OBSERVATIONS ON THE FATE OF SODIUM SULFATE INJECTED INTRAVENOUSLY IN MAN[1]

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The present work was carried on in an attempt to investigate the fate of sodium sulfate injected intravenously in man as regards its diffusion into body fluids, its effect on some of the electrolytes of blood, and its excretion. Before these subjects can be discussed, however, certain consideration must be given to the distribution of endogenous sulfate, and some experimental facts and observations should be quoted.

The concentration of inorganic sulfate in normal human serum has been studied by several investigators. Their findings, reviewed recently (17), range from 0.1 to 3.3 mgm. of sulfur per 100 cc. Using Cope's technique (2), we have found, for ten determinations on eight normal subjects, an average of 0.66 m.eq. per liter of total serum, with figures ranging from 0.37 to 0.92 m.eq. (1 m.eq. per liter = 1.6 mgm. of sulfur per 100 cc.). These figures are in exact agreement with those of Loeb and Benedict obtained gravimetrically on 10 cc. of serum (21). In pathological conditions, especially in chronic nephritis, this level may rise very high (5, 6, 8, 21, 29). According to Macy (22), it varies but little in the normal person in the course of the day.

There is still much doubt concerning the inorganic sulfate content of red cells. Reed and Denis (27) claim that blood inorganic sulfate is almost entirely confined to plasma; Cuthbertson and Tompsett (5), that one fifth is in the cells. We have confirmed Øllgaard's observation (23) that analytical methods involving benzidine are not suitable for corpuscles or whole blood. No accurate information is available in the literature. For the present investigation this uncertainty seems immaterial. More important is the question of the permeability of the red cells to added sulfate. It has been shown (30, 31) that red cells are practically impermeable to sulfate added *in vitro*. In experiments conducted in this laboratory it has been observed that human red cells become permeable to a variable degree only when added sulfate is left several days in contact with blood, or when blood is subjected to an extreme saturation with CO_2; in other words, under circumstances that are never realized in physiological or pathological conditions. That, *in vivo*, red cells should be rapidly permeable to inorganic sulfate seems, therefore, improbable.

Since no determinations of the inorganic sulfate content of transudates seem to have been published thus far, some figures obtained by us are given (Table I). Too much stress must not be placed on them, since most were not obtained in the postabsorptive state, and often blood and fluid were drawn a few hours apart. It is evident that they do not show a constancy of distribution comparable to that of chloride. For the present purpose, the figures suffice to show that endogenous sulfate is, roughly speaking, equally distributed between serum and transudates, and presumably also extracellular tissue fluids. This is supported by some figures given by Hayman (15), which show that the concentrations of inorganic sulfate in ultrafiltrates of serum and in native serum are the same. In three determina-

TABLE I

Distribution of chloride and sulfate between serum and transudates

Diagnosis	SO_4		Cl		Proteins	
	Per liter of fluid	Per liter of serum	Per liter of fluid	Per liter of serum	Per 100 cc. of fluid	Per 100 cc. of serum
	m.eq.	*m.eq.*	*m.eq.*	*m.eq.*	*grams*	*grams*
(Pleural fluid)						
1. Rheumatic fever, extreme heart failure...	1.61	2.05	105.0	109.0	1.7	
2. Acute anuria.....	3.04	4.19	109.9	102.8	1.5	5.0
3. Chronic nephritis.....	2.42	2.51	114.0	107.2		
Same, two weeks later..	2.98	2.96				
(Peritoneal fluid)						
4. Liver cirrhosis.....	.84	.71	111.6	102.5	1.1	5.7
5. Liver cirrhosis.....	.74	.67	100.2	93.8	1.4	6.5
6. Liver cirrhosis.....	.60	.64	99.6	91.8	0.9	5.8
7. Polyserositis.....	.80	.63	108.6	101.5	4.2	6.5

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tions we found that sulfate was decidedly lower in spinal fluid than in the corresponding sera.

