

# MEASUREMENT OF EXTRACELLULAR FLUID VOLUME IN NEPHRECTOMIZED DOGS<sup>1</sup>

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(Submitted for publication June 4, 1954; accepted July 9, 1954)

The volumes of distribution of a number of substances of widely varying molecular size and chemical properties, including sulfate, thiosulfate, mannitol, sucrose, and inulin, have been found to represent 15 to 25 per cent of body weight of man, dog, and other mammals. The apparently equal volumes of distribution of these dissimilar substances has suggested that the distribution of each of these substances measures the same portion of body fluid and has led to efforts to identify this volume of body fluid with the extracellular fluid volume.

In a series of individuals, however, there is a considerable range in the fraction of body weight which the volume of distribution of any one of these substances represents. In only a few instances have the distributions of two of these substances been compared simultaneously in the same individual. Schwartz (1) reported a ratio of thiosulfate volume to simultaneously measured mannitol volume of 0.90 (0.87 to 0.96) in four normal dogs, a ratio of 1.02 and 0.98 in two normal human subjects, and a ratio of inulin to simultaneously measured mannitol volume of 0.97 (0.93 to 1.02) in six normal human subjects (2). Walser, Seldin, and Grollman (3) reported a ratio of radiosulfate volume to simultaneously measured inulin volume of 0.95 (S.D.  $\pm$  0.11) in nine normal human subjects. Deane, Schreiner, and Robertson (4) measured volumes of sucrose and inulin in each of four normal human subjects on separate occasions and found an average ratio of sucrose volume to inulin volume of 0.97 (0.94 to 1.03). Most of these comparisons depend on quantitative recovery in the urine of infused inulin.

Kruhøffer's (5) data indicate a larger volume of distribution for sucrose than inulin in nephrectomized rabbits. Raisz, Young, and Stinson (6) have reported a ratio of inulin volume to thio-

sulfate volume of 0.76 (0.74 to 0.79) in four normal dogs and of sucrose volume to thiosulfate volume of 0.86 (0.86 to 0.87) in three normal dogs in which distributions of these pairs of substances were studied simultaneously. Nichols, Nichols, Weil, and Wallace (7) found that the ratio of inulin volume to thiosulfate volume averages 0.79 in five nephrectomized dogs.

To determine which, if any, of these substances distribute in equal volumes, the distributions of six substances: radiosulfate, thiosulfate, mannitol, sucrose, raffinose, and inulin, ranging in molecular weight from 96 to 5100 and including two anions, have been compared in nephrectomized dogs. These comparisons have been made under conditions which eliminate or minimize uncertainties regarding: a) extrarenal removal of these substances, b) changing plasma blank, c) completeness of recovery of the injected substance in the urine, and d) upper urinary tract dead space. The volumes of distribution of three of these substances, radiosulfate, thiosulfate, and mannitol, have been found to be equal. Sucrose, raffinose, and inulin volumes are smaller in inverse order to the molecular size of these substances.

Volumes of distribution of d-galactose, d-xylose, and l-arabinose have been reported to increase in nephrectomized dogs and rabbits after insulin administration, presumably the result of insulin facilitating transport of these substances across cell membranes (8, 9). The possibility that endogenous insulin release might alter volumes of distribution of the six substances studied has been investigated by observing the effect of intravenous insulin on their volumes of distribution.

## METHODS

### A. Experimental plan

1. In the analytical methods used for inulin, raffinose, and sucrose, these substances are mutually interfering. Therefore, the volumes of distribution of the six substances were compared in three groups of four or five

<sup>1</sup> This work was supported by a grant from the American Heart Association.

experiments each: a) A group in which inulin, mannitol, thiosulfate, and radiosulfate were infused; b) a group in which raffinose, mannitol, thiosulfate, and radiosulfate were infused; and c) a group in which sucrose, mannitol, thiosulfate, and radiosulfate were infused. In three control experiments sodium chloride solution equal in volume and approximately isosmotic with the solutions given in each of the first three groups of experiments was infused and changes in plasma blank for each of the six substances were determined under comparable experimental conditions.

2. The effect of insulin on the distribution of these six substances was determined in two experiments for each of the six substances. 0.2 units of crystalline zinc insulin per kilogram of body weight was injected intravenously four hours after infusion of these substances.

3. The distribution of inulin and radiochloride were determined simultaneously, as part of a separate study, under conditions essentially the same as those of the above experiments.

#### B. Experimental procedure

1. Healthy adult male dogs were anesthetized with sodium pentobarbital, weighed, and nephrectomized. Bilateral nephrectomy was accomplished through six cm. flank incisions by ligating capsular vessels, doubly ligating ureters, major branches of renal arteries and renal veins, and finally placing ligatures about the entire pedicles. The kidneys were left *in situ* and muscle and skin layers approximated with sutures. Following operation animals were kept lightly anesthetized. Adequacy of ligation was verified by autopsy following each experiment.

