DETERMINATION OF THE VOLUME OF THE EXTRACELLULAR FLUID OF THE BODY WITH RADIOACTIVE SODIUM

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In order to secure precise knowledge of the fluctuations of body water, it is necessary not only to obtain the concentration of electrolytes in the serum but also to know the volume of fluid in which they are dissolved and the apportionment of these fluids among the various compartments of the body. While there are now available several fairly accurate methods for measuring the volume of the vascular or plasma fluid, attempts to determine the volume of intracellular fluid have not proved too successful. On the other hand estimation of the extracellular volume by means of certain diffusible, non-metabolized substances such as thiocyanate, sucrose, and sulfate have been more fruitful (1–5). Most of these substances are not present in the body normally but resemble chloride in that they are restricted mainly to the extracellular fluids. The principle of the methods employed is not complicated. Some hours after the intravenous injection of the test substance, samples of blood and urine are obtained and the concentration determined in each. Assuming that the substance is uniformly distributed, the amount remaining in the body divided by the concentration in the serum water gives the volume of fluid in which it is dissolved. It appears that these substances are distributed throughout approximately 20 to 25 per cent of the body weight of man (1, 2, 5).

Lavietes, Bourdillon, and Klinghoffer (2) have shown that for practical purposes sucrose and sulfate are not suitable for determination of the extracellular fluid because of their speed of excretion. However, sucrose has an advantage in that it is a non-electrolyte and therefore its concentration in the water of serum and of interstitial fluid is probably the same. On the other hand thiocyanate has numerous advantages. It is rapidly distributed through the body, it is slowly excreted, and its concentration can be readily determined with accuracy in a small sample. It has several disadvantages in that it enters the red blood cells, attaining a concentration approximately the same as that of serum, and it enters the cells of certain glandular organs. Lavietes and coworkers (2, 6) and Gilligan and Altschule (7) believe that it is 'bound'

somewhat to some relatively non-diffusible substance in the serum, so that the average concentration in the transudate is 100/110 that in the serum.

In view of the fact that none of these substances proved to be ideal for the purpose, a substance such as sodium which is normally present in the body and is mainly limited to the extracellular phase, should afford a convenient measure of the volume of this compartment. This can be accomplished readily by determining the volume of body fluid through which radioactive sodium (Na^{24}) is distributed after its intravenous injection. The purpose of this paper is to describe such a method and to report the results of determinations of the sodium space on normal subjects and to compare these results with those obtained by estimating the volume of fluid available for the distribution of sodium thiocyanate.

Methods and Procedures

Radiosodium was prepared in the cyclotron in the Department of Physics of the University of Rochester. It was formed by the bombardment of sodium chloride with deuterons. The reaction was $Na^{23} + H_2 \rightarrow Na^{24} + H^1$. The chloride isotope was also produced but it has a very short half life of 37 minutes. Radiosodium decays with the emission of free electrons or β-rays, $Na^{24} \rightarrow Mg^{24} + e^-$. The half life of Na^{24} is 14.8 hours. The amount of the radioactive isotope present in a sample was determined by measuring its β-ray activity by means of a modified Geiger-Müller counter. The counting tube described by Bale, Haven, and LeFevre (8) was employed. The radio activity measurements were recorded in terms of scale-of-four counts per minute per milliliter. Each sample was corrected for decay of its β-ray activity to the time of the counting of the original radiosodium standard.

The method is based on dilution of a known quantity of Na^{24} (in counts) by the extracellular water. The volume in which the sodium is diluted is found by dividing the quantity of radiosodium present in the body by the concentration in a sample of serum. The quantity in the body at any given time is the quantity injected minus the urinary loss. Here the assumption is made that radiosodium does not diffuse into the cells, but is exclusively limited to the extracellular fluid. It will be shown later that there is an excess of sodium in the body that cannot be ascribed to the extracellular compartment.

The active material (approximately 300 mg.) was dissolved in 13 to 20 ml. of distilled water and the solution autoclaved. A measured volume (usually 9.16 ml.) of this solution was injected into the antecubital vein from a specially calibrated syringe. The syringe was rinsed with blood several times to insure delivery of all the sodium. From the remaining portion of the radiosodium solution, 1 ml. samples were delivered into three separate volumetric flasks and diluted with physiological saline to 2 liters. These are referred to as the radiosodium standards. It was found that three samples were necessary for accurate determination of the number of counts injected.

Simultaneously, the thiocyanate method for the measurements of extracellular fluid was carried out as described by Gregersen and Stewart (4). Immediately following the injection of the radioactive sodium, 16 to 18 ml. (0.8 to 0.9 gm.) of sterile 5 per cent sodium thiocyanate solution were injected slowly into the ante-

cubital vein of the subject from a calibrated syringe. Thiocyanate was determined as ferric thiocyanate by the use of the spectrophotometer in preference to the colorimeter (1, 4). The readings were made at a wave length of 480 millimicrons.

Blood volume determinations were made in some of the subjects by the use of the blue azo dye (T-1824) as described by Gibson and Evans (9). Optical densities were determined by the spectrophotometer at wave lengths of 620 millimicrons for the dye and 574 millimicrons for correction for hemolysis. In order to detect possible change in the relationship of cells and plasma several hematocrits were taken during each experiment. Blood was also taken for plasma protein determinations.

The experiments fall into two groups; those carried out during the daytime ranging from 3 to 6 hours and those conducted at night for a 12 hour period. The subjects were kept under basal conditions. Control serum and urine samples were obtained from each subject prior to the injection of radioactive sodium and thiocyanate. Blood samples were taken at fairly regular intervals during each experiment to determine the mixing tine of Na^{24} and thiocyanate. In one group of patients the concentrations of Na^{24} and thiocyanate in blood were compared with those of various serous effusions. Two blood samples (6 ml. of whole blood) were taken in separate syringes through one needle. Such samples were put under oil, allowed to clot, centrifuged, and the serum pipetted off in preparation for the determination of β-ray activity, dye, and thiocyanate concentrations. Urine specimens were obtained following the venipuncture, in order to determine the excretion of radiosodium and thiocyanate. Duplicate transudate samples were taken immediately following the blood samples.

From each sample of the standard radiosodium solution, serum, urine, and transudate, a 2 ml. aliquot was pipetted carefully into a Geiger-Müller cup. These were counted for at least two 5 minute periods. Further periods were often counted to increase accuracy.[1] The optimum range for the Geiger-Müller counter used in most of the experiments was below 100 counts per minute for a 2 ml. sample. When the activity of samples exceeded this value, time was allowed for radioactive decay until the optimum counting range was reached. An improved counter with a very much higher optimum range was used in the more recent determinations.[2] The same urine, serum, and transudate samples were used for the thiocyanate determinations. No toxic reactions of any kind were observed with either thiocyanate or radiosodium.

