JCI The Journal of Clinical Investigation

THE VARIABILITY OF EXTRACELLULAR FLUID SPACE (SUCROSE) IN MAN DURING A 24 HOUR PERIOD WITH AN APPENDIX AN EXAMINATION OF A SEQUENCE OF RANDOMIZED ERRORS BY THE MONTE CARLO METHOD

Richard E. Peterson, … , Walter M. Kirkendall, Oscar Kempthorne

J Clin Invest. 1959;38(9):1644-1658. https://doi.org/10.1172/JCI103942.

Research Article

THE VARIABILITY OF EXTRACELLULAR FLUID SPACE (SUCROSE) IN MAN DURING A 24 HOUR PERIOD *†

By RICHARD E. PETERSON, JAMES J. O'TOOLE and WALTER M. KIRKENDALL

(From the Radioisotope and Medical Services, Veterans Administration Hospital and the Cardiovascular Research Laboratories, Department of Internal Medicine, State University of Iowa, Iowa City, Iowa)

WITH AN APPENDIX

AN EXAMINATION OF A SEQUENCE OF RANDOMIZED ERRORS BY THE MONTE CARLO METHOD

By OSCAR KEMPTHORNE

(From the Department of Statistics, Iowa State College, Ames, Iowa)

(Submitted for publication October 2, 1958; accepted May 21, 1959)

The water content of the human body averages 60 per cent of its total weight. Techniques for measurement and criteria for accuracy have been developed for total body water but there are no generally accepted standards for extracellular fluid volume (EFV). It is our purpose in this paper to describe our exploration of the erratic variations of extracellular fluid volume in normal subjects as measured by sucrose infusions and to discuss wide fluctuations in what had been thought hitherto to be a "steady state."

It is believed that most methods of estimating EFV using nonelectrolyte labels achieve rapid equilibration in, and measure mainly, interstitial and plasma water, and that the substances used diffuse incompletely into the cerebrospinal fluid, joint spaces, bone and tendon water, or into glandular lumina, or gastrointestinal tract water (2). This incomplete mixing of test substance in these spaces should not be expected to introduce large errors into this estimation since plasma and interstitial fluid must comprise the major portion of EFV. Walser, Seldin and Grollman (3) have proposed the term "functional EFV" for the volume thus measured. However, the accuracy of EFV estimates has been further questioned with reports that even large nonelectrolyte molecules, such as inulin and sucrose, may leave the EFV and penetrate cell membranes (4, 5).

Most methods for measuring extracellular fluid space are based on the assumption that concentrations of the labeling substance in plasma and interstitial pool are in equilibrium when the blood samples are taken. The chief criticism of the single injection technique is that such equilibrium cannot be achieved (6). This shortcoming of the single injection method was removed in a constant infusion technique described by Schwartz, Schachter and Freinkel (6). With this technique, equilibrium of the labeling substance in the "functional" extracellular fluid pool was achieved at the time of measurement. The proof of equilibrium was the demonstration of a constant value for the plasma concentration of the test substance when measured over several consecutive periods of approximately one hour each. A later modification, the "calibrated infusion difference" method described by Deane, Schreiner and Robertson (7, 8) offers the advantage of several EFV estimates in one study.

In a study of the distribution of body water, we decided to measure EFV by the calibrated infusion difference method using sucrose as the indicator. Our experiences with six hour sucrose infusions using this technique suggested that EFV varied from period to period more than has been described previously in control situations (7). To explore these variations, and to make a critical examination of the calibrated infusion difference technique, we decided to extend these infusions over 15 to 24 hour periods. We have attempted to estimate the error of the method and speculate on the significance of the observed EFV changes. Because of the popularity and

* A preliminary report has been published (1).

† This work was supported in part by Grants H1909 and H3619 from the National Heart Institute of the National Institutes of Health.

convenience of the six hour infusion EFV measurement, we have compared the mean and variation of EFV in six hour studies with longer ones.

METHODS

A. Subjects. The subjects for these studies were volunteers. The majority were normal individuals (staff or student), but six studies were made on nonedematous hospitalized patients. The diagnoses of the patients are given in Table I.

B. Procedure. Since equilibrium between plasma and other extracellular fluids cannot occur if the intravenous infusion rates vary or are erratic, we used a calibrated delivery pump.[1] The pump motor moved a syringe plunger in a 1 ml. tuberculin syringe via an eccentric cam. The pump was adjusted to deliver a stroke volume of approximately 0.05 ml. and a minute volume of from 1.5 to 3.0 ml. Two counters built into the machine recorded the elapsed operating time in 0.1 second intervals and the number of times the syringe was emptied. The pump was calibrated for each study by pumping some of the infusion fluid into successive 25 ml. volumetric flasks for a period exceeding one hour. Under these conditions, the pump delivered a constant volume with a maximal error of ± 0.5 per cent and standard error of ± 0.3 per cent. The pump was fitted with small gauge polyethylene tubing (2.0 mm. O. D.) attached to the valve inlet and outlet. The inlet tubing was put directly into the sterile sucrose solution and the distal end of the outlet tubing was fitted by a plastic ferrule to a 20 gauge needle in a vein of the patient's forearm. The entire apparatus and tubing were sterilized before use by filling the system with aqueous Zephiran® (1:1,000) for at least 12 hours. Sterile water was used to flush the Zephiran® from the system just before priming with sucrose.

The entire apparatus was wheeled to the patient's bedside. The lightweight polyethylene tubing which carried the infusion fluid was seven feet long so the patient could move his arm during the infusion. A short polyethylene catheter for blood sampling was secured in the antecubital vein of the other arm.

The concentration of sucrose in the infusion fluid was approximately 5 per cent in water or, in a few instances, normal saline. Specimens for blank values were obtained for blood and urine within the hour immediately preceding the start of the infusion. The initial infusion rate[2] of 3 ml. per minute was maintained until after the first hour blood sample was collected. Then the rate was reduced and maintained constant at a value approximating half the initial rate for the rest of the study period. All samples were collected at approximately one hour intervals. Urine was collected by an indwelling Foley catheter, and the bladder was washed with three 75 ml. volumes of sterile saline at each collection. The beginning of the third saline bladder wash indicated the termination of one period and the beginning of another. At that instant the readings of the stroke and time counters were recorded. Five ml. oxalated blood samples were collected similarly at the beginning and end of each period. After the end of the infusion, the patient's urine was collected for another 24 hours. It contained most of the remaining sucrose.[3] All samples were refrigerated until analyzed, usually on the following morning.

C. Chemical methods. Filtrates of blood and urine were prepared by the barium hydroxide-zinc sulfate method of Nelson and Somogyi (10). The sucrose in blood plasma and urine was determined in duplicate by the resorcinol method of Roe as modified by Higashi and Peters (11). The blank values ranged from 1 to 2 mg. per cent sucrose equivalent for blood plasma, and from zero to 1 per cent for urine. In the studies reported in this paper, the analytical error of the various sucrose measurements was estimated from the mean difference in optical densities of the duplicate determinations. The standard error was essentially the same for blood and urine samples (2.0 per cent). The exact sucrose concentration of the infusion was found by analysis of an aliquot at the same time the blood and urine filtrates were analyzed.

To calculate sucrose space during the infusion, we used the calibrated infusion difference method described by Deane, Schreiner and Robertson (7) with slight modifications. At any one period the sucrose volume of distribution is $Vx = \frac{\Sigma iv - \Sigma uv}{P_t}$, where Vx is the sucrose volume of distribution, Σiv is the total amount of sucrose infused, Σuv is the total amount of sucrose excreted, and P_t the plasma sucrose concentration. Besides estimating sucrose space in this manner, we also calculated the space after adjusting Σuv for sucrose not recovered and P_t for plasma sucrose fluctuations. Complete recovery of infused sucrose was not always obtained, nor was a constant plasma concentration achieved consistently. Therefore, we corrected Σuv for unrecovered sucrose by dividing the measured Σuv by the per cent sucrose recovered, and by this maneuver adjusted Σuv to 100 per cent recovery (see Discussion for details and example). This correction assumed that the unrecovered sucrose disappeared at a rate which was a constant

[1] Brewer Automatic Pipetter, Model 40, Baltimore Biological Laboratories.