2. Polyethylene tubing was introduced into an iliac vein or inferior vena cava through the femoral vein and connected to a 50 cc. burette. Substances to be infused were transferred quantitatively to the burette, following which burette and tubing were washed with 3 per cent glucose. At 30-minute intervals for two hours after infusion and at hourly intervals for four additional hours, 20 cc. arterial blood samples were drawn into heparinized syringes. Plasma was promptly separated.

Ampouled inulin (U. S. Standard Products Co., lot 2-110A1) was heated in boiling water for 20 minutes, cooled and diluted to 100 cc. after adding 0.9 gm. NaCl. Fifty cc., containing approximately 2.2 gm. of inulin, were transferred to the infusion burette. An aliquot of the remainder was appropriately diluted for analysis. Quantities of raffinose, sucrose, and mannitol to give plasma concentrations of 75 to 100 mg. per 100 cc. were accurately weighed out shortly before infusion and dissolved separately in distilled water to make slightly hypertonic solutions. These were quantitatively transferred to the infusion burette. At the same time, and for each experiment, a sample of each substance to be infused was weighed out and diluted to volume to serve as a standard. A volume of 2.5 per cent hydrated sodium thiosulfate calculated to give an initial plasma concentration of 35 to 45 mg. per 100 ml. was pipetted into the infusion burette. An aliquot of the same solution

was titrated with standard KIO<sub>3</sub> solution. Radiosulfate, obtained from Oak Ridge National Laboratory as carrier-free H<sub>2</sub>S<sup>35</sup>O<sub>4</sub>, was appropriately diluted in isotonic saline and sulfuric acid added to give a concentration of 0.001 N sulfate in the solution infused. From 4 to 15 ml. of this solution, approximately 2.5 microcuries per kilogram of body weight, were infused. Cl<sup>36</sup>, obtained from Oak Ridge National Laboratory in 0.1 to 0.2 N HCl, was neutralized and diluted to 0.5 microcuries per ml. Approximately 0.25 microcuries per kilogram of body weight were infused.

In the first three groups of experiments, the four substances were infused in sequence over a 15-minute period. The mid-point of the thiosulfate infusion, the third substance infused, was taken as zero time. The infusions are estimated to have increased extracellular fluid by 2 to 4 per cent of pre-infusion volume.

3. Plasma volume was estimated two and six hours after infusion by dilution of T-1824 dye using the procedure described by Chinard (10). The mean of the two determinations was used in the calculations.

#### C. Analytical methods

Inulin concentration in aliquots of infusion fluid and in Somogyi filtrates of plasma was determined after 30-minute exposure to yeast by the method of Harrison (11). Dilutions of a solution of an accurately weighted sample of Difco inulin served as standards.

Plasma glucose was determined by the method of Nelson (12). Raffinose in an aliquot of the same filtrate was hydrolyzed in 0.1 N HCl at 80° C. for 25 minutes. After neutralization with NaOH, reducing sugar was determined by the method used for glucose. Raffinose concentration was calculated from the difference in raffinose equivalents of reducing substances present before and after acid hydrolysis. Acid hydrolysis did not increase the apparent glucose concentration of plasma not containing raffinose.

Sucrose was determined in cadmium filtrates by method of Schreiner (13), the optimal conditions for hydrolysis and color development described for inulin determination having been found to apply equally well for sucrose. Mannitol was determined in cadmium filtrates by the method of Corcoran and Page (14). In experiments in which sucrose was infused, sucrose and mannitol were determined in the same filtrates.

Plasma blanks of inulin, sucrose, and mannitol were determined additively.

Thiosulfate was determined by the indirect method of Newman, Bordley, and Winternitz (15). Concentrations are expressed as mg. per cent sodium thiosulfate.

Sulfate was precipitated by addition of BaCl<sub>2</sub> to trichloroacetic acid filtrates of plasma and standards at 90° C. after addition of sufficient carrier sulfate to give a density thickness of 25 mg. per cm.<sup>2</sup> when the precipitate was transferred with 95 per cent alcohol to No. 42 Whatman, paraffin-rimmed filter discs. Benzidine was not used to precipitate sulfate since it also precipitates thiosulfate. Each duplicate sample was counted in a flow counter for a total of 10,000 counts at a counting rate of approxi-

mately 1,000 counts per min. with a background of approximately 20 counts per min.

Except for inulin, raffinose, and sucrose, which are mutually interfering, recoveries from plasma of each of the test substances in the presence of the others were satisfactory (96 to 104 per cent).

Cl<sup>-</sup> in plasma and standards was determined after digestion in concentrated nitric acid containing excess AgNO<sub>3</sub>. The AgCl precipitate was transferred in glycerine-alcohol washes to No. 1 Whatman paraffin-rimmed filter discs. Each duplicate sample was counted with a 1.5 mg. per cm.<sup>2</sup> mica end window Geiger tube for a total of 10,000 counts at a counting rate of approximately 500 counts per min. and a background of approximately 18 counts per min. Variations in plasma chloride concentration encountered in these experiments do not introduce significant error.

Plasma and whole blood chloride concentrations were determined by the method of Van Slyke and Hiller (16). Hematocrits were measured in Wintrobe tubes centrifuged at 4,000 rpm. for 30 minutes (radius, 17 cm.). Concentration of water in plasma was estimated from plasma specific gravity (17).