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[1] The procedure for counting the β-ray activity of all the samples for one experiment is as follows: (1) Distilled water background. (2) Potassium acetate standard. (3) Radiosodium standard number 1; radiosodium standard number 2; radiosodium standard number 3. (4) Control serum sample. (5) Duplicate serum samples (taken at varying intervals following the injection of Na^{24}). (6) Duplicate transudate samples. (7) Control urine sample. (8) Urine samples (taken at varying intervals following the injection of Na^{24}). (9) Radiosodium standard (one, selected at random). (10) Potassium acetate standard. (11) Distilled water background.

[2] It was found that neither the specific gravity nor any of the constituents of serum and urine had any effect upon the penetration of β-rays. Equal amounts of radiosodium solution were added to equal amounts of serum, distilled water, and urine and these solutions exhibited the same β-ray activity.

The fluid available for distribution of radiosodium (sodium space) was calculated as follows:

$$\text{Sodium space, liters} = \frac{\text{Counts injected} - \text{counts lost in urine}}{\text{Concentration counts per minute per liter of serum}}$$

The interstitial fluid (extravascular extracellular fluids) is equal to the sodium space minus the plasma volume.

The fluid available for the distribution of thiocyanate was calculated by the formula:

$$\text{Thiocyanate space, liters} = \frac{\text{Mg. CNS injected} - \text{mg. lost in urine}}{\text{Mg. CNS per liter of serum}} \text{ (Formula A).}$$

Thiocyanate space was also calculated according to the formula of Lavietes, Bourdillon, and Klinghoffer (2);

$$\text{Thiocyanate space corrected} = \frac{\text{Mg. CNS retained} - (\text{concentration in serum} \times \text{blood volume})}{100/110 \times \text{concentration in serum}} + \text{serum volume} \quad \text{(Formula B)}$$

Gregersen and Stewart (4) found that the available fluid inside the red blood cells is equal to their water content, which is about 70 per cent of the cell volume. Furthermore, when the cell volume is calculated from the plasma volume and the hematocrit, it is somewhat higher than the cell volume determined directly by the carbon monoxide method. This formula then attributes too much thiocyanate to the erythrocytes.

Accuracy of the Method.—In order to determine the value of this procedure for use in clinical investigation, it is necessary to establish the magnitude of errors inherent in the method. The technical errors resolve themselves into (*a*) pipetting, sampling, and counting the β-ray activity of the standard, serum, and urine and (*b*) the measuring and injection of the radioactive substance into the body. There is ample data on the former to describe the limits of errors with reasonable accuracy. All counts referred to in this paper represent scale-of-four counts on the impulse recorder and therefore one-fourth the total number of discharges in the counting tube. It was found that three times the standard error of a single determination of β-ray activity was the same for individual standard solutions and serum samples, amounting to 2.3 counts per minute per milliliter. There was no correlation between the magnitude of the error and the degree of activity of the same over a range of 10 to 100 counts per minute per milliliter, therefore, the error in counting a single sample is properly expressed in counts per minute per milliliter rather than in per cent. The error in the sodium space due to counting errors in standard, serum, and urine samples depends, because of this, on the magnitude of activity of the various samples, with accuracy increasing as the activity increases. From the analysis of the data available, the error in the sodium space due to errors in counting the activity of these samples is $\pm$2520 ml. when the count is equal to 10 per minute per milliliter; $\pm$644 ml. when the count equals 40; and $\pm$266 ml. when the count is 100. The average count in this series of experiments was 40 per minute per milliliter. Therefore, with an average sodium space of 18.3 liters, the error, due to counting, should not exceed $\pm$0.644 liter or $\pm$3.5 per cent in 95 per cent of the cases.

This takes into consideration most of the technical errors that can be determined, except for the measuring and the injecting of radiosodium. In order to get an idea of the magnitude of error involved in these procedures, a series of five *in vitro* experiments was done. The procedure was as follows: 14 liters of normal saline were placed in a large container, and 9.16 ml. of a radioactive sodium solution were injected. The measurement and injection of the sodium were carried out in the same manner as though it was to be injected into a subject. After thoroughly mixing the contents of the container, samples were taken in order to determine the β-ray activity. Then 2 more liters of saline were added to the container and the process repeated. The total counts injected divided by the activity of the sample per minute per cubic centimeter gave the estimated volume in liters. The mean difference between the theoretical and the estimated volumes was -0.013 liter with a standard deviation of ± 0.4146. Correcting for errors of distribution due to the small size of the sample at a level of significance of $P = 0.05$, the deviation of the sodium space from the "truth" should not exceed ± 0.896 liter (10). The percentage error, with an average sodium space of 16 liters, is ± 5.6. Since the average count in this series of experiments was 75 counts per minute per cubic centimeter, the error to be expected from counting the β-ray activity should not be greater than ± 0.268 liter. The difference between the values (± 0.896 and ± 0.268 liter) theoretically represents the error in measuring and injecting the radiosodium.

In order to define the limits of error in the thiocyanate method, the volume of fluid in the above experiments was also estimated by means of sodium thiocyanate. These errors include most of the technical ones associated with this method. The mean difference between the actual and estimated volumes was $+0.052$ liter with a standard deviation of ± 0.193 liter. Again correcting for the errors of distribution due to the small size of the sample, the deviation of the thiocyanate space from "truth" should not exceed ± 0.425 liter in 95 per cent of the cases. This represents a percentage change of ± 2.7 with an average volume of 16 liters.

Material.—Twenty-two measurements of the extracellular fluid were made on thirteen normal subjects, whose ages varied between 20 and 56 years, body weight between 58 and 106 kg., and body surface area between 1.6 and 2.3 square meters. All the subjects were in good health.

In order to determine the rate of diffusion of radiosodium and thiocyanate from the blood stream into the interstitial fluid, serous effusions into the pleural cavity caused by congestive heart disease, Hodgkin's disease, and pulmonary tuberculosis were obtained ten times from 5 patients; ascitic fluid from a patient suffering with congestive heart failure; synovial fluid from a patient with rheumatoid arthritis; and spinal fluid from four subjects whose fluid showed no abnormalities. Saliva was collected from three normal subjects and gastric juice from two.