[2] This constituted the priming dose and differed from the method of Gaudino and Levitt (9) as well as from that of Schwartz, Schachter and Freinkel (6) in being an accelerated rate of infusion for one hour, rather than a single injection. The intent was to accelerate equilibrium as evidenced by a constant plasma sucrose level. The method of Deane, Schreiner and Robertson (7) did not include a priming dose.

[3] Urine collection of the test molecule after the end of the infusion was not used in calculation of EFV by Deane, Schreiner and Robertson (7), but was used by Schwartz, Schachter and Freinkel (6). We have used this urine sucrose recovery in the previously described manner (6) as well as in corrections for sucrose losses.

TABLE I

Summary of 17 prolonged sucrose infusion studies

Study No.	Subject initials	Age	Height	Body wt.	Remarks	Duration of infusion	Post priming infusion rate	Average plasma sucrose level	Coeff. variat.* plasma sucrose	Average urine sucrose ex.	Coeff. variat. sucrose ex.	Duration of post-infusion recovery	Total sucrose recovered
		yrs.	*in.*	*Kg.*		*hrs.*	*mg./min.*	*mg. %*		*mg./min.*		*hrs.*	*as % infused*
31	R. P.-1	34	72	75	Normal	24	219.4	160.0	6.3	210.6	11.7	72	97.3
34	A. K.	26	67	55	Normal†	24	138.4	166.0	11.0	135.2	17.5	72	103.0
36	W. L.	32	71	86	Chronic meningoencephalitis	25	148.7	109.0	8.1	139.4	14.3	24	99.8
37	H. J.	66	72	64	Possible lateral sclerosis	24	167.8	164.5	17.8	140.8	14.0	48	93.0
38	W. F.	31	69	59	Multiple sclerosis	24	61.9	46.9	8.0	58.4	11.9	24	93.9
39	J. O'M.	34	66.25	75	Tension headaches	24	69.2	51.2	19.3	60.0	13.9	24	91.3
49	D. S.	29	67	78	Normal	17	28.0	20.5	9.6	23.4	16.6	24	86.0
51	C. P.	30	70.5	70	Normal	18	68.7	61.3	9.6	60.9	34.4	24	91.5
54	R. B.	25	69	63	Normal	18	82.3	61.9	13.9	72.4	10.0	24	91.4
55	L. M.	35	69.75	86	Angina pectoris	18	78.4	58.8	14.7	68.8	17.5	48	92.5
70	R. S.	35	73	83	Normal	14	103.0	84.6	8.6	82.8	25.5	24	83.4
73	R. H.-1	28	70	91	Normal	15	80.8	60.3	10.9	67.8	23.6	48	85.0
78	R. H.-2	28	70	91	Normal	15	72.1	57.2	7.3	63.5	20.6	48	97.6
88	R. H.-3	28	70	92	Normal	15	77.9	63.3	9.6	68.1	13.4	48	95.6
111	R. H.-4	28	70	96	Normal	14	96.0	75.7	13.6	79.7	18.5	24	91.7
125	R. P.-2	35	72	70	Normal	10	98.0	63.4	9.3	90.6	24.2	24	99.2
137	B. B.	28	71	87	Normal	14	100.4	64.9	10.5	76.9	19.9	24	85.9
Average				77.7					11.1		18.1		92.8

Study No.	Subject initials	Age	Height	Body wt.	Remarks	Sucrose space (corrected for recovery)				Final interval space uncorrected	Final interval space corrected for recovery	Post-infusion recovery space	Δ Corrected recovery space‡
						Mean	Range as % mean		Coeff. variat. 3–24 hrs.				
							Entire	Beyond 3 hrs.					
		yrs.	*in.*	*Kg.*		*as % body wt.*				*% body wt.*	*% body wt.*	*% body wt.*	
31	R. P.-1	34	72	75	Normal	23.1	29	20	4.3	28.9	22.3	21.6	0.6
34	A. K.	26	67	55	Normal†	23.1	59	45	13.1	15.7	22.0	22.6	−0.6
36	W. L.	32	71	86	Chronic meningoencephalitis	18.0	43	43	7.9	24.6	23.9	23.9	0
37	H. J.	66	72	64	Possible lateral sclerosis	29.3	28	28	9.7	38.4	28.2	26.1	2.0
38	W. F.	31	69	59	Multiple sclerosis	13.7	87	42	9.1	32.4	14.7	13.9	0.8
39	J. O'M.	34	66.25	75	Tension headaches	20.3	33	33	11.7	45.8	23.8	21.7	2.1
49	D. S.	29	67	78	Normal	25.5	28	28	9.0	46.8	22.2	20.2	1.9
51	C. P.	30	70.5	70	Normal	26.0	20	20	5.1	38.2	25.7	22.8	2.9
54	R. B.	25	69	63	Normal	19.6	42	42	11.9	34.3	18.5	17.2	1.3
55	L. M.	35	69.75	86	Angina pectoris	18.4	31	25	6.8	27.8	17.8	16.3	1.5
70	R. S.	35	73	83	Normal	20.1	55	47	16.7	35.9	17.9	16.3	1.7
73	R. H.-1	28	70	91	Normal	19.0	27	27	7.9	38.3	17.0	17.1	−0.1
78	R. H.-2	28	70	91	Normal	17.7	24	21	7.6	21.6	19.3	19.2	0.1
88	R. H.-3	28	70	92	Normal	17.8	16	11	3.7	29.0	18.5	17.0	1.5
111	R. H.-4	28	70	96	Normal	15.3	27	27	6.7	23.7	16.7	15.4	1.3
125	R. P.-2	35	72	70	Normal	19.5	20	18	5.0	22.4	21.4	23.3	1.8
137	B. B.	28	71	87	Normal	19.1	31	20	6.5	38.1	21.4	20.0	1.5
Average				77.7		19.4 (or 15.1 L.)	35.3	29.3 (or 4.4 L.)	8.4 (or 1.3 L.)	31.8	20.7	19.7	1.2

* Coefficient of variation $= \frac{\text{standard deviation} \times 100}{\text{mean}}$.

† Difference between final interval space corrected for recovery and postinfusion recovery space.

‡ Only woman.

fraction of the sucrose excreted in each period throughout the test and for the following 24 hours. A postinfusion sucrose recovery space also was calculated by dividing the postinfusion urine sucrose recovery by the final plasma sucrose level. This latter calculation did not contain the correction for incomplete sucrose recovery.

Because unexplained fluctuations occurred in plasma concentrations of sucrose throughout our studies we believed that a better approximation of the plasma concentration over any one period could be obtained by averaging the plasma sucrose concentrations of successive periods. Hence, P_t was obtained from $\frac{P1 + P2}{2}$, where P1 was the plasma level at the beginning and P2 at the end of a collection period. For simplicity, the correction for plasma water was not applied until the space calculation was made. The correction used was based on an assumed value of plasma water content of 92 per cent.

Throughout this paper the term "essentially constant" includes minimal changes that do not exceed the 95 per cent confidence interval for the factor under discussion. The 95 per cent confidence intervals of the various aspects of the technique are as follows: *1*) Blood sucrose, standard error [4] is equal to 2 per cent so that a 95 per cent confidence interval on measured value is approximately 96 per cent to 104 per cent, with a range of 8 per cent; *2*) urine sucrose, standard error is ± 2 per cent and 95 per cent confidence interval is ± 4 per cent; *3*) sucrose space,[5] standard error is ± 2.7 per cent or 95 per cent confidence interval is ± 5.5 per cent. We interpret changes exceeding the 95 per cent confidence limits in any factor between intervals as evidence of physiologic changes.