All determinations were made in duplicate.

#### CALCULATIONS

All plasma concentrations have been corrected for plasma water content and in the case of inulin, sucrose, and mannitol for the average increment with time in plasma blank as determined in control experiments (Figure 1). No significant changes in thiosulfate or raffinose plasma blanks were observed. Volumes of distribution of the non-electrolytes were calculated as the amount infused divided by the theoretical concentration at zero time obtained by extrapolation of the rectilinear

portion of the curve of the logarithm of plasma concentration plotted against time. Assuming a Donnan factor of 1.1 for the divalent anions, sulfate and thiosulfate (3), their volumes of distribution ( $V_D$ ) were calculated as follows:

$$V_D = V_{pw} + \left( \frac{Q - P \times V_{pw}}{P \times F} \right)$$

where  $V_{pw}$  equals T-1824 volume  $\times$  fraction of water in plasma,  $Q$  equals quantity infused,  $P$  equals concentration of the substance in plasma water, and  $F$  equals the Donnan factor.

The virtual chloride volume was calculated by the equation above where  $F$  equals 1.05 and  $Q$  equals total exchangeable chloride minus total chloride in erythrocytes. Total exchangeable chloride was calculated from total activity infused and mean specific activity of plasma samples obtained between two and one-half to four hours after infusion. Total chloride in erythrocytes was calculated from plasma and whole blood chloride concentration, plasma volume and hematocrit.

The extrarenal clearance of each substance from its volume of distribution was calculated by the equation of Newman, Bordley, and Winternitz (15) as the product of the volume of distribution times the slope of the rectilinear portion of the plot of logarithm of concentration in plasma water against time. It is expressed as cc. per hour per Kg. body weight.

#### RESULTS

Volumes of distribution of mannitol, thiosulfate and radiosulfate, simultaneously compared with either inulin, raffinose, or sucrose, are shown in Table I.

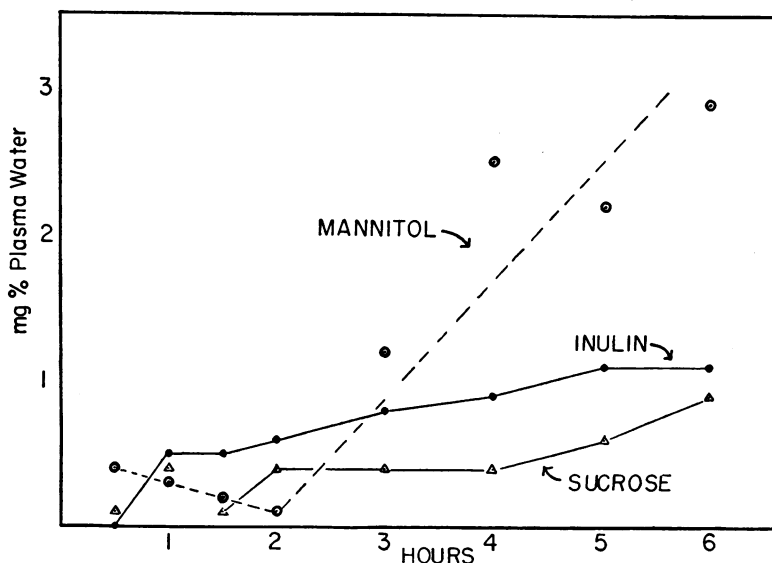


FIG. 1. INCREMENTS IN PLASMA BLANKS (AVERAGE OF THREE CONTROL EXPERIMENTS)

TABLE I  
Comparisons of volumes of distribution

Exp. No.	Body Wgt.	Volume of Distribution											
		% of Body Weight						% of Mannitol Volume					
		kg.	Inulin	Raffinose	Sucrose	Radiosulfate	Thiosulfate	Mannitol	Inulin	Raffinose	Sucrose	Radiosulfate	Thiosulfate
5	20.0	15.0			21.6	20.9	21.4	70				101	98
6*	26.5	13.4			18.1	+	19.2	70				94	+
7	22.9	17.1			20.4	21.7	21.1	81				97	103
21	16.6	18.5			not done	22.9	24.3	76				not done	94
11	20.6		20.2		23.9	+	23.2		87			103	+
12	24.2		21.0		24.0	23.9	23.8		89			101	100
13*	25.9		16.6		18.5	+	18.7		89			99	+
20	22.5		19.8		24.2	23.5	23.8		83			102	99
18	18.2			20.2	24.8	23.4	23.9			84		104	98
28	11.7			25.0	26.6	25.0	26.6			94		100	94
29	22.1			19.4	20.4	20.4	19.6			99		104	104
30	22.6			18.8	20.5	20.3	20.5			92		100	99
31	18.3			23.3	27.2	+	27.2			86		100	+
					Average		22.6	74	87	91		100	99

\* Relatively fat dogs.  
+ Fall in conc. not a simple exponential function of time.