EXPERIMENTAL

Sulfate was in most cases injected with thiocyanate in order to afford a means of comparison for the extent and velocity of its diffusion. Doses of Na_2SO_4 ranged from 1.3 to 19 grams. Thiocyanate was injected as NaCNS in doses of 1 to 1.5 grams added to the sulfate. The solution was made up to about 4 per cent with freshly distilled water, boiled 10 minutes for sterilization, and injected at the rate of 10 to 20 cc. per minute. In some cases KCNS was taken perorally (1 to 1.5 grams) the evening before. The subjects were in the postabsorptive state; they were the two authors, in good health, convalescent patients, and a few nephritics in a state of advanced renal failure. No meal, except a little coffee or fruit juice in some cases, was taken during the experiment. No toxic effects were noted.

All determinations were made on venous serum, the blood being drawn without stasis and allowed to clot under oil. Inorganic sulfate was determined after Cope (2), the same method proving quite convenient for dilute urine; thiocyanate after Crandall and Anderson (4); CO_2 with the Van Slyke manometric apparatus (25); total base and sodium in serum after Hald (14); urine sodium after Butler and Tuthill (25); serum chloride after Hald's modification of the Volhard-Harvey method (25); O_2 capacity by the Van Slyke and Hiller carbon monoxide method (25); proteins by macro-kjeldahl (25), red cell volume with Daland hematocrit tubes.

Recently Hoffman and Cardon (17) have claimed that all analytical methods involving benzidine previously published, including Cope's, are quite misleading, the results obtained being always far too high, sometimes as much as 300 per cent in nephritic sera. The objections raised by these authors certainly lack convincing evidence. That phosphate does not interfere practically with the procedure has been shown several times (2, 5, 18, 23). The values we have obtained for normal sera are exactly comparable to those found by Loeb and Benedict (21) (from 0.4 to 1.0 m.eq., average 0.7) in thirteen determinations made on 10 cc. of serum with the $BaCl_2$ gravimetric method. This is also the range of figures obtained by Øllgaard (23) (from 0.7 to 1.5 mgm. of sulfur per 100 cc. in twenty determinations), who used a procedure involving benzidine sulfate titration with $BaCl_2$, $MgCl_2$ being added in order to eliminate possible interference of phosphate. The high figures we have often found in nephritic cases are quite in agreement with those of Loeb and Benedict. We can not certify that the precipitate that forms in Cope's method is strictly pure benzidine sulfate; but the fact that we have been able to recover small amounts of Na_2SO_4 added to normal and nephritic sera with an error no greater than 4 per cent should be sufficient proof that the method is quite suitable for the experiments here recorded.

The original method of Cope has been slightly simplified by the substitution for the Rehberg burette of a 0.15 ml. microburette of the ordinary type, while the titration tube is heated directly with a microburner instead of a steam-jacket.

When checking the method on theoretical solutions of Na_2SO_4, containing from 0.15 to 2.25 m.eq. per liter, it was observed that a certain constant loss occurred, which was independent of the amount determined. The minimum for this loss was equivalent to 0.002 or 0.003 ml. of 0.02 N NaOH, but it would sometimes rise to 0.010 ml., the striking feature being that the loss was always exactly the same for all the tubes of one batch of determinations. The conditions of the whole procedure were always kept rigorously alike, and it has not been possible thus far to determine the exact source of error. The acetone and trichloroacetic acid used were sulfate free. When distilled water was subjected to the whole procedure the titration did not differ from the titration on distilled water alone; i.e., the same negative correction of a few c. mm. only of NaOH solution was necessary for the end-point of phenolphthalein. Varying the length of time the tubes were left standing before centrifugation, from 0 to 1.5 hours, did not seem to make any difference. It is suggested that a variable amount of benzidine sulfate, comprising that originally contained as impurity in the benzidine, plus a small amount of the benzidine sulfate formed in the reaction, remains in solution and can not be recovered. For the determinations given in this paper, a correction of + 0.006 ml. of NaOH was habitually used to offset this loss. Whatever may be the cause of the error, it is too small to affect in any appreciable fashion the results presented here.