RESULTS

The range and frequency of sucrose space variations

Sucrose infusions were continued for over 24 hours in six subjects (see Figure 1), for 18 hours in four subjects and for 10 to 15 hours in seven (Table I). Sucrose space was calculated at hourly intervals, giving a mean sucrose space for the entire group of 19.4 per cent body weight or 15.1 L., for the average individual in this study. The average of the ranges of variation from the third interval (when equilibration had probably been achieved) until the end of the infusion was 29.3 per cent (4.4 L.) of the mean sucrose space. The average of the coefficient of variation [6] over this same period was 8.4 per cent (1.3 L.) of the mean sucrose space. In a single study, the smallest range of variation in sucrose spaces from the third hour until the end of the infusion was 11 per cent of the mean sucrose space (Study No. 88 in Tables I and II, and Figure 2). The largest range of variation between the third hour and the end of the infusion was 47 per cent of the mean sucrose space (Study No. 70, Table I). Some large shifts (3.0 L.) were seen to occur within approximately 60 minutes (Studies No. 34 and 37, Figure 1). On other occasions progressive shifts (3.0 to 5.0 L.) of sucrose space were found to extend over six hour periods (Studies No. 38 and 39, Figure 1). These shifts in sucrose space were not predictable as to direction, magnitude or duration.

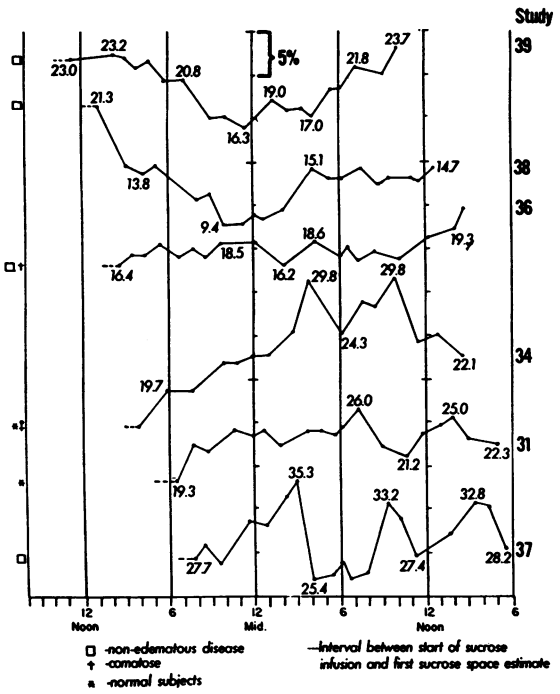

FIG. 1. DIURNAL VARIATIONS IN SUCROSE SPACE
Space in units of per cent body weight.

The experimental model: An examination for random compounding of analytic errors

We sought to determine if the wide variability in sucrose space observed was due to errors or to artifacts in the technique. We investigated this by carrying out several mock experiments. A standard sucrose solution was prepared by weighing the pure sugar and diluting it to a measured

[4] Standard error is used as synonymous with standard deviation.

[5] See Appendix for standard error estimate by Monte Carlo Method.

[6] Coefficient of variation $= \frac{\text{standard deviation} \times 100}{\text{mean}}$.

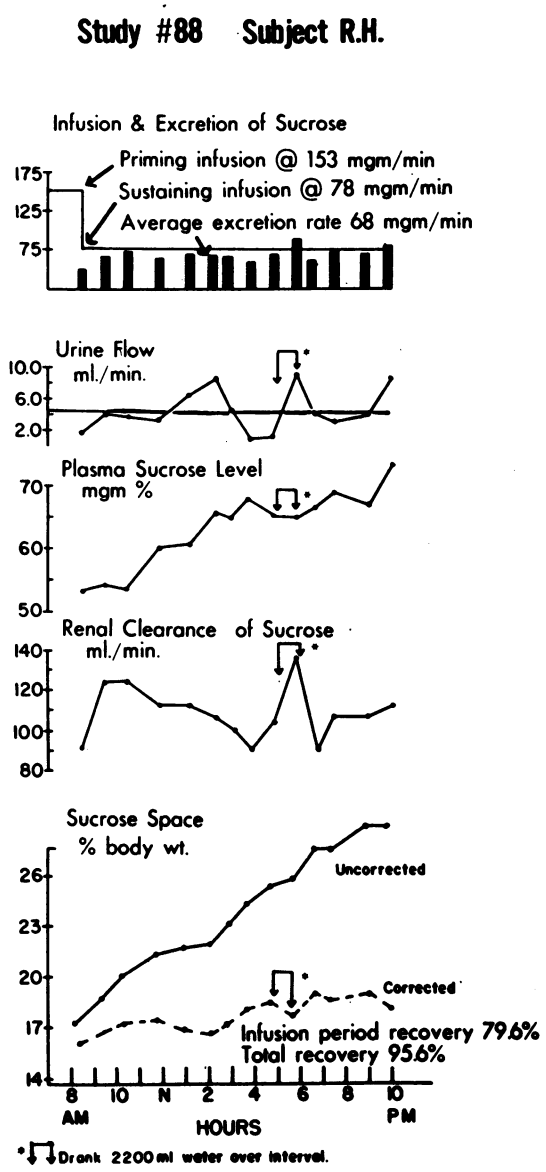

FIG. 2. SUCROSE DISTRIBUTION IN STUDY 88

Although the average sustaining infusion rate of sucrose was 78 mg. per minute, it should be noted, as is shown in Table II, that the rate from 8 a.m. to 4 p.m. was 76 mg. per minute, while that from 4 p.m. to the end of the study was 81 mg. per minute.

volume. This solution was then pumped through the infusion machine for measured intervals of one hour, and the measured volume of each interval was collected in tared volumetric flasks. The volume delivered at each interval was then weighed and compared to the volume that was calculated from the stroke counter. At the end of the experiment, each volumetric flask was brought to volume with distilled water and an aliquot taken for chemical analysis, along with an aliquot of the infusion fluid. The amounts of sucrose delivered at each interval were compared gravimetrically and chemically. Assuming the gravimetric measurement of sucrose as 100 per cent, the chemical recovery of sucrose for each experiment was 99.2, 102.1 and 97.8 per cent, respectively. The difference between the hourly amounts of sucrose determined chemically compared to the same amounts calculated from the stroke counter showed a coefficient of variation of $\pm$ 2.58 per cent in one six hour experiment and $\pm$ 2.13 per cent and $\pm$ 1.44 per cent, respectively, in each of two 10 hour experiments. The coefficient of variation for these three experiments when pooled was $\pm$ 1.8 per cent. The total amount of infusion delivered during a patient's study was checked on six occasions, including one 24 hour infusion, by weighing the infusion solution and container at the beginning and end of the infusion period. In these studies, the mean discrepancy between metered infusion delivery and weighed infusion delivery was 1.7 per cent, with a maximum discrepancy of 3.6 per cent.

The possibility that the compounding of analytic errors and pump errors could account for the occurrence of apparent shifts of sucrose space was also explored by using the Monte Carlo Method (see Appendix). Our observed errors of 0.3 per cent for the pump, 2.0 per cent for blood sucrose analysis and 2.0 per cent for urine sucrose analysis were randomized to observe the consequences of these additive errors on successive estimates of sucrose space. The coefficient of variation of a series of "25 intervals" was less than 3.0 per cent of the mean sucrose space. In the unlikely circumstance that all errors (magnitude of one standard error) increased the calculated space at one interval and decreased the space at the next interval, the difference in space would have been 6 per cent of the mean sucrose space. Consequently, the observed variations of EFV in our subjects greatly exceeded those which could be attributed to random compounding of analytic and pump errors.