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In 13 dogs, 11 of which were lean and 2 of which relatively fat, the mannitol volume averaged 22.6 per cent of body weight (18.7 to 27.2 per cent). In 12 comparisons the ratio of radiosulfate volume to mannitol volume averaged 1.00 (0.94 to 1.03). In 9 comparisons, the ratio of thiosulfate volume to mannitol volume averaged 0.99 (0.94 to 1.05). In the other four experiments the thiosulfate volume could not be calculated because, for unknown reasons, the logarithm of thiosulfate concentration continued to decrease curvilinearly, convex toward the coordinates, rather than decreasing rectilinearly after two hours.

In four experiments the ratio of inulin volume to mannitol volume averaged 0.74 (0.70 to 0.81) as the mannitol volume ranged from 19.2 to 24.3 per cent of body weight. In four experiments the ratio of raffinose volume to mannitol volume averaged 0.87 (0.83 to 0.89) as the mannitol volume ranged from 18.7 to 23.8 per cent of body weight. In five experiments the ratio of sucrose volume to mannitol volume averaged 0.91 (0.84 to 0.99) as the mannitol volume ranged from

19.6 to 27.2 per cent of body weight. These comparisons, expressed as per cent of the simultaneously measured mannitol volume, are shown graphically in Figure 2. In Figures 3 to 5 are shown data from a representative experiment in each of the three groups. The concentration in plasma water of each of these six substances apparently decreases as a simple exponential function of time beginning one and one-half to three hours after infusion.

#### Extrarenal clearance

The extrarenal clearances of these substances are shown in Table II. The clearance of mannitol is within the range observed in nephrectomized dogs by Houck (18), but averages somewhat less. The extrarenal clearances of inulin, sucrose, and radiosulfate are within the same range, that of raffinose is slightly higher, and that of thiosulfate is several times as high.

#### Insulin effect

The effect of insulin on the distribution of these substances is shown in Figure 6. Insulin in an

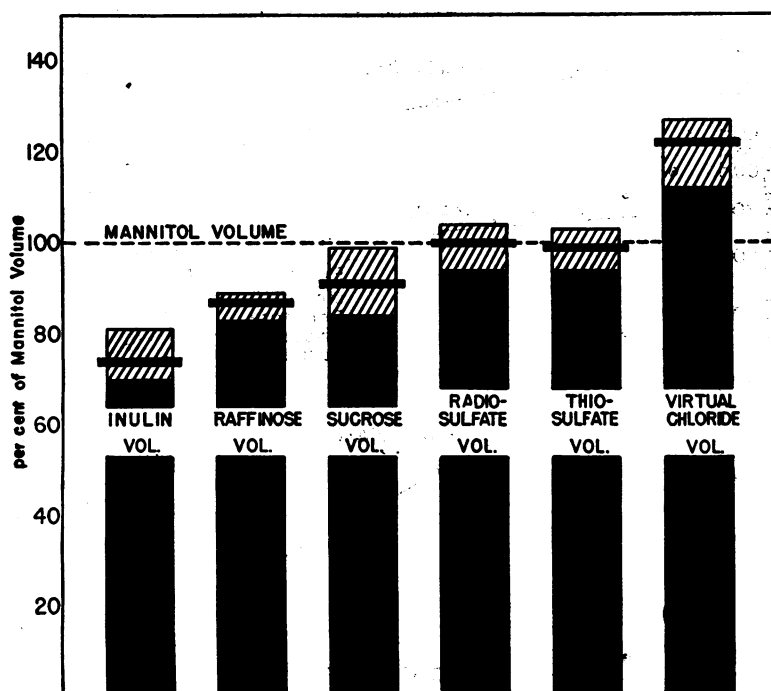


FIG. 2. COMPARISON OF VOLUMES OF DISTRIBUTION EXPRESSED AS PER CENT OF SIMULTANEOUSLY MEASURED MANNITOL VOLUME

Black horizontal line near top of each column represents average of four or five experiments. Cross-hatched area represents the range in the four or five experiments.

TABLE II  
Total extrarenal clearance

Exp.	CLEARANCE					
	Inulin	Raffinose	Sucrose	Mannitol	Radiosulfate	Thiosulfate
5	5.0			4.3	2.5	36
6	3.5			4.3	1.7	
7	3.7			4.8	5.8	29
21	5.3			10.9		51
11		5.1		3.2	6.6	
12		13.2		5.5	2.9	37
13		7.4		3.8	1.3	
20		9.2		5.0	4.0	30
18			5.0	4.2	1.9	32
28			3.8	7.8	6.8	71
29			3.5	3.0	4.4	36
30			4.0	5.3	4.8	27
31			5.6	5.2	3.1	
AV.	4.4	8.7	4.4	5.4	3.8	39