RESULTS

Rate of Diffusion of Radiosodium and Thiocyanate.—After the intravenous injection of the radioactive isotope of sodium the β-ray activity of the serum gradually fell with time until equilibrium was reached. In Fig. 1 are plotted the volumes of extracellular fluid calculated from the concentration of counts

in the serum against time. These volumes were corrected for loss of radiosodium in the urine. In general from 1 to 3 hours after the intravenous injection, the volume increased rather rapidly (Fig. 1A), while from 3 to 9 hours

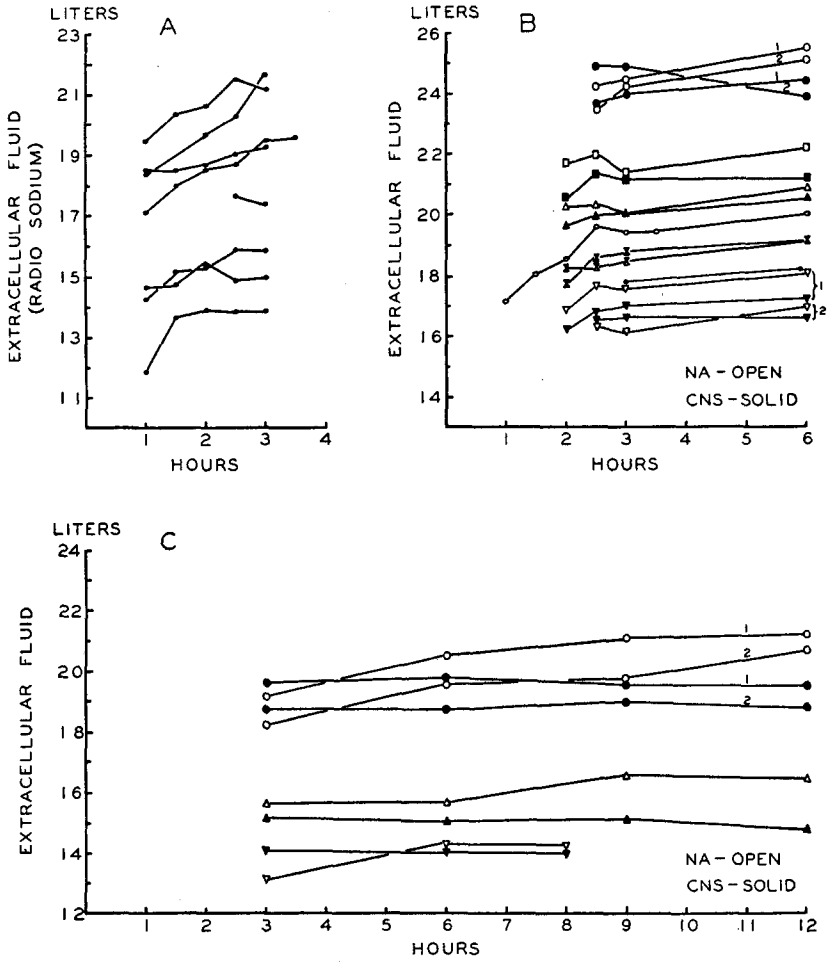

FIG. 1. The rate of diffusion of radiosodium and sodium thiocyanate in the body of normal subjects after intravenous injection.

after the administration the rate of increase was much slower, indicating that the marked sodium was diffusing more slowly into certain parts of the body (Fig. 1 B and C). Measurements of the extracellular fluid on eight normal subjects showed that the average value for the volume 3 hours after the injection was 18.8 liters as compared with 19.6 liters at 6 hours or an average increase of 800 cc. A similar increase was noted from 6 to 9 hours after the

injection, while from 9 to 12 hours the values approximated each other more closely (Fig. 1 C). Serial determinations of the sodium space on two normal subjects at 3, 6, 9, and 12 hours gave average values of 17.4, 18.1, 18.9, and 18.9 respectively. The rapidity with which the β-ray activity of the serum fell during the first 3 hours after the injection suggests that the tagged sodium diffused rapidly into certain portions of the body (24.8 per cent of the body weight) while the slower rate of decline during the next 6 hours indicates that there are certain portions of the body into which the sodium penetrates less easily.

In some instances the diffusion rate of thiocyanate was studied simultaneously with that of Na^{24}. The rate of diffusion for thiocyanate was more rapid than that for sodium. Equilibrium was reached 3 hours after the administration (Fig. 1 B and C). In eight normal individuals the average value for the volume of fluid available for the distribution of thiocyanate was 18.8 liters 3 hours after the injection and 18.9 liters at 6 hours. When samples of serum were obtained every 3 hours for a 12 hour period, the average values for the thiocyanate space (not corrected) in two subjects were 17.4 liters at 3 hours, 17.3 liters at 6 hours, 17.3 liters at 9 hours, and 17.0 liters at 12 hours.

The rate of attainment of diffusion equilibrium for Na^{24} and thiocyanate between the blood stream and serous effusions was also studied. The results are given in Table I. All the subjects had rather large accumulations of fluid. In the case of pleural fluid, diffusion equilibrium for both Na^{24} and thiocyanate appeared to be reached in most cases between 9 and 12 hours after the injection of the substances. This was also true in the case of ascitic fluid (7). 10 liters of ascitic fluid were withdrawn from patient V.C. The observations with respect to thiocyanate are not in accord with the results of previous workers (2, 6, 7), who found the concentration of thiocyanate in transudates was 100/110 of the concentration in serum. This discrepancy in results is due in all probability to the fact that the effusions in the cases herein reported are not strictly speaking normal interstitial fluid because of their high protein content.

In contrast to other ultrafiltrates of serum, thiocyanate does not enter the spinal fluid to any great extent. This confirms the observation of Wallace and Brodie (11) that thiocyanate does not enter the central nervous system, unless the concentration in the blood stream is much higher than that used in these experiments. It required about 12 hours for Na^{24} to attain diffusion equilibrium between spinal fluid and serum (Table I). If, as has been suggested (12), the extracellular fluid of the central nervous system has the same relationship to spinal fluid as that of other tissues has to serum, then radiosodium diffuses slowly into nervous tissue and even 12 hours after injection it may not yet be in ionic equilibrium with spinal fluid. In the dog the radio-

sodium has not penetrated the central nervous system to its maximum concentration (Table V), for the apparent extracellular volume calculated from Na^{24} was smaller (29.3) than that calculated from the chloride (44.6). According to Manery and Hastings (13) the ratio Na:Cl is slightly higher for spinal cord than for ultrafiltrate of serum.