Sucrose losses

An attempt was made to account for all of the sucrose infused by measuring its recovery in the

TABLE II

The calculation of sucrose space and correction for sucrose losses *

Interval	Time	Infusion†	Plasma sucrose end of int.	Mean plasma sucrose level	Sucrose dose infused	Cumulative dose	Urine sucrose	Cumulative excretion	Dose retained	Uncorrected sucrose space		Corrected urine sucrose§	Corrected cumul. excretion	Corrected dose retained	Corrected sucrose space	
	min.	*mg./min.*	*mg./100 ml.*	*mg./100 ml.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*L.*‡	*% body wt.*	*Gm.*	*Gm.*	*Gm.*	*L.*‡	*% body wt.*
1	87.2	152.6	53.6	53.6	13.309	13.309	4.348	4.348	8.961	15.37	16.71	4.547	4.547	8.762	15.02	16.33
2	67.4	75.2	54.2	53.9	5.069	18.378	4.512	8.860	9.518	16.24	17.65	4.718	9.265	9.113	15.54	16.89
3	49.3	75.3	53.6	53.9	3.711	22.089	3.323	12.183	9.906	16.89	18.36	3.475	12.740	9.349	15.94	17.33
4	88.4	75.6	60.1	56.9	6.684	28.773	5.671	17.854	10.919	17.67	19.21	5.930	18.670	10.103	16.35	17.77
5	72.7	75.7	60.6	60.4	5.506	34.279	4.878	22.732	11.547	17.60	19.13	5.101	23.771	10.508	16.02	17.41
6	67.6	76.6	66.5	63.6	5.179	39.458	4.604	27.336	12.122	17.54	19.07	4.814	28.585	10.873	15.74	17.11
7	47.5	75.9	65.3	65.9	3.604	43.062	3.049	30.385	12.677	17.70	19.24	3.189	31.774	11.288	15.76	17.13
8	44.5	75.9	68.2	66.8	3.380	46.442	2.744	33.129	13.313	18.34	19.94	2.869	34.643	11.799	16.26	17.67
9	64.8	76.4	64.5	66.4	4.952	51.394	4.482	37.611	13.783	19.12	20.77	4.687	39.330	12.064	16.73	18.18
10	59.0	79.5	64.1	64.3	4.693	56.087	5.183	42.794	13.293	19.02	20.67	5.420	44.750	11.337	16.22	17.63
11	58.7	81.3	67.1	65.6	4.774	60.861	3.506	46.300	14.561	20.42	22.20	3.666	48.416	12.445	17.45	18.97
12	51.5	81.4	69.4	68.2	4.194	65.055	3.829	50.129	14.926	20.13	21.88	4.004	52.420	12.635	17.04	18.52
13	84.0	81.8	66.8	68.1	6.868	71.923	6.189	56.318	15.605	21.08	22.91	6.472	58.892	13.031	17.60	19.13
14	63.5	81.0	72.7	69.7	5.141	77.064	5.030	61.348‖	15.716	20.74	22.54	5.260	64.152	12.912	17.03	18.51
0–24 hr. post-infusion urine							11.312	72.660‖				11.829	75.981			
24–48 hr. post-infusion urine							1.039	73.699‖				1.086	77.067			

* These data are from Study No. 88 (a 28 year old normal male weighing 92.0 Kg.).

† Infusion A (4.30 per cent sucrose) was used through Interval 9; infusion B (4.54 per cent sucrose) was used for Intervals 10 through 14.

‡ Correction for plasma water (0.92) was applied at this point. Sucrose space $= \frac{\text{grams retained}}{\text{mean plasma level, Gm. per L.}} (0.92)$.

§ These values were obtained by dividing the uncorrected urine sucrose for each interval by 0.956, the fraction of the total sucrose infused that was excreted. Then the sucrose dose retained within the body at each interval was recalculated for the cumulative results of this correction. Finally, sucrose space was recalculated on the basis of the corrected sucrose dose retained.

‖ Initial recovery = 79.61 per cent; 24 hour recovery = 94.29 per cent; and 48 hour recovery = 95.63 per cent.

TABLE III

Variability of sucrose recovery in repetitive studies (normal subjects)

Subject initials	Study No.	Infusion interval	Sucrose			Recovery during infusion period	Recovery 0–24 hrs. post infusion	Recovery 24–48 hrs. post infusion	Total recovery
			Infused	Average plasma level	Highest plasma level	Total sucrose infused			
		hrs.	*Gm.*	*mg./100 ml.*	*mg./100 ml.*	%	%	%	%
R. H.	73	15	81.9	60.9	71.7	74.6	9.5	0.9	85.0
	78	15	66.6	57.5	63.6	81.3	15.3	0.95	97.6
	88	15	77.0	65.0	72.7	79.6	14.7	1.3	95.6
	111	15	86.0	75.7	90.7	76.8	15.0		91.8
	176	6	34.1	50.8	59.2	55.7	30.3		86.0
R. S.	70	15	97.4	84.0	95.3	71.6	11.8		83.4
	75	6	26.9	52.4	57.1	67.7	29.9		97.6
	77	6	33.3	64.1	69.4	63.1	29.0		92.1
	87	6	42.3	76.9	83.7	63.7	27.9		91.6
	90	6	40.9	58.7	72.6	63.7	30.4		94.1
E. H.	52	6	36.2	50.0	57.7	68.4	23.3		91.7
	59	6	39.5	57.9	71.0	66.2	23.8		90.0
	68	6	42.4	57.7	68.6	70.5	23.5		94.0
	69	6	31.2	43.2	46.5	71.2	22.8		94.0
J. S.	74	6	31.1	51.8	56.8	59.6	22.1		81.7
	79	6	31.5	53.3	60.1	60.6	24.6		85.2
	80	6	11.6	63.2	74.9	67.1	29.2		96.3

urine. In 17 sucrose infusions extending over 10 to 24 hours, the mean urine recovery was 91.4 per cent (S.D. ± 8.5). The completeness of urine sucrose recovery was unrelated to the concentration of sucrose infused or the plasma sucrose level achieved (Table I). In the same individual in different studies (Table III), the degree of sucrose recovery was unrelated to plasma sucrose concentration, indicating that factors other than those measured were most important in determining loss of sucrose. The total sucrose recovered varied as much as 10 per cent in the same person in successive studies (Table III).

The urines were pooled from the infusion period and, in addition, collected for at least the following 24 hours. Twenty-four to 48 hour postinfusion urines were collected after studies on 28 nonedematous individuals. These urines were found to have a mean sucrose content of 2 per cent of the infused dose.[7] Forty-eight to 72 hour postinfusion specimens were collected from three persons, and sucrose in this urine accounted for 0.1 to 0.5 per cent of that infused with a mean of 0.3 per cent. Because of the insignificant amounts of sucrose excreted after 24 hours post infusion, urine was collected routinely for only the first 24 hours post infusion, and this amount was added to the quantity recovered during the infusion period to determine the total sucrose recovery.

In 10 patients with biliary fistulas, six hour sucrose infusion studies were done to learn if sucrose losses in the bile could account for our incomplete recovery. Biliary sucrose could be detected in trace amounts in each study, but the maximum of sucrose loss, with an assumed 24 hour bile flow of 1.5 L. would have been 0.6 per cent of the sucrose infused.

Plasma from two individuals having poor sucrose recovery was incubated for six and eight hours, respectively, in a sucrose solution, and no change in sucrose content was found, either by copper reduction or resorcinol methods. On six occasions, blood specimens were split into two fractions immediately after collection. One was immediately centrifuged and plasma was separated from the red blood cells and blood filtrates prepared immediately; the other specimen was stored in the refrigerator overnight as whole

[7] Six hour infusion studies in 33 edematous individuals had a mean total sucrose recovery of 71.1 per cent (S.D. ± 18.3). The urine collected between the twenty-fourth and forty-eighth hour post infusion of five edematous patients averaged 3.9 per cent of the infused sucrose.

blood. The plasma sucrose level in the whole blood specimen was identical with that of the separated plasma sample in each case.

The possibility was explored that the degree of unexplained sucrose loss was related to the range of sucrose space variations. In Table I, it can be seen that the range in space variation was unrelated to the magnitude of sucrose loss. In five studies where there was a total sucrose recovery of 97 to 103 per cent, there was a range of sucrose space variation (considering only spaces calculated after three hours of infusion) averaging 29.5 per cent of the mean sucrose space. Seven studies with total sucrose recoveries of 91 to 94 per cent showed an average range of 31 per cent of the mean sucrose space for each study. The five other studies had a total sucrose recovery of 83 to 90 per cent, and they had an average range of variation of 26.5 per cent of their respective, mean sucrose space.