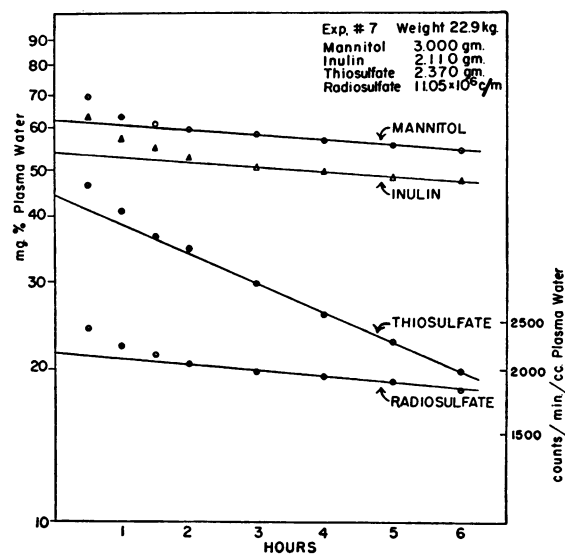


FIG. 3. CONCENTRATIONS IN PLASMA WATER OF MANNITOL, INULIN, THIOSULFATE AND RADIOSULFATE FOLLOWING INFUSION OF THESE SUBSTANCES IN AMOUNTS INDICATED INTO THE NEPHRECTOMIZED DOG AT ZERO TIME

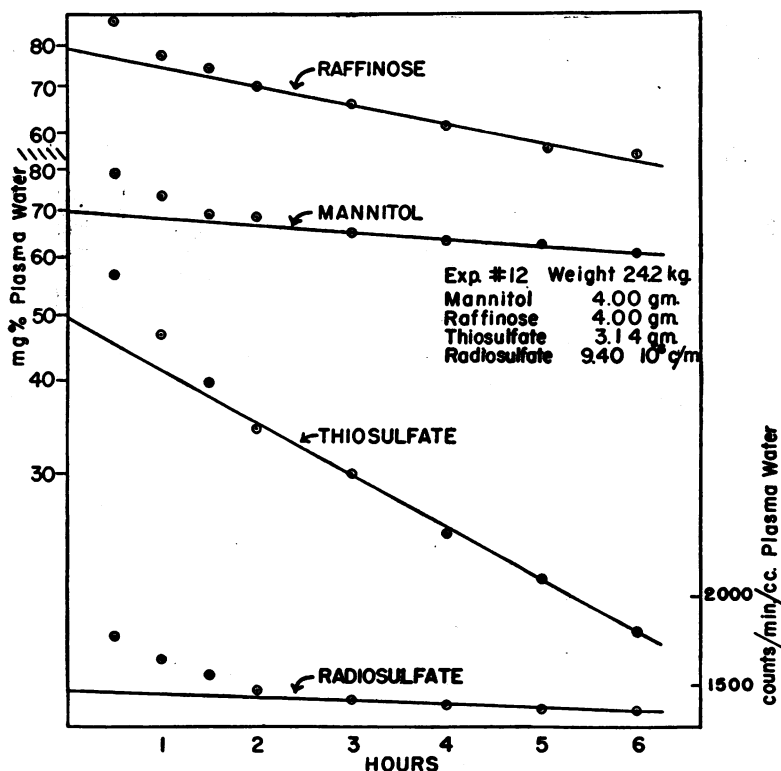


FIG. 4. CONCENTRATIONS IN PLASMA WATER OF MANNITOL, RAFFINOSE, THIOSULFATE AND RADIOSULFATE FOLLOWING INFUSION OF THESE SUBSTANCES IN AMOUNTS INDICATED INTO THE NEPHRECTOMIZED DOG AT ZERO TIME

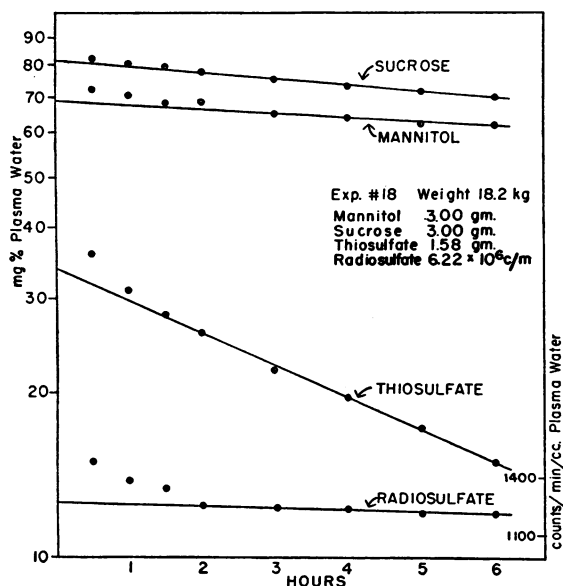


FIG. 5. CONCENTRATIONS IN PLASMA WATER OF MANNITOL, SUCROSE, THIOSULFATE, AND RADIOSULFATE FOLLOWING INFUSION OF THESE SUBSTANCES IN AMOUNTS INDICATED INTO THE NEPHRECTOMIZED DOG AT ZERO TIME

amount sufficient to depress plasma glucose concentration more than 50 per cent reduced the raffinose concentration abruptly to approximately 60 per cent of the original concentration and increased the apparent volume of distribution to 30 per cent of body weight. Insulin did not change plasma concentrations of inulin, sucrose, mannitol, and radiosulfate. The steep slope of falling thiosulfate concentration makes an evaluation of the effect of insulin less reliable. These data are omitted.