TABLE I

Concentration of Radiosodium and Thiocyanate in Serous Effusions Expressed as Percentage of the Serum Concentration

Subject	Serous effusion	Time after injection	Protein		Sodium concentration	Thiocyanate concentration	Condition
			Serum	Effusion			
		hrs.	*gm. per cent*	*gm. per cent*	*per cent*	*per cent*	
M. M.	Pleural fluid	3	4.7	2.7	61	101	Hodgkin's disease
	" "	6			87	130	
E. H.	" "	6	6.4	4.8	90	87	Congestive heart failure
	" "	9			101	99	
	" "	12			95	101	
G. J.	" "	6.5	6.4	2.5	103	94	Congestive heart failure
	" "	9			99	103	
A. W.	" "	3	6.3	3.0	87	—	Congestive heart failure
	" "	6			94	—	
	" "	24			97	—	
N. H.	" "	9	8.8	6.3	78	78	Pulmonary tuberculosis
A. J.	Synovial fluid	12	7.0	5.4	101	95	Rheumatoid arthritis
V. C.	Ascitic fluid	12	6.3	3.3	95	100	Congestive heart failure
	" "	13			97	99	
B. L.	Spinal fluid	3			22	—	Normal
J. O.	" "	6			23	9	"
M. E.	" "	9			68	—	"
F. B.	" "	12			101	0	"
P. M.	Saliva	3			7	870	Normal
L. Y.	"	3			7	1150	"
F. B.	"	3			13	1280	"
N. H.	Gastric juice	3			39	111	
P. Q.	" "	6			28	770	

It has been shown that thiocyanate enters the cells of the glands of the digestive tract, and that it is secreted into the alimentary tract (1). The results of this study showed that the concentration of thiocyanate in saliva and gastric juice was very much higher than that in the serum. On the other hand, the concentration of radiosodium in such fluids was in the expected range (Table I).

Volume of Extracellular Fluid in Normal Subjects.—The results of measurements made on fourteen subjects are given in Table II. The average value for the sodium space calculated from the counts in the serum sample taken 3 hours after the injection of labeled sodium was 18.8 liters or 24.8 per cent of

the body weight and 9.7 liters per square meter of body surface area. The extreme values for the volume were 14.2 and 24.4 liters. At 6 hours the average

TABLE II

The Fluid Available for the Distribution of Radiosodium and Thiocyanate in Normal Subjects

Experiment No.	Normal subject	Date	Weight	Time after injection	Plasma volume	Extracellular fluid "sodium space" volume	Extracellular fluid "thiocyanate space"	
							A*	B*
		1939	*kg.*	*hrs.*	*liters*	*liters*	*liters*	*liters*
1	H. H.	Apr.	69.6	3	3.16	19.0		
2	J. Z.	Sept.	67.1	3	2.77†	13.9		
3	J. De.	Aug.	80.6	3	2.82	19.4		
				6		20.0		
		1940						
4	J. D.	Mar.	64.6	3	3.07	16.7		
				6		17.1		
5	J. T.‡	June	51.0	12	2.41†	17.5	17.5	17.0
6	J. P.	Jan.	69.8	12	2.58	18.4	17.8	16.8
7	L. Y.	Mar.	84.5	3	3.31	20.1	20.2	18.9
				6		20.9	20.6	19.4
8	F. B.	"	102.5	3	2.84	21.3	21.2	20.0
				6		22.2	21.0	19.8
9	V. D.	Apr.	61.3	3	2.99	17.6	17.0	15.8
				6		18.2	17.1	16.0
10	J. A.	Mar.	67.3	3	2.73	17.6	17.8	16.8
				6		18.3	18.2	17.2
11	G. M.	Apr.	106.3	3	4.33	24.4	24.0	22.5
				6		25.5	24.4	22.9
12	S. G.	"	56.8	3	2.43†	14.2	15.2	14.3
				6		15.3	15.1	14.2
				8		15.2	15.1	14.1
13	N. K.	"	77.6	3	3.86	19.2	19.6	18.1
				6		20.5	19.7	18.2
				9		21.1	19.6	18.1
				12		21.2	19.5	18.0
14	J. C.	Mar.	58.6	3	2.35	15.6	15.2	14.2
				6		15.6	15.0	14.1
				9		16.6	15.1	14.2
				12		16.5	14.7	13.8
Mean§ standard deviation				3	3.10 ±6.24	18.8 ±3.04	18.8 ±2.83	17.6 ±2.53
Mean§ standard deviation				6		19.6 ±3.22	18.9 ±2.98	17.7 ±2.96

* See "Methods."

† Plasma volume predicted on the basis of surface area from the data of Gibson and Evans.

‡ Recent weight loss.

§ Includes Experiments 7 to 14.

value for the sodium space was 19.6 liters or 25.9 per cent of the body weight. Subject V. D., a tall, slender individual had the highest extracellular volume relative to his body weight, 29.7 per cent of the body weight; while the individual F. B. with an excessive amount of fat had the smallest volume relative to his body weight, 21.7 per cent. These values calculated from the sample of

serum withdrawn 6 hours after administration of the substance are somewhat too low because diffusion of the marked sodium was not complete at this time. 12 hours should be allowed for establishment of complete diffusion equilibrium between serum and tissues.

The average value for the interstitial fluid (extravascular extracellular) 6 hours after the injection of radiosodium was 16.5 liters or 21.8 per cent of the body weight. 84 per cent of the sodium space was represented by the interstitial fluid and 16 per cent by the vascular compartment.

In three normal subjects, N. K., J. C., and J. P., the average value for the sodium space determined from the 12 hour sample was 18.7 liters representing 27.4 per cent of the body weight and 10.5 liters per square meter of surface area.

Simultaneous determinations of the fluid available for the distribution of thiocyanate were made on eight of these subjects. When the thiocyanate space was calculated simply as the amount of thiocyanate retained in the body divided by the concentration of substance in the serum (Formula A, Table II), the average value was 18.8 liters or 24.9 per cent of the body weight at 3 hours and 18.9 liters or 25 per cent of the body weight at 6 hours. The volume of the extracellular fluid calculated according to the formula of Lavietes *et al.* (Formula B, Table II) was somewhat lower than that found for the sodium space. The average value of eight normal individuals for the thiocyanate space was 17.6 liters and 17.7 liters calculated from the 3 and 6 hour sample of serum respectively. If in Formula B we substitute 70 per cent of the total cell volume (its water content) as the amount of fluid available for thiocyanate rather than 100 per cent of the cell volume, then the average values for the thiocyanate space for these eight normal subjects are about the same as those calculated according to Formula A.; *i.e.*, 18.7 liters at 3 hours and 18.8 liters at 6 hours. In view of this we agree with Gregerson and Stewart (4) and Stewart and Rourke (14) that the best way to calculate the thiocyanate space is according to Formula A. These figures for the thiocyanate space are very similar to those reported previously (1, 2).

Repeated Volumes.—Repeated determinations of the extracellular fluid by means of both radiosodium and sodium thiocyanate were made on normal subjects at varying intervals of time and under similar conditions. In many of these subjects, at the time of the second measurement, a small amount of thiocyanate and radioactive sodium was still present in the body from the first injection. In such cases the change in concentration in the serum was used in calculating the volume. The results are given in Table III. The average difference between the duplicates was -0.43 liter with a standard deviation of ± 0.40 liter for the sodium space. Taking into consideration in the calculation the small size of the sample, differences between duplicate determinations of the sodium space should not exceed ± 0.98 liter or ± 4.9 per cent with an average volume of 19.9 liters. This error in the sodium space,

providing the volume of extracellular fluid remained constant during the interval, represents the sum total of errors involved in this method. Of this error, approximately ±0.64 liter was due to errors in pipetting and counting the β-ray activity in the Geiger counter, since the average count in this group of experiments was 45 per minute per cc. Two determination on subject N. K. done at an interval of 1 year gave values of 19.3 and 19.2 liters. During the interval the subject gained 3.8 kg. in weight.