Role of fluid intake, urine flow and changes in body weight

During the course of these tests the infused water averaged from 95 to 115 ml. per hour and the total 24 hour infusion volume ranged from 2,300 to 3,300 ml. An additional 300 to 1,000 ml. of oral fluid intake was ingested by six subjects studied for 24 hours. Consequently, the total fluid intake during the six 24 hour infusions varied between 2,300 and 4,000 ml. There was no evidence that sucrose space was increased by intravenous fluids given at this rate or by oral fluids given at a rate not exceeding 250 ml. per hour. However, with greater water loads administered rapidly, larger, but inconsistent, sucrose space changes occurred in the following two instances. In Study No. 55 (Figure 3), a 1,000 ml. water load given over 20 minutes by mouth was associated with a 500 ml. (approximately 0.6 per cent body weight) increase in sucrose space. In Study No. 88 (Figure 2), a 2,200 ml. oral water load given over 30 minutes was associated with a 700 ml. (0.8 per cent body weight) decrease in sucrose space during that period, and was followed by a 1,300 ml. (1.4 per cent body weight) increase in sucrose space in the next hour when 10 mU. of vasopressin was given intravenously.

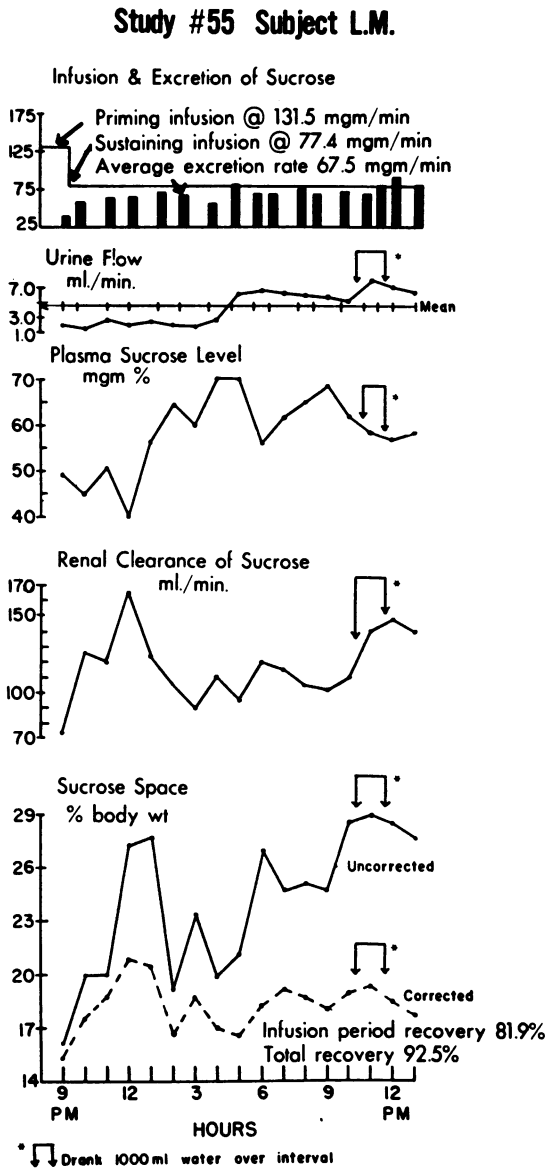

FIG. 3. SUCROSE DISTRIBUTION IN STUDY 55

Urine flow varied widely among individuals and within a single study. In Study No. 34 (Figure 4), the urine flow varied between 0.75 and 1.5 ml. per minute throughout the first 20 hours of infusion with no constant relationship to sucrose space shifts. However, during the last four hours of the same study a sharp increase in urine flow did coincide with a significant decrease in sucrose space. In Study No. 88 (Figure 2), urine flow varied from 0.75 to 9 ml. per minute, changing inversely with the minimal shifts in sucrose space observed. The

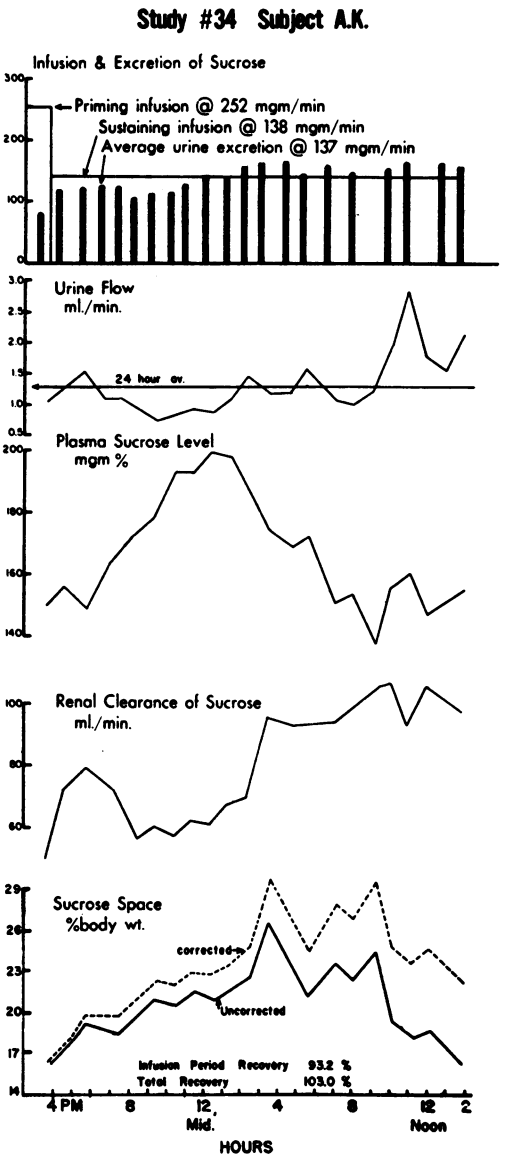

FIG. 4. SUCROSE DISTRIBUTION IN STUDY 34

remaining studies showed lack of a consistent relationship between urine flow and space changes. Changes in urine volume and renal sucrose excretion appeared to account for only a minor portion of the changes in EFV observed.

Changes in body weight were not checked during the six 24 hour sucrose infusions. In four 17 to 18 hour infusions (Studies No. 49, 51, 54 and 55) done in a similar fashion, body weight was determined by having the subject weighed on an in-bed scale at each interval when sucrose space was calculated. During these later four studies, body weight varied from + 0.5 to − 5.5 pounds during the infusion. In the two studies in which weight losses of 4.0 and 5.5 pounds were observed, no significant correlation could be made between changes in body weight and changes in sucrose space.

Role of time of day

These studies were started at various hours of the day. In Figure 1, the possibility was examined that the variations in space represented a diurnal pattern. The studies were arranged in the order of the hour of the day when the infusion was started. No recognizable pattern of space change was correlated with the time of day, or the duration of infusion.

DISCUSSION

The calibrated infusion difference method, like all techniques which estimate extracellular fluid volume, is based on certain premises, the first being that EFV is a discrete volume although made up of subcompartments. The simplest view is of the vascular pool communicating with the major extravascular pool. Actually, the different tissues and organs may vary widely in the relative size of their vascular as well as their extravascular pools, and consequently, estimates of extracellular fluid volume represent sums of these different pools.

Following the injection of an indicator into the vascular compartment, there is an initial rapid vascular mixing phase followed by a slower phase during which the indicator diffuses into the extravascular pools. Since the body tissues vary both in blood volume and perfusion rate, this second extravascular diffusion phase probably differs among organs as to time required for indicator equilibration (12, 13). This major extravascular portion is sampled indirectly through the vascular pool; therefore, to estimate extracellular volume reliably in a short time, rapid diffusion of the indicator must be achieved between the vascular and extravascular pools.

The calibrated infusion difference method as described by Deane, Schreiner and Robertson (7) had four theoretical conditions intended to insure equilibration of the indicator throughout EFV. They felt that: *1*) The rate of indicator infusion

should be constant and accurately measured; *2*) the rate of loss of the indicator should be accurately known from urine analysis; *3*) the rate of diffusion of the indicator should be accurately evaluated from serial measurements of its concentration in the blood and from serially calculated EFV values (a constant blood level was not considered an essential criterion for equilibrium); and *4*) the EFV was assumed to be a pool of constant size. Equilibration of the indicator was thought to be demonstrated when space measurements remained unchanged in successive estimates. In our studies, infusion of the indicator was controlled and renal losses were accurately measured. However, we did not observe a constant EFV and consequently had difficulty evaluating the time at which indicator equilibrium was achieved.