How insulin reduces raffinose concentration has not been further investigated. Conceivably, as suggested by Goldstein, Henry, Huddleston, and Levine (8), the galactose portion of the raffinose molecule may be involved.

#### Comparison of radiochloride and inulin volumes

Data derived from the group of experiments in which inulin and radiochloride distributions were compared are shown in Table III. Total ex-

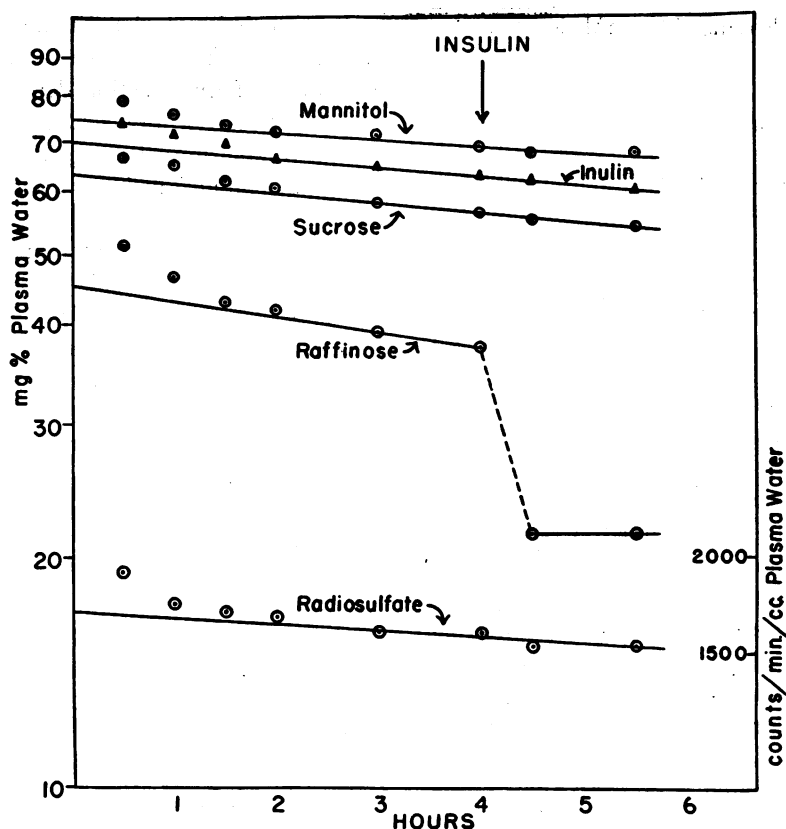


FIG. 6. EFFECT OF INTRAVENOUS INSULIN ON CONCENTRATIONS IN PLASMA WATER OF MANNITOL, INULIN, SUCROSE, RAFFINOSE AND RADIOSULFATE (COMPOSITE OF SEVERAL EXPERIMENTS)

changeable chloride averages 35.4 mM per Kg., or 637 mM in an 18 Kg. animal. When the difference between total exchangeable chloride and total erythrocyte chloride (637 minus 32 mM) is partitioned between plasma and an ultrafiltrate of plasma, the virtual chloride volume averages 27.3 per cent of body weight, while the simultaneously determined inulin volume averages 16.7 per cent

of body weight. Assuming the ratio of inulin volume to mannitol volume to be 0.74, the average value obtained in the first group of experiments, the ratio of virtual chloride volume to mannitol volume can be estimated to average 1.22 under the conditions of these experiments. Of the 637 mM of total exchangeable chloride, 32 mM can be located in erythrocytes and 495 mM in the

TABLE III  
Comparison of inulin and chloride volumes of distribution

Exp. No.	Wgt. Kg.	Plasma Water Volume cc.	Chloride Conc. in Plasma Water mM./L.	Total Exchangeable Chloride mM./Kg.	Total RBC Chloride mM.	Virtual Chloride Volume		Inulin Volume		Measured $V_{Cl^-}/V_{In.}$	Calculated $V_{Cl^-}/V_{Mann.}$
						cc.	% Body Wgt.	cc.	% Body Wgt.		
22A	19.6	1110	119.5	34.5	42	5600	28.5	3035	15.5	1.85	1.37
23A	23.6	1160	118.0	36.5	35	6725	28.5	4220	17.9	1.60	1.18
24A	16.5	750	118.5	32.5	38	4040	24.5	2670	16.2	1.52	1.12
25A	16.3	770	113.6	37.2	28	4540	27.9	2940	18.0	1.55	1.15
28A	14.1	620	115.0	36.7	25	3860	27.3	2210	15.7	1.75	1.29

mannitol volume, leaving 110 mM located elsewhere.

#### DISCUSSION

These data demonstrate that the volumes of distribution of three chemically dissimilar substances, radiosulfate, thiosulfate, and mannitol, are consistently within 6 per cent of each other as body weight varies between 12 and 27 Kg. and as the per cent of body weight which these volumes represent ranges from 19 to 27 per cent. This observation suggests that the volumes of distribution of these three substances represent the same portion of body water.