Duplicate determinations of the thiocyanate space on these subjects varied somewhat more than those for the sodium space (Table III). The average difference between duplicate measurements was −0.63 liter with a standard deviation of ±0.848. Therefore, in 95 per cent of cases the difference between

TABLE III

Repeated Measurement of the Sodium Space and Thiocyanate Space in Normal Subjects

Normal subjects	Date	Time after injection	Serum proteins	Cells	Sodium space		Thiocyanate space (A)	
					Liters	Difference	Liters	Difference
		hrs.	*gm. per cent*	*per cent*		*liters*		*liters*
V. D.	Apr. 16, 1940	6	6.3	44.8	18.2		17.1	
	" 21, 1940	6	6.2	43.9	17.0	−1.20	16.7	−0.4
G. M.	" 17, 1940	6	6.4	48.1	25.5		24.4	
	" 20, 1940	6	6.7	46.9	25.2	−0.30	23.8	−0.6
N. K.	" 1939	3	6.0	42.0	19.3		—	
	" 1940	3	6.4	43.9	19.2	−0.10	19.6	—
N. K.	" 16, 1940	12	6.4	44.1	21.2		19.5	
	" 19, 1940	12	5.9	45.1	20.7	−0.50	18.8	−0.7
C. T.	June 5, 1940	12	6.9	47.2	19.0		—	
	" 6, 1940	12	7.0	46.5	18.8	−0.02	—	—
J. T.	" 4, 1940	12	6.8	40.0	17.5		17.5	
	" 7, 1940	12	7.4	43.1	17.2	−0.30	16.8	−0.7

duplicates will not exceed ±1.33 liters or a variation of ±7.6 per cent with an average volume of 17.5 liters.

The results recorded in Table III show one other interesting observation, *i.e.* the second determination in all the subjects was lower than the first. This was observed consistently when the two determinations were made at such short intervals, so that at the time of the second determination there were present residual sodium and thiocyanate in the body from the first measurement. This observation was too consistent to be due to chance. Obviously then it must be due to some systematic error. In two cases when duplicates were done at an interval of almost a year, the values were essentially the same in one individual, and in the other the value for the second determination was 2.7 liters higher than that for the first. This systematic error cannot be adequately explained. However, one may submit at least a theoretical pos-

sibility. If there were certain tissues from which sodium and thiocyanate disappear at a slower rate than from other portions including the serum, then, following the second injection, less radiosodium and thiocyanate will diffuse into these particular tissues than after the first injection, and the concentration in the serum will be higher than it should be. As will be pointed out later, the skeleton and central nervous system may serve as such tissues for sodium.

Effect of Seasonal Variation upon the Volume of the Blood and Extracellular Fluid.—Measurements of the plasma volume and the extracellular fluid were made on five normal subjects in Rochester during the warm season and again during the winter months. In the month of March, 1940, during which time all but one of our observations were made, the mean daily high temperature

TABLE IV

The Effect of Seasonal Variations upon the Volume of the Blood and the Sodium Space

Normal subjects	Date	Time after injection	Serum protein	Cells	Plasma volume		Blood volume		Interstitial fluid		Sodium space	
					Liters	Difference	Liters	Difference	Liters	Difference	Liters	Difference
		hrs.	*gm. per cent*	*per cent*		*liters*		*liters*		*liters*		*liters*
L. Y.	Mar., 1940	3	6.7	45.0	3.31		6.02		16.8		20.1	
	Aug., 1939	3	6.6	43.8	3.51	+0.20	6.25	+0.23	17.6	+0.80	21.1	+1.00
F. B.	Mar., 1940	3	6.4	49.6	2.84		5.64		18.5		21.3	
	Sept., 1939	3	6.2	48.0	2.97	+0.13	5.72	+0.08	18.7	+0.20	21.7	+0.40
J. D.	Mar., 1940	3	6.7	47.5	3.07		5.85		13.6		16.7	
	Sept., 1939	3	6.9	46.8	3.06	−0.01	5.75	−0.10	14.2	+0.60	17.3	+0.60
V. D.	Apr., 1940	3	6.2	44.0	2.99		5.34		14.6		17.6	
	Sept., 1939	3	5.7	42.6	3.22	+0.23	5.61	+0.27	11.7	−2.90	14.9	−2.70
J. A.	Mar., 1940	3	6.3	45.2	2.73		4.98		14.9		17.6	
	Sept., 1939	3	6.3	44.8	2.50	−0.23	4.53	−0.45	13.3	−1.60	15.8	−1.80

was 33°F. and the mean of the lows 22°F. The mean relative humidity at 7:30 a.m. was 84 per cent; at 1:30 p.m., 69 per cent; and at 7:30 p.m., 78 per cent. The percentage of sunshine was 47. Between August 9, and September 9, 1939, the mean daily high temperature was 80°F. and the mean of the lows, 63°F., with an average relative humidity of 86 per cent at 7:30 a.m.; 51 per cent at 1:30 p.m.; and 66 per cent at 7:30 p.m. The percentage of sunshine was 74.

During this study the subjects followed their ordinary activities and were exposed to the outside temperature only several hours a day. The results are given in Table IV. During the warm season, all the subjects weighed less than during the winter months (average difference 1.9 kg.). During the warm weather the plasma volume increased in three subjects, remained the same in one, and decreased in two. The mean difference in the plasma volume was +0.064 liter or +2.2 per cent with a range of from −11 per cent to +8 per

cent. The mean difference was not significantly different from zero. There was no significant change in the blood volume, the mean difference being +6 ml. The changes in the sodium space were no more consistent than those found for the volume of the blood. The volume during the warm season as compared with the cold increased in three subjects and decreased in two. The mean difference was −0.50 liter, a value not significantly different from zero.

These results are not in agreement with the marked changes in the volume of the blood found by Barcroft *et al.* (15) and Bazett and coworkers (16), during seasonal variation. The former investigators using the CO-method for determining the volume of the blood found an increase of about 35 per cent in this volume as they sailed through the tropics and Bazett *et al.* observed equally large increases in the blood volume during the summer heat. There was an increase in both the plasma and the cell volume in the summer, so that the hematocrit readings did not change. More recently Forbes, Dill, and Hall (17) found much less marked changes in the blood volume with changes in climate. A group of laboratory workers on moving to a hot damp climate for the summer showed an increase in the volume of the blood and plasma of only 4.5 and 4.2 per cent, with a range from −6 per cent to + 12 per cent.