When we began these studies, we prolonged the infusions to explore the possibility that a six hour infusion was not long enough to allow for indicator equilibration throughout EFV. The classic study of Cotlove (14) had suggested that EFV in rats has two distinct compartments: the rapidly equilibrated "functional" EFV plus the slowly equilibrated connective tissue water compartment. Conway (15), in a careful re-examination of Cotlove's data, concluded that the evidence did not support the existence of a slowly equilibrating connective tissue water compartment. Cheek, West and Golden (16), in studies on rats, added further evidence to support this latter view. They found agreement between a six hour inulin space and sodium and chloride spaces after corrections were made for intracellular and skeletal fractions of these ions. For all our studies, when the corrected sucrose space during the fourth interval was compared with the corrected space for the thirteenth interval, a mean decrease of 0.7 per cent body weight was noted. A similar trend was noted in those studies extending to 24 hours. Although this difference is not statistically significant, it certainly is contrary to what should occur with such prolonged infusions, if a significant slowly equilibrating compartment were present.

Even with infusions extending to 24 hours, EFV did not become constant and the plasma sucrose concentration continued to vary significantly, thus precluding the achievement of constant absolute equilibration of sucrose throughout EFV. However, since we found evidence supporting previous reports (*7*) that sucrose equilibrates rapidly, the inability to achieve absolute equilibration is believed to be the result of the continuously changing EFV.

Problems of equilibration

Indirect evidence for the equilibration of inulin throughout the EFV, using the constant infusion technique, has been based on three observations: *1*) the achievement and maintenance of a constant EFV volume in successive measurements by the calibrated infusion difference method with *2*) a plateau in plasma concentration and/or *3*) a reproducible disappearance curve of plasma concentration at the cessation of infusion (6, 8). Using one or more of these criteria, Schwartz, Schachter and Freinkel (6) and Deane (8) presented evidence for a five hour equilibration period for inulin in the EFV of normal men. On the basis of the ratio of the free diffusion coefficients of inulin and sucrose, one might expect sucrose to equilibrate in about one-third of the inulin time. In the hind limb preparation of the cat, Pappenheimer, Renkin and Borrero (12) report sucrose disappearance at a rate somewhat greater than can be accounted for by its diffusion rate relative to inulin.[8]

The view that such substances as sucrose diffuse slowly into connective tissue was substantiated by White and Rolf (17) in tendon, where there is an absence of capillary network. Skin, however, which contains a large percentage of connective tissue and an adequate blood supply, has been shown to be readily permeable to sucrose inasmuch as sucrose space equilibrated with sodium space in 30 minutes (17).

On the basis of having achieved a plateau in serial EFV values, Deane, Schreiner and Robertson (*7*) indicated that sucrose had reached equilibration in the body water within two to three hours. In our studies, EFV variations

[8] The Einstein-Stokes diffusion radii (effective molecular radii, cm. $\times 10^8$) of glucose, sucrose and inulin are 3.6, 4.4 and 15.2, respectively. The free diffusion coefficients at 37° C. (cm.^{2} per sec. $\times 10^{-5}$) are: glucose, 0.90; sucrose, 0.75; and inulin, 0.21 (12).

ranged around a mean sucrose space value which was usually close to that space calculated at two hours. Similarly, the sucrose space estimate made by measuring the final interval blood sucrose and the 24 hour postinfusion urine sucrose was close to the two hour value. Thus, virtual sucrose equilibration was achieved early but, because of changing EFV, we cannot claim to have observed absolute or complete equilibration of sucrose throughout this space.

Extrarenal sucrose losses

The extrarenal sucrose losses mentioned in the Results are another factor to be considered in this sucrose space variability. The reports (18, 19) of "sucrose nephrosis" after massive doses of this sugar in man and dog suggest that part of the sucrose loss might be secondary to intracellular accumulation of this agent in the renal parenchyma. White and Rolf (20) also mentioned the kidney as a major site of intracellular accumulation, since losses were greater in the intact dog than in the nephrectomized dog. Intracellular sequestration in liver and spleen is one type of extrarenal loss which has already been reported in nephrectomized rats by White and Rolf (17). They found no evidence that this was a cyclic phenomenon and concluded from their own data and that of Giebisch, Berger and Pitts (21) from nephrectomized dogs that this intracellular penetration was at a constant rate.[9] Sucrose once within the cell is apparently retained or metabolically degraded. This type of intracellular loss seemed to be the basis for the majority of the extrarenal losses we observed. Moreover, as cited in Results, our studies showed that the magnitude of this extrarenal loss had no relationship to the variability of sucrose space.

Our modifications of the calibrated infusion difference method

Because of the changing level of plasma sucrose and the probable lag in consequent changes in interstitial sucrose, plasma samples at the end of each interval seemed an inappropriate basis for EFV calculation. As a correction for this, we have substituted the mean of the plasma sucrose at the beginning and end of each interval. This change has modulated the extremes of change in EFV previously calculated, but has left intact the major evidence for a continuously varying EFV.

The other important modification was the correction for the constant rate of extrarenal sucrose loss or metabolism. Gaudino and Levitt (9) reported that inulin was quantitatively recovered in the urine within four to six hours following the end of the infusion. Although Schwartz, Schachter and Freinkel (6) found that 95 per cent of the inulin eventually recovered was excreted within the first 12 hours, they did not recover all of the infused inulin even after 36 hours of urine collection. Levy, Ankeney and Berne (22) proposed a correction for this inulin as yet unexcreted which was based on the observation that the logarithmic plot of excretion is linear for the second two and one-half hours of a five hour postinfusion urine collection. Using this linear slope for the excretion rate, they extrapolated to infinity the amount that would eventually be excreted. They made no correction for metabolic loss. Deane and Smith (5) reported that sucrose as well as inulin was apparently lost through metabolism in prolonged infusions but did not propose any correction for this factor in EFV measurements by the constant infusion difference method.

In our studies, extrarenal loss was suggested by the observation that sucrose space increased with time up to the magnitude of 60 per cent body weight. Even with urine collections for 72 hours post infusion, we were frequently unable to recover more than 90 per cent of the infused sucrose. Ikkos (23) reported the finding that sucrose space increased during the course of infusion to an unlikely size, but did not report the degree of sucrose recovery in urine. The inability to recover all of the infused sucrose, if not taken into consideration, resulted frequently in spuriously large estimates of EFV where this space was calculated by the calibrated infusion difference method. This loss of sucrose might be due

[9] This assumption can eventually be tested in studies similar to ours, by using C^{14}-labeled sucrose. This could be done by checking the plasma for evidence that C^{14} was appearing in glucose, fructose or other metabolic fragments at a constant rate. It might also be possible to check the expired CO_2 to determine the degree of variation in specific activity.

to metabolism or to long-term sequestration in some tissue without immediate degradation. We have used a correction factor which is based on the assumption that the total urinary sucrose recovery, including urine of the 24 hours post infusion, divided by the total sucrose infused, described a constant rate of sucrose recovery. An example of a study with the correction applied is illustrated in Table II and Figure 2. This correction, which is based on the constant rate of loss appears to be an empirically valid assumption on the basis of the slope of the uncorrected sucrose space plot. Furthermore, graphs of the successive changes in sucrose space, when corrected over 24 hours, appear to describe variations over a constant mean space. Yet another calculation of sucrose space which does not involve this correction for sucrose metabolism was made by dividing the amount of sucrose recovered in the urine during the 24 hours after the infusion by the plasma sucrose level found at the end of the infusion. This postinfusion recovery space was calculated in the 17 infusions of over 10 hours' duration, and the average difference of this space from the final interval space corrected for recovery was found to be approximately 6 per cent (Table I). This average difference of 6 per cent is less than the standard deviation for successive intervals beyond the third hour corrected for recovery (8.4 per cent). Since the mean of postinfusion recovery spaces was smaller than the mean of final interval corrected spaces, collection of urines for 48 hours post infusion would have increased the postinfusion recovery space and materially decreased the difference between them.