Sulfate excretion

For the purposes of these experiments it is important to know that exogenous sulfate acts as a foreign substance and can be recovered *in toto* from urine. This has been shown to be true for thiocyanate (26). The fact that sulfate is apparently a natural waste product makes it unlikely that it should undergo chemical transformation; any considerable increase of phenol conjugation is unlikely under the conditions of these experiments (16). Consideration must, of course, be given to the excretion of endogenous sulfate

which, in the fasting subject, according to Macy (22) amounts to about 1 m.eq. per hour. This may rise to as much as 3 m.eq. per hour in the course of the day in persons receiving meals, paralleling rather closely the urinary nitrogen, since urinary sulfate is largely derived from protein (25). It may also be increased by profuse diuresis. In these experiments, however, feedings containing protein were avoided and diuresis was never evident. It has, therefore, been assumed that endogenous sulfate excretion regularly amounted to 1 m.eq. per hour. On this basis, in Experiment 3b, Table II, at the end of 6.5 hours, $51.7 - 6.5 = 45.2$ m.eq. of exogenous sulfate had been recovered in the urine after the injection of 47.3 m.eq., a reasonable agreement. Serum sulfate, meanwhile, had returned to its original level.

A glance at Table IV shows that sodium and sulfate were excreted in approximately equivalent amounts without any considerable excess of water or chloride. This may be taken as contributory evidence that the salt is treated as a single foreign substance and subjected to no metabolic transformations.

Sulfate concentration in serum

Serum sulfate levels after injection, recorded in the first column of Table II, decrease rapidly in normal, slowly in nephritic subjects. This is illustrated by Figure 1, in which the concentration of exogenous sulfate in serum (concentration observed minus fasting concentration) in all experiments of Table II have been plotted logarithmically against time. It has been observed in dogs (15) that the rate of excretion of injected sulfate is proportional to the concentration of sulfate in serum. Dominguez and Pomerene (11) have shown that the decreasing serum level and excretion rate of exogenous creatinine both yield satisfactory exponential curves. That in man sulfate is excreted in the same fashion seems likely from Experiments 2, 3b, 5, 6, and 8a plotted logarithmically in the figure, since all yield three points approximately on a straight line. Experiments 1, 3a, and 6 show that in the first ten or twenty minutes after the injection there is a sharp drop. (In Experiments 1 and 3a, blood was drawn one minute after the end of the injection, and from the other arm; in Case 6, from the same arm,

TABLE II

The distribution of intravenous SO_4

Experiment number	Time from beginning of injection	Serum SO_4	Volume of distribution of		Urinary SO_4
			SO_4	CNS	
	minutes	*m.eq. per liter*	*liters*	*liters*	*m.eq.*
1. Normal male. Body weight 52 kgm. 19 m. eq. injected * in 2 minutes	0	0.3			
	3	3.0	8.2	10.5	
	4	2.6	9.6	10.4	
	5	2.6	9.6	11.9	
	15	2.1	12.2	13.1	
	180	0.8			
2. Normal female. Body weight 88 kgm. 64.9 m.eq. injected * in 5 minutes	0	0.5			
	20	4.4	16.6	16.8	
	40	4.0		18.1	
	125	2.4		19.7	
3a. Normal male. Body weight 85 kgm. 39.7 m.eq. injected * in 4 minutes	0	0.7			
	6	5.3	8.8	12.1	
	9	3.9	12.4	14.6	
	20	3.4	14.7	15.7	
b. Body weight 85 kgm. 47.3 m.eq. injected in 6 minutes	0	0.9			
	35	2.9	16.7		14.6
	135	1.9	18.0		17.0
	255	1.2			10.4
	390				9.7
c. Body weight 85 kgm. 41.8 m.eq. injected * in 8 minutes	0	0.8			
	15	3.7	12.2	13.9	6.7
	45	2.5	15.3	16.9	9.9
4. Normal female. Body weight 54 kgm. 50.7 m.eq. injected in 8 minutes	0	0.9			
	25	5.2	9.3		11.0
	120	2.2			
	180				30.0
5. Normal male. Body weight 65 kgm. 29.9 m.eq. injected ‡ in 5 minutes	0	0.9		17.0	
	15	2.8	11.7		7.3
	45	2.1	14.3		6.3
	105	1.5			5.2
6. Female, chronic nephritis. Body weight 44 kgm. 19 m.eq. injected * in 5 minutes	0	2.2			
	6	4.4	8.6	11.3	
	20	3.6	13.6	14.5	
	65	3.6	13.6	14.8	
	360	3.4	15.8	15.8	
7. Female, chronic nephritis, edema. Body weight 49 kgm. 30.5 m.eq. injected *	0	2.4			
	20	4.6	13.9	17.0	
	185	4.0	19.1	22.0	
8a. Male, chronic nephritis. Body weight 54.4 kgm. 28.1 m.eq. injected ‡	0	2.5		16.8	
	25	4.6	13.1		
	50	4.4	14.8		
	100	4.1			
b. Body weight 56.8 kgm. 40.8 m.eq. injected ‡	0	1.8		17.9	
	50	4.2	17.0		
	100	3.9			