We have employed essentially the same methods for the blood volume as Forbes and his group. The results in the two groups are very similar. The discrepancy between the results of Barcroft and Bazett and Forbes and ours cannot be explained at the present time.

DISCUSSION

The results of this investigation indicate that the fluid available for the distribution of radiosodium (sodium space) represents approximately 25 per cent of the body weight. The sodium space will be equal to the volume of extracellular fluid providing that either sodium is exclusively limited to the extracellular phase, or if not, it does not exchange with intracellular sodium, and further, providing that the radioactive isotope is equally distributed throughout the sodium phase. There is good evidence to suggest that neither sodium nor chloride is exclusively limited to the extracellular compartment. Manery and Hastings (13) have shown that the Na:Cl ratio varies in different organs. The ratio for most tissues is approximately the same as that of an ultrafiltrate of serum, but in certain tissues (stomach, tendon, and testes) there is excess chloride while in certain other tissues (spinal cord, cartilage, and bone) there is extra sodium, which suggests the existence of both intracellular sodium and chloride. The results of Harrison, Darrow, and Yannet (18) on the analysis of whole bodies of animals support these findings with respect to sodium. They found an excess of sodium amounting to ap-

proximately 25 per cent of the total sodium in the dog, 85 per cent of which was limited to the skeleton.

Several questions then arise in reference to the use of marked sodium as a means of measuring extracellular fluid. First: is the tagged sodium distributed in the same proportion as total sodium, and second: does the radiosodium diffuse into the skeleton and reach its maximum concentration during the 12 hours allotted for mixing in man? In answer to the first question, Greenburg *et al.* (19) and Manery and Bale (20) observed that radiosodium penetrates all the tissues and is distributed in the same proportion as total sodium. Radiosodium is not concentrated in any tissue. A definite answer to the second query cannot be given. Manery and Bale (20) found that in certain tissues of rabbits and rats the penetration of radiosodium was complete 20 minutes after the injection and remained constant for at least 12 hours, and that in certain other tissues (testes, brain, and bone) the penetration was delayed but gradually proceeded to completion in 3 to 12 hours. Their data on bone were very sparse. Greenburg *et al.* (19) using rats found that the marked sodium diffused rapidly throughout the tissues. Their studies did not include bone. Hahn, Hevesy, and Rebbe (21) observed that 67 hours after the subcutaneous injection of radiosodium in a rabbit the concentrations of marked sodium in tibia epiphyses and tibia diaphyses were 59.1 and 50.9 per cent of that in plasma respectively.

These observations then suggest that in the series of experiments reported above the total sodium of the body was measured, and therefore the figures given for the volume of extracellular fluid are too high, because of excess sodium attributed to the extracellular volume.

In order to determine how rapidly sodium diffuses into bone and what concentration it reaches, the experiments of Harrison *et al.* (18) have been repeated in part on dog and man using radioactive sodium rather than chemical methods.

After securing a sample of serum to serve as control, radiosodium and sodium thiocyanate were injected intravenously. At suitable intervals thereafter samples of blood and bone were obtained, the β-ray activity counted, and the chloride concentration chemically determined. The bone was freed from excess tissue (fat, marrow) and weighed. One portion was used for the determination of chloride, and another was dissolved in concentrated nitric acid on a steam bath. 2 ml. of the resultant solution were placed in a Geiger-Müller counter and its activity measured. The first two dogs were sacrificed 12 hours after the injection of the substances and numerous samples of bone were obtained. In the third dog, after the substances were injected, the animal was kept in an anesthetized state with nembutal during the remainder of the experiment. Every 3 hours for 12 hours after the administration of sodium, samples of blood were obtained and at each period corresponding sections of tibia or radius were removed using a different limb at each period.

The counts of the tissues alone mean very little, but their relative values give information desired, when related to the number of counts in the serum. The ratios of the tissue concentration to the plasma concentration for radioactive sodium and the chemically determined chloride were calculated. When the ratios are corrected for the water content of serum and the Gibbs-Donnan equilibrium, the resulting values give the apparent volume of the extracellular water. In keeping with the symbols used by Manery and Bale (20) the calculated value has been designated $(H_2O)_E$.

The results of these experiments along with data secured from humans are presented in Table V. The values for $(H_2O)_E$ Na^{24} in bone in every instance were much higher than those for $(H_2O)_E$ Cl, indicating that there is extra sodium in bone which cannot be attributed to the extracellular compartment. The average values for $(H_2O)_E Na^{24}$ in bone correspond very closely to the value of 52 calculated from the results of Harrison *et al.* (18). In general the figures for $(H_2O)_E Na^{24}$ were higher in compact than in cancellous bone. Another finding of interest is that the value for $(H_2O)_E Na^{24}$ in bone of dog 3, 3 hours after the injection was just as high as that found at the end of 12 hours. At least in the dog, radiosodium penetrates bone and diffuses into it rather rapidly.

The nature of the "excess sodium" in bone is not known. It is felt that the extra sodium must contribute little, if any, to the osmotic pressure of skeletal water and presumably is present as an insoluble or undissociated compound serving to form the matrix of bone. Harrison *et al.* (18) felt that the excess sodium of bone was associated with the inorganic part. We attempted to find out the percentage of radiosodium associated with the organic and inorganic portions of bone. Counts were made on adjacent portions of bone, after treating one part with nitric acid and the other after extracting the organic part of the bone with ethylene glycol. The results show that the counts associated with the organic part of dense bone 12 hours after injection represented 56.5 per cent of the total count, while those of spongy bone represented on an average 73 per cent of the total activity. However, these data do not throw much light on the nature of the extra sodium in bone because of the relatively short duration of these experiments. The rate of penetration of the radiosodium into the inorganic compound may be governed by the rate at which sodium is renewed.

From the information at hand it is possible to calculate the excess radiosodium that diffused into bone of the dog. Using 24.3 per cent of the body weight as skeleton (18) and 60 for $(H_2O)_E$ Na^{24} in bone in dog 1, it was found that 41.6 per cent of the number of counts present in the body were in bone. This compares favorably with Harrison's value of 46 per cent determined chemically. Assuming that the chloride space of bone (20 per cent of its weight) represents its extracellular volume, then the extra sodium in bone

TABLE V

Ratios of Tissue (Bone) Concentration to Plasma Concentration for Radioactive Sodium and Chemically Determined Chloride

	Compact bone							Cancellous bone		Compact and cancellous bone		Spinal cord	
	Femur		Scapula	Radius tibia		Humerus		Femur epiphysis scapula		Vertebra			
	$(H_2O)_E Na^{24}$	$(H_2O)_E Cl$	$(H_2O)_E Na^{24}$	$(H_2O)_E Na^{24}$	$(H_2O)_E Cl$	$(H_2O)_E Na^{24}$	$(H_2O)_E Cl$	$(H_2O)_E Na^{24}$	$(H_2O)_E Cl$	$(H_2O)_E Na^{24}$	$(H_2O)_E Cl$	$(H_2O)_E Na^{24}$	$(H_2O)_E Cl$
Dog 1	66.4	23.5	72.0					45.2	32.9				
	59.5	22.1	75.3						26.3				
	66.8							53.9					
Dog 2	56.9	20.1						31.4	17.1	39.9		29.3	44.6
	58.4	21.8						36.7	17.7				
	51.2												
	62.2												
Dog 3	72.6	20.0		68*		62.9	18.2	46.3	20.4	51.0	24.4	—	46.0
		21.0		64.2		62.9							
				68.7†									
				64.3									
				71.4‡									
				75.4									
				66.4	20.0								
				64.5									
Patient										Skull			
H. H.										48.9			
										34.6			
M. A.		19.6											
		22.8											
			Rib										
A. S.			51.4										
			41.5										
			44.0										
										Spine			
W. S.										56.5			
										62.5			

* Portion of tibia, diaphysis removed at 3 hours.