Clinical utility of six hour infusions

The variability in EFV in prolonged infusion studies naturally raised questions concerning the clinical usefulness of sucrose space studies of five to six hours' duration. The mean sucrose space of our 17 prolonged infusion studies was 19.4 per cent body weight. When only the eight normal subjects with 12 studies were considered, the mean was 20.5 per cent body weight. These means are somewhat higher than the 17.6 per cent found for four normal individuals by Deane, Schreiner and Robertson (7). However, in view of the small normal populations being compared and their probable variation in body fat content, these differences are probably not significant.[10] A more important question posed is: How well does an infusion of five to six hours define the EFV as revealed by prolonged infusions? Although the coefficients of variation for sucrose spaces beyond the third hour averaged 8.4 per cent, in 11 of 17 studies the average sucrose spaces calculated for the fourth, fifth and sixth hours agreed within 5 per cent of the mean values for the entire infusion. In only one study did the mean of the fourth, fifth and sixth hours differ more than 15 per cent from the mean of space values for the entire infusion. For this reason, we concluded that a six hour sucrose infusion, using the calibrated infusion difference method, usually described the mean EFV as found by prolonged infusion, but it does not describe the range of EFV variation observed with prolonged infusions. The mean values for the EFV of the first three hours varied more widely from the mean of the entire infusion than did the mean EFV of the fourth, fifth and sixth hours. This would indicate that equilibrium of sucrose was not attained during the first two periods. Nevertheless, the mean of all one to six hour values in a study agrees very well with the mean of an entire prolonged infusion (13 of 17 agree within 5 per cent, but three of 17 differ by more than 15 per cent). Thus, in nonedematous subjects, a six hour sucrose infusion provides a reasonable estimate of EFV, although there is a chance for significant deviation in any one set of observations.

Evidence for EFV change

Our evidence suggests that EFV, as measured by sucrose, changes within the short intervals necessary for space measurement. The reported experience of others using a variety of labels and techniques provides evidence for variability in EFV over short intervals. However, the lack of sufficient control information in normal subjects

[10] We have found, in data from a group of 46 adult males, a meaningful correlation between sucrose space and body fat. The following equation has a negative regression coefficient of 0.441 with a p value < 0.01. Sucrose space (per cent body weight) = 20.1 per cent − 0.173 (per cent fat − 18.7 per cent).

makes the evaluation of these data difficult. Stewart and Rourke (24) reported on the single injection method using a thiocyanate label. Their report gave early evidence of large increases in EFV in postoperative patients having had ether anesthesia. Later evidence (25, 26) using similar techniques reported decreases as well as increases in EFV when ether, as well as other anesthetic agents, was used. The single injection method with sodium thiosulfate as indicator has shown unpredictable changes in EFV in postoperative patients (27). When this same technique was used in normal subjects, some of the results suggested the possibility that EFV could change within the period of measurement (2, 28). Ryan, Pascal, Inoye and Bernstein (29), using single injections of sodium sulfate-S^{35}, found evidence of space increasing and decreasing within the few hours of a study. Recently, Poulos (30) reported on his "constant-change" modification of the single injection technique (using sucrose and inulin) and observed in normal subjects shifts equivalent to 30 per cent of the mean space. Crawford and Gaudino (31), using constant rate infusions of inulin, found unpredictable fluctuations from minus 16 to plus 83 per cent of the initial EFV, following the administration of anesthetic agents or saline infusions. The random nature of these shifts made causal connection with the treatment seem doubtful.

However, any EFV estimate is a function of the indicator and technique used, the body composition and the net effects of physical and biochemical forces acting upon the indicator. This is emphasized here to point out the difficulties that might be encountered in using two or more indicators simultaneously for the purpose of verifying the volume, or changes in volume, of EFV. A promising intercomparison could be made by the use of radioactive indicators, which would permit EFV calculation, both from chemical and from radioassay data of the same indicator. Thus, an important problem raised by these studies is the need for a technique to verify and measure precisely the changes in EFV as reported here.

We believe that the changes observed in sucrose space are due to physiologic shifts in body water which occur from unknown forces. These variable shifts have been observed in previous reports, but have been ignored or ascribed to induced factors and to laboratory and clinical errors (24–27, 30, 31). Since our subjects (except Study No. 88) were not manipulated during the studies and our errors were small, these explanations do not fit our data.

SUMMARY AND CONCLUSIONS

A) A calibrated sucrose infusion technique with extracellular fluid volume (EFV) calculated by the difference method has been described and evaluated in 17 studies of 10 to 24 hours' duration.

B) Indirect evidence is provided that parenterally administered sucrose is metabolized in man. An empirically satisfactory correction for the rate of sucrose metabolism has been applied to the calculation of EFV by the difference method and compared with EFV calculated by the post-infusion recovery method.

C) EFV, as measured by the calibrated infusion difference method, has been observed to exhibit frequent variations of significant magnitude in control subjects, and it is considered reasonable that these measurements describe real fluctuations in EFV.

D) A six hour sucrose infusion usually describes the mean EFV of an individual, but probably does not describe the normal variability of EFV changes for the individual.

E) This variability of EFV demonstrates the complexity of the equilibration problem for any test molecule used in similar techniques and the difficulty in comparing EFV measured by different techniques.

F) There is need for the development of a better technique to verify and measure precisely the changes in EFV as reported here.

ACKNOWLEDGMENTS

We wish to thank Miss Arvilla Kobernusz and Mrs. Ruth Records for their interest in this project and their diligent and careful work in support of it.

APPENDIX

AN EXAMINATION OF A SEQUENCE OF RANDOMIZED ERRORS BY THE MONTE CARLO METHOD

By OSCAR KEMPTHORNE

The important question that has arisen throughout the present study is whether or not the fluctuations that are observed in the sucrose space of an individual are real fluctuations or are merely artifacts arising from the facts that the ingredients in a computed value are subject to error in the statistical sense, and that the computational procedure incorporates these errors in such a way as to give the appearance of nonrandom fluctuations. An example familiar to students of statistics is the so-called Slutsky effect which may be observed by taking a sequence of random numbers, such as are given in tables of random numbers, and calculating a moving average. It will be found that the resulting sequence of averages exhibits a very marked periodicity and it will generally be accepted that such periodicity is purely an artifact.

It will be seen from the description of the procedure of calculating the sucrose space that the ingredients are the cumulated infused amount less the cumulated excreted amount divided by the blood plasma level, apart from the constant adjustment of incomplete recovery. It is conceivable that a compounding of errors in the measurement of amounts infused and excreted would lead to fluctuations of the magnitude observed. If this were so, the compounding of errors would be a simpler explanation of the observed fluctuations than hypothesizing variations in the true space, defined as the result of the calculation process with observations so replicated as to contain essentially no error.

It is not easy to calculate mathematically the effects of compounding errors (though possibly someone more expert in probability theory could do so) and for such cases a standard procedure is to use the so-called Monte Carlo method (32). This method consists essentially of constructing artificial data subject to the known errors and then observing how a computed value of interest varies. A primary ingredient in such a trial is a sequence of random numbers, which follow the hypothesized random distribution. We assumed that errors of measurement were normally distributed and could have used a table of random normal deviates. Alternatively, as we did, one can take two figure random numbers from a table such as Table XXXIII of Fisher and Yates (33). Converting these to decimals by dividing by 100, we can read from Table IV of Fisher and Yates (or similar tables) the probit corresponding to this proportion, and after subtracting 5 we have a random normal deviate which will have an average value of zero and a standard deviation of unity. Denoting this random number by R, with, for example, an infusion rate of 3.27 Gm. per hour and a relative standard deviation of 0.005 (or 0.5 per cent), we take the amount infused in a particular hour interval to be $3.27\ (1 + 0.005\ R)$. The cumulative amount infused is the sum of the amounts for the successive periods.