* NaCNS injected at the same time.

‡ KCNS given perorally 12 hours earlier.

after about 30 seconds.) This is evidence that the substance takes an appreciable time to reach its final distribution between blood and tissues.

The three curves obtained from Subject 3, after injection of comparable amounts of sulfate, follow diffuses approximately evenly into all the fluids except the water in tissue cells; this volume of fluid represents a definite, and, in the normal individual, little changing entity (19). It may be calculated by dividing the amount of thiocyanate

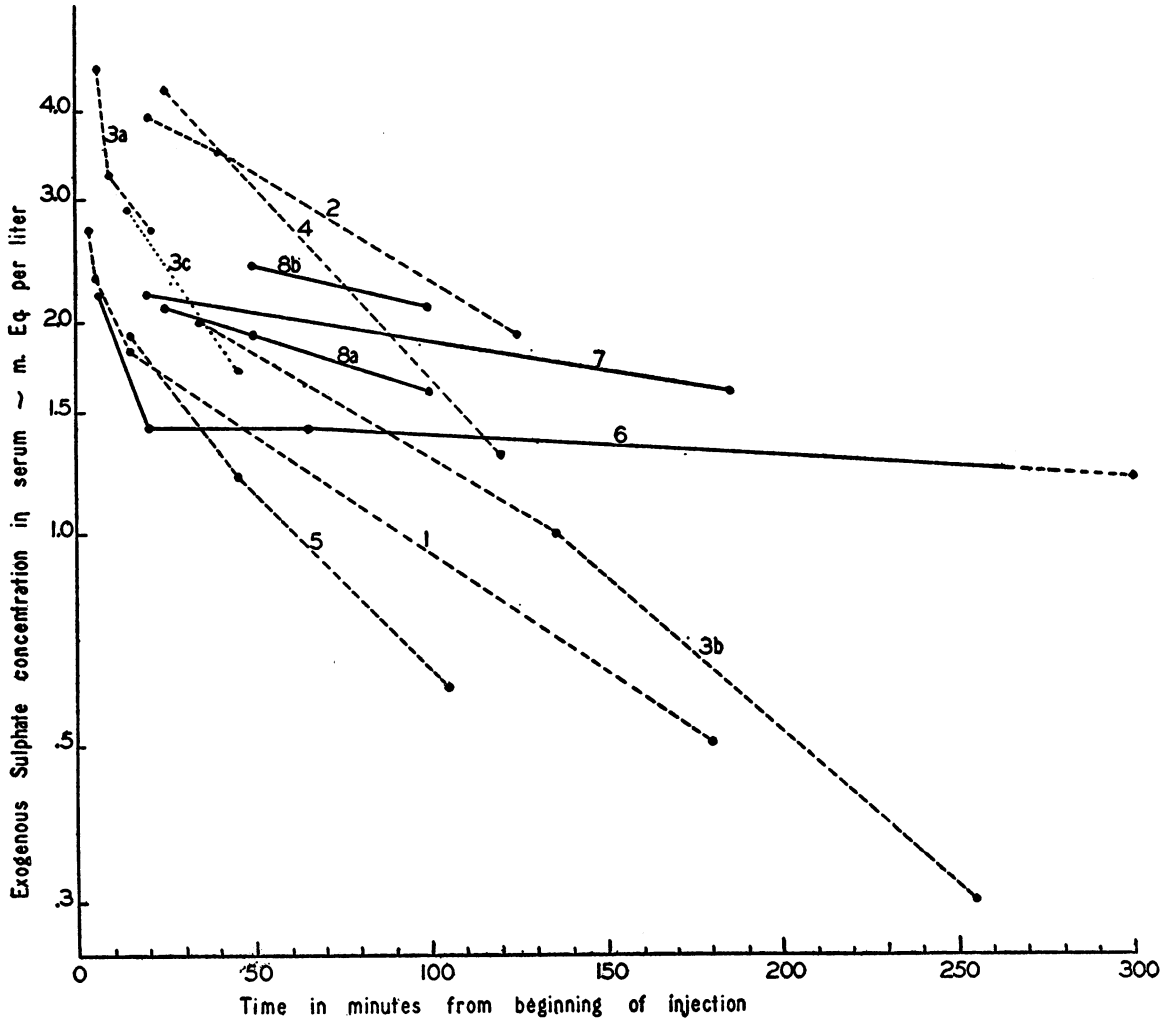

FIG. 1. THE RATE OF DISAPPEARANCE OF INJECTED SULFATE FROM SERUM IN NORMAL SUBJECTS (·-------·) AND IN PATIENTS WITH CHRONIC NEPHRITIS (·————·).

each other closely, showing that the reaction of the same individual to the repeated procedure is quite constant. Curves 6, 7, 8a and 8b, obtained from nephritics, are much less steep than the normal ones.

Sulfate distribution

There are strong reasons for believing that when thiocyanate is introduced into the body, it injected, minus the amount excreted, by the concentration found in serum.

To apply the same calculation to injected sulfate is a little more complicated, since it is necessary to deduct, from the serum level observed, the fasting level, and, from the sulfate excreted, a certain amount for endogenous excretion. In the experiments recorded here, this amount was assumed for reasons already given, to be 1 m.eq.

per hour. In some cases urinary excretion was not determined.

In Table II, it appears that distribution figures for sulfate are in rather close agreement with the corresponding thiocyanate figures, but always somewhat lower. It should be noticed that only in the experiments in which sulfate excretion was determined and taken into account in the calculation are the sulfate distribution figures exact. In the other experiments, they are too large, the error becoming greater as time elapses. Nevertheless, even as they are given here, they do not reach the thiocyanate figures as late as 15 or 20 minutes after the injection in normal cases, and much later in nephritic subjects, in which renal excretion must have been considerably retarded (especially in Experiment 6). Consequently, the extent of sulfate distribution in all cases, although it follows that of thiocyanate in its progressive increase, always falls short of it by a certain proportion. The water of red cells, into which thiocyanate only penetrates, would account for part of the difference. This conclusion holds only, of course, if the distribution ratios between serum and extravascular fluids are always the same for SO_4 and CNS, which is only approximately true. Roughly speaking, sulfate and thiocyanate must diffuse into approximately the same volume of fluid. That changes in this volume are reflected by changes in the distribution of both salts is illustrated by Experiments 8a and 8b, made 6 days apart; an increase of 2.4 kgm. in body weight, induced in the meantime by forcing NaCl and water, is accompanied by an increase in the distribution figures of both thiocyanate and sulfate.