† Portion of tibia, diaphysis removed at 6 hours.

‡ Portion of radius, diaphysis removed at 9 hours. All other samples obtained 12 hours after injection of radiosodium.

$$(H_2O)_E Na^{24} = \frac{\text{Tissue counts per min. per kilo}}{\text{Serum counts per min. per liter}} \times \frac{0.93}{0.95} \times 100.$$

$$(H_2O)_E Cl = \frac{\text{Tissue Cl (m.eq. per kilo)}}{\text{Serum Cl (m.eq. per liter)}} \times 0.93 \times 0.95 \times 100.$$

was equal to 32 per cent of the total counts in the body. The average value for three dogs was 28.5 per cent. If Skelton's value (22) of 17.4 per cent of the body weight as skeleton is employed, then 29.8 per cent of the total number of

counts was found in the bone (dog 1) and the excess counts amounted to 20 per cent of the total counts remaining in the body. The average value (three dogs) for excess counts was 18.1 per cent. The extent then to which the excess sodium in bone will correct the sodium space, depends on the value selected to represent the percentage of skeleton to body weight. The value given by Skelton (22) probably represents a closer approximation to the conditions of these experiments than Harrison's values, since the bone used for analysis was free of excess tissue and fat.

Employing Skelton's figure, the sodium space of the dogs was corrected for the excess sodium existing in bone (Table VI). The corrected volumes were similar to those determined by the distribution of thiocyanate. The average corrected sodium space of the three dogs was 4.8 liters or 27.6 per cent of the

TABLE VI

Fluid Available for the Distribution of Radiosodium Corrected for the Amount of Extra Sodium in Bone (Dogs)

Dog No.	Time after injection	Weight	$(H_2O)_E Na^{24}$ (Bone)	Sodium space	Sodium space corrected*		Thiocyanate space	
					Liters	Body weight	A†	B†
	hrs.	*kg.*		*liters*		*per cent*	*liters*	*liters*
1	12	15	60.0	5.26	4.22	28.1	5.28	5.01
2	12	19	47.6	6.14	5.23	27.5	5.81	5.32
3	3	17.9	58.4	6.35	5.11	28.6	5.25	4.80
	6			6.22	5.00	27.9	5.50	5.08
	9			6.38	5.12	28.6	5.59	5.19
	12			6.06	4.86	27.2	5.57	5.17

* Corrected for excess sodium in skeleton.

† See "Methods."

body weight. These values are similar to those derived by more direct methods (18). No correction has been applied for the diffusion of labeled sodium into the erythrocytes (23).

Similar results were found in man. The values for $(H_2O)_E$ Na^{24} were higher than those for $(H_2O)_E$ Cl. In two instances (A. S. and W. S.) in which ample samples of bone were obtained, the values for $(H_2O)_E$ Na^{24} were similar to those found for the dog (Table V). In one case (A. S.) in which the data were complete, it was found that 37.4 per cent of the total number of counts in the body was in bone and that the excess sodium in bone was equivalent to 21 per cent of the total sodium counts.

Corrections for the sodium space were made in ten normal subjects (Table VII). It was assumed that the skeleton represented 16 per cent of the body weight (22) and the values of 50 and 20 were taken for $(H_2O)_E$ Na^{24} and $(H_2O)_E$ Cl respectively. In eight normal subjects in whom both sodium and thiocyanate spaces were determined, the correction applied reduced the volume

determined by sodium an average of 3.7 liters or 18.9 per cent. The average corrected value for the sodium space was 15.9 liters (21.1 per cent of the body weight) compared with 17.7 liters (23.5 per cent of the body weight) for the corrected thiocyanate space. These values then represent the closest approximation to the volume of extracellular fluid that one can make with the available data. Both the sodium and thiocyanate spaces require large corrections, the former for excess sodium in bone and the latter for thiocyanate in erythrocytes.

TABLE VII

Fluid Available for the Distribution of Radiosodium Corrected for the Amount of Extra Sodium in Skeleton (Normal Subjects)

Experiment No.	Subject	Time after injection	Weight	Interstitial fluid (radiosodium)		Extracellular fluid sodium space		Extracellular fluid thiocyanate space	
				Volume	Corrected* volume	Volume	Corrected† volume	A*	B*
		hrs.	*kg.*	*liters*	*liters*	*liters*	*liters*	*liters*	*liters*
5	J. T.	12	51.0	14.8	12.3	17.5	15.0		
6	J. P.	12	69.8	15.8	12.5	18.4	15.0		
7	L. Y.	6	84.5	17.6	13.6	20.9	16.9	20.6	19.4
8	F. B.	6	102.5	19.4	14.5	22.2	17.3	21.0	19.8
9	V. D.	6	61.3	15.2	12.2	18.2	15.2	17.1	16.0
10	J. A.	6	67.3	15.6	12.4	18.3	15.1	18.2	17.2
11	G. M.	6	106.3	21.2	16.1	25.5	20.4	24.4	22.9
12	S. G.	6	56.8	12.9	10.2	15.3	12.6	15.1	14.2
		8		12.8	10.1	15.2	12.5		
13	N. K.	6	77.6	16.6	12.9	20.5	16.8	19.7	18.2
		9		17.2	13.5	21.1	17.4		
		12		17.3	13.5	21.2	17.4		
14	J. C.	6	58.8	13.2	10.4	15.6	12.8	15.0	14.1
		9		14.2	11.4	16.6	13.8		
		12		14.1	11.3	16.5	13.7		
Average		6		16.5	12.8	19.6	15.9	18.9	17.7
Body weight, *per cent*				21.8	17.0	25.9	21.1	25.0	23.5

* See "Methods."

† Corrected for excess sodium in skeleton.