The basis of the model used is $V = \frac{\Sigma iv - \Sigma uv}{P_t}$, which assumes EFV to be a single compartment (see Methods).

The figures used in this hypothetical case were selected to give the best approximation of the various factors in the volume estimates as observed in our human studies. Σiv, the grams infused, was set at 10.0 for the first interval and 3.27 for all subsequent intervals. The grams infused for each interval was multiplied by the pump error of 0.5 per cent which was randomized as previously described. P_t, the blood level in grams per liter, was set at 0.52 with an increment of 0.01 for each interval, multiplied by a randomized standard error of 2.0 per cent. Σuv, the grams excreted for each interval, was set at 3.0 with an increment of 0.01 for each interval, also multiplied by a randomized standard error of 2 per cent. V, calculated with the randomized errors in each factor, was then compared with that V calculated without errors in the same factors. The model was calculated for 25 intervals.

This process was carried out with two complete sets (total, 150) of two figure random numbers. Thus, having each of the three components in the space calculation available in two series with randomized errors, it was possible to calculate for eight cases over 25 intervals, the permutations of randomized errors on sucrose space. The situation was that we have eight sequences as follows:

time	1	2	3	n
"true" space	t_1	t_2	t_3	t_n
"observed" space	0_1	0_1	0_3	0_n

The relative error P_n was calculated as follows: $P_n = 100\frac{0_n}{t_n} - 100$. Then the standard deviation of P_n was calculated, giving the relative compounded error (in this instance, synonymous with coefficient of variation), which ranged from 1.75 to 2.94 for the eight sequences. Using the same representation as above, the largest deviation from zero for the eight sequences ranged from −8.3 to +10.5 per cent. The maximum range of deviation from true space in a series of 25 intervals varied from 7 to 11 per cent. The maximum change over one interval for the eight sequences ranged from 5.1 to 6.1 per cent. Confidence limits were established by constructing a histogram of changes from one time to the next. Over 95 per cent were found to be less than 5.5 per cent and 99 per cent were found to be less than 6.0 per cent.

The coefficient of variation for sucrose space in the 17 patients (313 intervals) was approximately three times the magnitude of the coefficients of variation for space in the eight cases (200 intervals) of randomized errors. Similarly, the range of deviation from mean sucrose space in the 17 patients was approximately three times the similar figure for the cases of randomized error. Only Study No. 88 had sucrose space deviations which approach those of randomized errors, and it had one interval shift that exceeded the 99 per cent confidence interval. Consequently, the deviations in sucrose space found in these patients cannot be attributed just to random analytic and pump errors. Biologic factors must play the major role in the sucrose space deviations described.

REFERENCES

1. Peterson, R. E., O'Toole, J. J., and Kirkendall, W. M. The variability of extracellular fluid space (sucrose) in man during a 24-hour period (abstract). J. clin. Invest. 1956, **35**, 730.
2. Cardozo, R. H., and Edelman, I. S. The volume of distribution of sodium thiosulfate as a measure of the extracellular fluid space. J. clin. Invest. 1952, **31**, 280.
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. Finkenstaedt, J. T., O'Meara, M. P., and Merrill, J. P. Observations on the volume of distribution of inulin in anuric subjects. J. clin. Invest. 1955, **32**, 209.
5. Deane, N., and Smith, H. W. Fate of inulin and sucrose in normal subjects as determined by a urine reinfusion technique. J. clin. Invest. 1955, **34**, 681.
6. Schwartz, I. L., Schachter, D., and Freinkel, N. The measurement of extracellular fluid in man by means of a constant infusion technique. J. clin. Invest. 1949, **28**, 1117.
7. 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.
8. Deane, N. Methods of study of body water compartments (space techniques). Meth. med. Res. 1952, **5**, 159.
9. Gaudino, M., and Levitt, M. F. Inulin space as a measure of extracellular fluid. Amer. J. Physiol. 1949, **157**, 387.
10. Nelson, N. A photometric adaptation of the Somogyi method for the determination of glucose. J. biol. Chem. 1944, **153**, 375.
11. Higashi, A., and Peters, L. A rapid colorimetric method for the determination of inulin in plasma and urine. J. Lab. clin. Med. 1950, **35**, 475.
12. Pappenheimer, J. R., Renkin, E. M., and Borrero, L. M. Filtration, diffusion and molecular sieving through peripheral capillary membranes. Amer. J. Physiol. 1951, **167**, 13.
13. Best, W. R. Relation of plasma time-concentration curves to plasma volume, interstitial fluid volume, vascular permeability, and renal clearance of substances confined to the extracellular fluid. Report No. 125, Medical Nutrition Laboratory, United States Army, Feb. 20, 1954.
14. Cotlove, E. Mechanism and extent of distribution of inulin and sucrose in chloride space of tissues. Amer. J. Physiol. 1954, **176**, 396.
15. Conway, E. J. Nature and significance of concentration relations of potassium and sodium ions in skeletal muscle. Physiol. Rev. 1957, **37**, 84.
16. Cheek, D. B., West, C. D., and Golden, C. C. The distribution of sodium and chloride and the extracellular fluid volume in the rat. J. clin. Invest. 1957, **36**, 340.
17. White, H. L., and Rolf, D. Whole body and tissue inulin and sucrose spaces in the rat. Amer. J. Physiol. 1957, **188**, 151.
18. Anderson, W. A. D., and Bethea, W. R., Jr. Renal lesions following administration of hypertonic solutions of sucrose: Report of 6 cases. J. Amer. med. Ass. 1940, **114**, 1983.
19. Raisz, L. G., Young, M. K., Jr., and Stinson, I. T. Comparison of the volumes of distribution of inulin, sucrose, and thiosulfate in normal and nephrectomized dogs. Amer. J. Physiol. 1953, **174**, 72.
20. White, H. L., and Rolf, D. Comparison of various procedures for determining sucrose and inulin space in the dog. J. clin. Invest. 1958, **37**, 8.
21. Giebisch, G., Berger, L., and Pitts, R. F. The extrarenal response to acute acid-base disturbances of respiratory origin. J. clin. Invest. 1955, **34**, 231.
22. Levy, M. N., Ankeney, J. L., and Berne, R. M. Kinetics of inulin distribution and excretion following a constant infusion. Amer. J. Physiol. 1952, **169**, 363.
23. Ikkos, D. Comparison of the volume of distribution of sucrose, inulin, and thiosulfate in human subjects. Metabolism 1955, **4**, 19.
24. Stewart, J. D., and Rourke, G. M. Changes in blood and interstitial fluid resulting from surgical operation and ether anesthesia. J. clin. Invest. 1938, **17**, 413.
25. Ariel, I. M., and Kremen, A. J. Compartmental distribution of sodium chloride in surgical patients pre- and postoperatively. Ann. Surg. 1950, **132**, 1009.
26. Bonnycastle, D. D. Effect of some anesthetic agents on volume of body fluid. J. Pharmacol. exp. Ther. 1942, **75**, 18.
27. Berry, Robert. Personal communication.
28. Friis-Hansen, B. The extracellular fluid volume in infants and children. Acta paediat. 1954, **43**, 444.
29. Ryan, R. J., Pascal, L. R., Inoye, T., and Bernstein, L. Experiences with radiosulfate in the estimation of physiologic extracellular water in healthy and abnormal man. J. clin. Invest. 1956, **35**, 1119.
30. Poulos, P. P. A constant-change (single injection) method for the estimation of the volume of distribution of substances in body fluid compartments. J. clin. Invest. 1956, **35**, 921.
31. Crawford, E. J., and Gaudino, M. Changes in extracellular fluid volume, renal function, and electrolyte excretion induced by intravenous saline solution and by short periods of anesthesia. Anesthesiology 1952, **13**, 374.
32. Monte Carlo Method (Applied Mathematics Series 12), A. S. Householder, Ed. Washington, D. C., National Bureau of Standards, 1951.
33. Fisher, R. A., and Yates, F. Statistical Tables for Biological, Agricultural and Medical Research, 4th revised ed. New York, Hafner Publishing Co., 1953, p. 128.