Effects of large injections of sulfate

It has been shown (4) that thiocyanate, when introduced into the body, diffuses into a fraction of fluid which represents on the average 24 per cent of the body weight, in man. This is the fraction of the body fluid which has been called above the volume of distribution. There is reason to believe that it consists chiefly of the extracellular or interstitial portion of the water of the body (19). That injected sulfate diffuses through approximately the same portion as thiocyanate is evident from the experiments just discussed. If this is extracellular fluid it should contain also the major portion of the sodium and chloride of the body (5a, 14a). As far as thiocyanate, sulfate and chloride are concerned this deduction finds support in certain data collected from the literature which are presented in Table III. In the first column the ratio, tissue Cl: blood Cl, has been calculated from tissue analyses of Cameron and Walton (1) with the assumption that blood Cl is 300 mgm. per 100 cc. Column 2 gives similar ratios taken from Corper (3) for the distribution of CNS after intravenous injection. The figures in Column 3 were calculated from the data of the first experiment of Denis and Leche (9). In all cases the animals used for analysis were killed without exsanguination. The three sequences, considering their varied sources, are sufficiently similar to warrant the assumption that Cl, CNS and SO_4 are distributed through approximately the same volume of fluid. Experiments of Greenwald (13) indicate that intravenously injected inorganic phosphate distributes itself like sulfate. A recent experiment in this department indicates that magnesium, injected as magnesium sulfate, behaves in the same manner.

TABLE III

Distribution of chloride, thiocyanate and sulfate in dogs

	$\frac{\text{Tissue Cl}}{\text{Blood Cl}}$	$\frac{\text{Tissue CNS}}{\text{Blood CNS}}$	$\frac{\text{Tissue } SO_4}{\text{Serum } SO_4}$
Lung	.77	.81	.80
Kidney	.84	.53	.86
Heart	.40	.61	.35
Liver	.45	.42	.47
Muscle	.22	.13	.08

The experiments in Table IV were devised to test in another manner the distribution of sulfate and its accessibility to the cells. For this purpose rather large amounts of hypertonic sodium sulfate were injected intravenously. Blood was withdrawn before the injections and shortly after the injections which took from 25 to 45 minutes. Time was not, therefore, allowed for complete distribution of the sulfate. Urine was voided before the injection and collected at the time of the second venous puncture in order that correction could be made for injected salt lost by excretion. If the hypotheses which have been expressed are correct, injection of such a hypertonic

solution should cause water to be transferred from the cells to the interstitial fluids in which the injected sodium sulfate should remain, while endogenous sodium and chloride should be diluted by the water injected and that derived from the cells. Thiocyanate, which was administered the preceding night to provide a further criterion of volume changes, should be diluted to an equivalent extent.

Attention may be turned first to the changes in the blood, with Experiment 14 as an example. If it is assumed that the total circulating hemoglobin did not change, the blood volume, from the dilution of hemoglobin (oxygen capacity), expanded $100\left(\frac{18.1-16.7}{16.7}\right)=8$ per cent. Since the ratio, oxygen capacity: cell volume, rose from $18.1/39.4=0.459$ to $16.7/34.7=0.481$, the original cells must have shrunk $100\left(\frac{0.481-0.459}{0.481}\right)=4.6$ per cent. As cells contain only 70 per cent of water by volume, this means, in actual point of fact, that the cells yielded 6.6 per cent of their water to the serum. The serum, meanwhile, esti-