Is this volume coextensive with extracellular fluid when the latter is defined, essentially as Edelman, Olney, James, Brooks, and Moore have (19), as plasma plus plasma ultrafiltrate bounded by capillary membranes, cell membranes, and extracellular solid structures (*e.g.*, hydroxyapatite crystals of bone or macromolecules in collagen and elastic fibers)? For cerebrospinal fluid, gastrointestinal secretion, and other smaller volumes of fluid which are separated from extracellular fluid by epithelium, Edelman, Olney, James, Brooks, and Moore suggest the term "transcellular fluid." Cerebrospinal fluid is not included in extracellular fluid as here defined. Neither is it included in the calculated volumes of distribution of these substances, because under the conditions of these experiments, concentrations of radiosulfate, mannitol, sucrose, and inulin in cerebrospinal fluid obtained from a lateral ventricle or the cisterna magna six hours after infusion are less than 10 per cent of the simultaneous plasma concentrations (20). Gastrointestinal secretions are not included in extracellular fluid as here defined. Under these experimental conditions, radiosulfate, mannitol, and sucrose appear in bile and pancreatic juice in concentrations approaching plasma concentration, while inulin is excreted in bile in a concentration approximately one-third that of plasma (20).

Inulin and sucrose are hydrolyzed in the gut and presumably their calculated volumes of distribution do not include gastrointestinal secretion or contents.

If mannitol (21) and radiosulfate are reabsorbed from the gut at these concentrations, a

fraction of their calculated volumes of distribution may include a fraction of gastrointestinal fluid, the magnitude of this fraction depending on the rate and site of reabsorption (22).

The volume measured by radiosulfate, thiosulfate, and mannitol includes approximately 80 per cent of the body chloride. The remainder, minus the chloride in erythrocytes and estimated chloride in transcellular fluid, is consistent with the fractions of chloride which Amberson, Nash, Mulder, and Binns (23) and Yannet and Darrow (24) found to be present in tissues in a form other than as an ultrafiltrate of plasma, either intracellular or bound in some manner to extracellular structures.

The concept that the volumes of distribution of mannitol, thiosulfate, and radiosulfate are coextensive with extracellular fluid as here defined is not compatible with available information on their distribution as measured by tissue analysis. Nichols, Nichols, Weil, and Wallace (7) have found thiosulfate to be virtually equilibrated with only 45 per cent of Achilles or patellar tendon water and with 78 per cent of skin water three hours after infusion. The bulk of tendon water is certainly extracellular, but its total volume in studies on the whole animal probably is not significant. However, even the most generous estimate of intracellular water in skin can account for only one-half to one-third of the water of skin into which thiosulfate fails to distribute. The volume of this water is probably significant in studies on the whole animal. Studies of the distribution into water of skin and bone of radiosulfate or mannitol, substances whose plasma concentrations do not decrease as rapidly as does that of thiosulfate, should answer whether significant fractions of extracellular fluid can be shown to be excluded from these volumes of distribution.

A truly rectilinear decrease in the logarithm of the plasma concentration of radiosulfate, thiosulfate, mannitol, sucrose, raffinose, and inulin, with time after one and one-half to three hours of equilibration, would indicate that these substances are being removed from their calculated volumes of distribution by such processes as destruction within cells or destruction or sequestration in the gut. It would exclude diffusion into more restricted or remote regions of extracellular fluid at relatively slower rates. The latter possibility



is suggested by the studies of Cotlove (25) and Nichols, Nichols, Weil, and Wallace (7) on the distribution of inulin, sucrose, and thiosulfate in tissues. Our data do not exclude this possibility because the very slight curvilinearity in the plot reflecting such a process would not become apparent during the period of these observations.

Despite the above uncertainties regarding the identification of the volumes of distribution of radiosulfate, thiosulfate, and mannitol with extracellular fluid, we believe their distributions are the best available measure of extracellular fluid volume in the nephrectomized dog. Disadvantages are: variability in the plasma blank in the case of mannitol, the occasional failure to observe a linear decrease in the logarithm of plasma concentration with time in the case of thiosulfate, and the uncertainty regarding Donnan distribution across capillary membranes in the case of radiosulfate and thiosulfate.

We are unable to rationalize on a structural basis the smaller volumes of distribution of sucrose, raffinose, and inulin. In the nephrectomized dog these volumes are clearly smaller than those of radiosulfate, thiosulfate, and mannitol. The question whether similar relations obtain in man could be answered by similar comparisons in anuric patients.

#### SUMMARY

1. The volumes of distribution of radiosulfate, thiosulfate, mannitol, sucrose, raffinose, and inulin have been compared in nephrectomized dogs. Radiosulfate, thiosulfate, and mannitol volumes are apparently equal when measured simultaneously in the same animal. The volumes of distribution of sucrose, raffinose, and inulin are smaller in inverse order to the molecular size of these substances.

2. Intravenous insulin, which has been shown by others to affect the distribution of other slowly metabolized monosaccharides, increases the apparent volume of distribution of raffinose, but does not affect the distribution of radiosulfate, mannitol, sucrose, or inulin.

3. The possibility that the volume measured by radiosulfate, thiosulfate, and mannitol distribution is coextensive with extracellular fluid, as here defined, is discussed.