Since the major portion of the extra sodium of the body is contained in skeleton (18, 20), the correction applied to the sodium space will depend on the weight of the skeleton. Due to the variability of the relative weight of the skeleton to the body weight in different individuals and in the same individuals under different conditions, it is impossible to arrive at the absolute weight of the skeleton and in turn to apply to the sodium space proper corrections for extra sodium.

Radioactive sodium may be employed in making comparative measurements of the sodium space on the same individual at two different times, providing the store of extra sodium does not fluctuate with the expansion or the contrac-

tion of the extracellular volume or with changes in the concentration of sodium in the extracellular fluid. Due to the nature of bone, one should expect little change in its sodium content with fluctuations in the volume of extracellular fluid without changes in the sodium concentration. It is conceivable, however, that drastic changes in the concentration of sodium in the extracellular fluid may vary the amount of extra sodium in the body. In the salt deficiency experiments done by McCance it appears that the subject R. A. M. lost approximately 210 m. eq. of sodium which came from some other portion of the body than his extracellular fluid (24).

It appears then that neither radiosodium nor thiocyanate is an absolute measure of the extracellular fluid. Since sodium and chloride are not exclusively limited to the extracellular phase, the volume of fluid available for radiosodium and thiocyanate is larger by an undeterminable amount than the space which functions as the interstitial fluid. In general, in soft tissues, chloride exceeds sodium while in skeleton sodium exceeds chloride (13). Radioactive sodium affords then at least as accurate a method as any previously described. Since our main interest in measuring the extracellular compartment is for comparative purposes in the same individual at two different times and not necessarily the absolute volume, the most useful representation of this volume at the present time is as follows:

$$\text{Sodium space, liters} = \frac{\text{Counts injected} - \text{counts lost in urine}}{\text{Concentration of counts per minute per liter of serum}}$$

In the derivation of this equation correction for the Gibbs-Donnan effect and the water content of serum was considered, but the values for these factors, 0.95 and 1/0.94 respectively, for practical purposes cancel each other. Cancellation of these factors results in a sodium space which is 200 cc. too high, providing we assume that the interstitial fluid of the normal subject is free of proteins. The futility of attempting to correct the concentration of counts of serum more closely than this is obvious when one recalls that the protein content of interstitial fluid of normal man is not known and that in patients with edema and serous effusions the transudates contain variable amounts of proteins not only in different subjects but also in different parts of the extracellular compartment in the same patient.

Finally it is worth while to point out the relative merits of radiosodium and thiocyanate as measures of the volume of extracellular fluid. Thus far there has been no evidence presented to show that radiosodium differs either chemically or physiologically from the sodium which occurs in nature (25). The evidence indicates that thiocyanate and tagged sodium spread from the plasma into their respective phases of individual tissues in the same proportion as chloride and total sodium are distributed. In normal man diffusion equilibrium of thiocyanate is more rapidly attained than that for sodium. From

the simultaneous measurements made on serum and serous effusions, it appears that from 9 to 12 hours is necessary for complete diffusion of sodium and thiocyanate in the bodies of patients suffering from congestive heart failure (7). Radiosodium has the advantage over thiocyanate in that it measures the volume of extracellular fluid of the nervous system, and it is not concentrated in the digestive juices. Radiosodium does not enter the red blood cells to any great extent as does thiocyanate (26). Neither radiosodium nor thiocyanate is rapidly excreted, so sufficient time (12 hours) may be allowed between the administration of the substances and the drawing of blood samples in order to insure complete diffusion through large accumulations of fluid. The amount of sodium chloride that it is necessary to inject in order to make accurate measurements is not large enough to cause changes in the electrolyte balance. One disadvantage is that labeled sodium is not always available and that it requires considerable technical equipment which is expensive.

SUMMARY AND CONCLUSIONS

A method for measuring the volume of fluid available for the distribution of sodium (sodium space) by the use of its radioactive isotope (Na^{24}) has been described and the accuracy of the method has been discussed. Simultaneous determinations of the plasma volume by means of the blue dye T-1824 and the volume of the extracellular fluid by employing radiosodium and sodium thiocyanate have been made in normal subjects. Repeated measurements were made at varying periods of time in the same individuals. In order to establish the rate of diffusion equilibrium for the radioactive isotope of sodium and thiocyanate between serum and serous effusions, simultaneous samples of both were obtained at varying intervals after the intravenous injection of these substances.

Since evidence in the literature indicates that there is an excess of sodium mainly limited to bone, which cannot be attributed to the extracellular phase, experiments on dogs and man were so devised that the ratio of tissue concentration to plasma concentration for radiosodium and chemically determined chloride could be calculated.

The following conclusions may be drawn from the results of this investigation:

1. Radiosodium after intravenous administration spreads rapidly during the first 3 hours from the plasma into a volume of fluid which represents approximately 25 per cent of the body weight of man. Thereafter for 6 hours it diffuses more slowly into certain tissue spaces—the central nervous system and probably the skeleton. The plasma volume and interstitial fluid represent 15 and 85 per cent of the sodium space respectively.

2. Diffusion equilibrium for both radiosodium and thiocyanate is not established between serum and transudates in edematous patients until from 9 to 12 hours after the intravenous injection of these substances.

3. Until more complete information is available, it is concluded that unless the difference between repeated observations on the same individual exceeds ± 1.38 liters there is no significant change in the sodium space providing that the activity of the standard and serum samples are in the range of 40 counts per minute per milliliter with the counting apparatus used. As the activity of the samples increases, the error becomes less because there is no correlation between the magnitude of the error and the magnitude of the activity.

4. Climatic conditions produce no significant changes in the volume of the blood or extracellular fluid.

5. In the dog, following the intravenous injection of radiosodium, the concentration of the isotope in bone reaches its maximum rapidly (3 hours). The extra sodium in the skeleton of dog is equal to about $\frac{1}{4}$ of the total counts in the body, assuming that the chloride space of bone represents its extracellular volume. Similar amounts of excess sodium are found in the skeleton of man 12 hours after the administration of Na^{24}.

6. Correction of the sodium space of man for the excess sodium reduced the average value by 3.7 liters or 18.9 per cent. The average corrected volume for the normal subjects 6 hours after the injection is 15.9 liters or 21.1 per cent of the body weight compared with the thiocyanate space of 17.7 liters, representing 23.5 per cent of the body weight.

7. The most useful method for calculating the sodium space from the data obtained after intravenous administration of radiosodium is as follows:

$$\text{Sodium space, liters} = \frac{\text{Number of counts retained in the body}}{\text{Concentration of counts per minute per liter of serum}}$$

This space exceeds the volume of extracellular fluid by the amount of excess sodium in the body that cannot be attributed to the extracellular phase.

8. While neither the thiocyanate method nor the radiosodium method gives precise estimates of the extracellular fluid, the error is of the same order of magnitude in both. For clinical use, the thiocyanate method is superior because of the ready availability of the substance, and the apparatus required.

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