (16).

The extent of distribution and the dilution volume measured with sodium selenate labeled with ^{75}Se closely approximate (Tables II, III) established values obtained with sulfate labeled with ^{35}S on dogs—20.1 $\pm$ 2.1% of body weight (16). It is debatable whether the extended method of calculations based on a corrected count rate (A1-A7) is necessary in order to improve accuracy. The measured dilution is not so critical as to warrant taking into account all possible factors expressed in calculations (A1-A7); and the short simple formula (B1) might serve the purpose in this respect.

The advantage for substituting sodium selenate labeled with ^{75}Se over sodium sulfate labeled with ^{35}S are:

1) the simplicity by which one can detect a radioactive element having only gamma emissions.
2) the facility of accurately and quantitatively measuring ^{75}Se with commercially available and modestly priced equipment.
3) Selenium-75 lends itself for use in studies where two or more tracers are utilized.

It is interesting to note that following 48 hours fasting, Dog No. 18, Table IV, lost 1000 gms in weight; this weight loss is reflected, to a great extent, as loss in E.C.F., 744 mls. Plasma volume and hematocrit showed only minimal changes. Following laparotomy, exposure of abdominal contents to room air resulted in a marked loss of fluid by evaporation which is demonstrated in Dog No. 14. Similar changes in plasma and blood volume have been observed in patients undergoing gastrointestinal surgery (43). Dogs No. 13,15,16, and 17 show an increase in E.C.F. level following infusion of Ringer's lactate solution which closely approximates the amount administered. Close duplication of repeat measurement in Dog. No. 12 is worth noting.

SUMMARY AND CONCLUSION

A pilot study was performed to demonstrate the feasibility of measuring a functional E.C.F. volume as a selenate space with sodium selenate labeled with ^{75}Se. This volume of fluid closely approximated the established sulfate space. The facility with which ^{75}Se can be measured on whole blood warrants further studies with this nuclide as a tracer.

Single case studies on dogs demonstrate reproducibility of measurements and fluctuations observed in the volume of fluid measured as a selenate space under different experimental conditions.

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