#### ACKNOWLEDGMENT

We are indebted to our associate, Dr. Henry D. Lauson, for his criticism of the manuscript.

#### REFERENCES

1. Schwartz, I. L., The measurement of extracellular fluid by means of a constant infusion technique without collection of urine. *Am. J. Physiol.*, 1950, **160**, 526.
2. Schwartz, I. L., Breed, E. S., and Maxwell, M. H., Comparison of the volume of distribution, renal and extrarenal clearances of inulin and mannitol in man. *J. Clin. Invest.*, 1950, **29**, 517.
3. Walser, M., Seldin, D. W., and Grollman, A., An evaluation of radiosulfate for the determination of the volume of extracellular fluid in man and dogs. *J. Clin. Invest.*, 1953, **32**, 299.
4. Deane, N., Schreiner, G. E., and Robertson, J. S., The velocity of distribution of sucrose between plasma and interstitial fluid, with reference to the use of sucrose for the measurement of extracellular fluid in man. *J. Clin. Invest.*, 1951, **30**, 1463.
5. Kruhøffer, P., Inulin as an indicator for the extracellular space. *Acta physiol. Scandinav.*, 1946, **11**, 16.
6. Raisz, L. G., Young, M. K., and Stinson, I. T., Comparison of the volumes of distribution of inulin, sucrose and thiosulfate in normal and nephrectomized dogs. *Am. J. Physiol.*, 1953, **174**, 72.
7. Nichols, G., Jr., Nichols, N., Weil, W. B., and Wallace, W. M., The direct measurement of the extracellular phase of tissues. *J. Clin. Invest.*, 1953, **32**, 1299.
8. Goldstein, M. S., Henry, W. L., Huddleston, B., and Levine, R., Action of insulin on transfer of sugars across cell barriers: common chemical configuration of substances responsive to action of the hormone. *Am. J. Physiol.*, 1953, **173**, 207.
9. Wick, A. N., and Drury, D. R., Action of insulin on volume of distribution of galactose in the body. *Am. J. Physiol.*, 1953, **173**, 229.
10. Chinard, F. P., Estimation of plasma volume by dye dilution method *in* *Methods in medical research*. M. B. Visscher, Ed., Chicago, Year Book Publishers, 1951, vol. 4, p. 38.
11. Harrison, H. E., A modification of the diphenylamine method for determination of inulin. *Proc. Soc. Exper. Biol. & Med.*, 1942, **49**, 111.
12. Nelson, N., A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.*, 1944, **153**, 375.
13. Schreiner, G. E., Determination of inulin by means of resorcinol. *Proc. Soc. Exper. Biol. & Med.*, 1950, **74**, 117.
14. Corcoran, A. C., and Page, I. H., A method for the determination of mannitol in plasma and urine. *J. Biol. Chem.*, 1947, **170**, 165.

15. Newman, E. V., Bordley, J., III, and Winternitz, J., The interrelationships of glomerular filtration rate (mannitol clearance), extracellular fluid volume, surface area of the body, and plasma concentration of mannitol. A definition of extracellular fluid clearance determined by following plasma concentration after a single injection of mannitol. *Bull. Johns Hopkins Hosp.*, 1944, **75**, 253.
16. Van Slyke, D. D., and Hiller, A., Application of Sendroy's iodometric chloride titration to protein-containing fluids. *J. Biol. Chem.*, 1947, **167**, 107.
17. Van Slyke, D. D., Hiller, A., Phillips, R. A., Hamilton, P. B., Dole, V. P., Archibald, R. M., and Eder, H. A., The estimation of plasma protein concentration from plasma specific gravity. *J. Biol. Chem.*, 1950, **183**, 331.
18. Houck, C. R., Disappearance of mannitol and PAH from plasma of bilaterally nephrectomized dogs. *Am. J. Physiol.*, 1951, **165**, 102.
19. Edelman, I. S., Olney, J. M., James, A. H., Brooks, L., and Moore, F. D., Body composition: studies in the human being by the dilution principle. *Science*, 1952, **115**, 447.
20. Swan, R. C., and Madisso, H., Unpublished studies.
21. Jaffe, M., Ueber das Vorkommen von Mannit im normalen Hundeharm. *Ztschr. f. physiol. Chem.*, 1883, **7**, 297.
22. Everett, N. B. and Simmons, B. S., The distribution and excretion of S<sup>35</sup> sodium sulfate in the albino rat. *Arch. Biochem. and Biophys.*, 1952, **35**, 152.
23. Amberson, W. R., Nash, T. P., Mulder, A. G., and Binns, D., The relationship between tissue chloride and plasma chloride. *Am. J. Physiol.*, 1938, **122**, 224.
24. Yannet, H., and Darrow, D. C., The effect of depletion of extracellular electrolytes on the chemical composition of skeletal muscle, liver, and cardiac muscle. *J. Biol. Chem.*, 1940, **134**, 721.
25. Cotlove, E., Mechanism and extent of distribution of inulin and sucrose in chloride space of tissues. *Am. J. Physiol.*, 1954, **176**, 396.