TABLE IV

The effect of large intravenous injections of sodium sulfate

Time from beginning of injection	Serum					Blood		Volume of distribution of		Urine			
	SO_4	Cl	Total CO_2	Total base	Proteins	O_2 capacity	Cells	SO_4	CNS	Approximate volume	SO_4	Cl	Na
minutes	*m.eq. per liter*	*m.eq. per liter*	*m.eq. per liter*	*m.eq. per liter*	*per cent*	*volumes per cent*	*volumes per cent*	*liters*	*liters*	*cc.*	*m.eq.*	*m.eq.*	*m.eq.*
Experiment 9, Normal female, body weight 58 kgm., 85.2 m.eq. injected in 12 minutes													
0	0.9	97.6	26.7		7.3								
17	9.1	90.4	25.7		7.1			9.1					
Experiment 10, Normal male, body weight 86 kgm., 93 m.eq. injected in 10 minutes													
0	1.0	97.5	28.5										
45	5.5	92.4	27.4					13.5		140	33.0	10.7	30.9
105										80	19.4	5.3	16.5
Experiment 11, Female, mild diabetic, body weight 70 kgm., 270 m.eq. injected in 45 minutes													
0	0.6	96.0	28.0		6.9								
45	16.2	88.8	28.0		6.0			13.4		200	62.2	6.0	63.8
105	9.4	93.6	27.6		7.1			15.3		200	74.5	0.7	67.8
Experiment 12, Male, mild diabetic, body weight 71 kgm., 270 m.eq. injected in 25 minutes													
0	(0.5)*	96.0	29.0	135.6‡	6.7				20.1				
30	14.5	96.2	27.4	144.5‡	5.5			16.5	20.5		40.3	18.0	55.0
130									19.9		95.5	8.0	90.0
Experiment 13, Normal male, body weight 59 kgm., 258 m.eq. injected in 30 minutes													
0	(0.5)*	98.5	26.0	148.8	6.4	20.5	47.5		16.2				
40	15.3	93.1	25.2	154.5	5.6	18.6	41.8	12.9	17.1	245	67.4	13.0	63.0
Experiment 14, Normal female, body weight 55 kgm., 266 m.eq. injected in 45 minutes													
0	(0.5)*	104.9	26.2	148.6	7.3	18.1	39.4		13.1				
50	20.3	99.2	22.5	155.9	6.5	16.7	34.7	9.6	14.1	260	76.9	9.0	80.0

* Assumed figure.
‡ Sodium only.

mated from the change of serum proteins, expanded $\frac{7.3-6.5}{6.5}=12$ per cent. From cell volume and oxygen capacity measurements its expansion can be calculated as follows: 100 cc. of whole blood, which contained 39.4 per cent of cells, became, after the injection, 100 cc. $\times$ 18.1/16.7 = 108 cc., containing 34.7 per cent of cells, or 108 cc. $\times$ 34.7/100 = 37.5 cc.; therefore the change in serum volume was from 100 cc. — 39.4 cc. = 60.6 cc. to 108 cc. — 37.5 cc. = 70.5 cc., which is an increase of 16 per cent. The agreement by the two methods is highly satisfactory considering the double assumption that neither the cells nor the protein in the circulation changed during the experiment. The concentration of base in the serum, if this may be taken as a measure of osmotic pressure, rose from 148.6 to 155.9, or about 5 per cent. The results, as far as the blood itself is concerned, then, are quite consonant with theory. The hypertonic solution withdrew, perhaps only temporarily, some interstitial fluid into the blood stream and at the same time stole enough from the cells to reestablish osmotic equilibrium without necessitating any transfer of base. The results in Experiment 13 are quite comparable. The blood volume increased 10 per cent; the cells contracted 3 per cent, cell water 4 per cent; serum volume expanded 14 per cent (from proteins) or 22 per cent (from cell volume and oxygen capacity); the concentration of base in serum rose 4 per cent.

Calculations of the exchanges of fluids and solutes in the other body fluids are more complicated. In Experiment 14, from the change of the concentration of chloride in serum the interstitial fluids would seem to have expanded $100\left(\frac{104.9-99.2}{99.2}\right)=5.7$ per cent (if the loss of 9 m.eq. of Cl in the urine is neglected). If sodium were diluted to the same extent, the concentration of endogenous sodium in the serum at the end of the experiment should have been 148.6/105.7 = 140.6 m.eq. The difference between this and the 155.9 m.eq. of sodium actually found amounts to 15.3 m.eq., which agrees fairly well with the increment of sulfate, 20.3 — 0.5 = 19.8 m.eq. The changes can be evaluated in a slightly different manner. Since the amounts of SO_4 and of Na excreted are so nearly identical it may be assumed that exogenous increments of Na and SO_4 are the same. Because endogenous SO_4 is negligibly small, the magnitude of these increments can be estimated from SO_4 as 19.8 m.eq. In this case the final endogenous Na must have been 155.9 — 19.8 = 136.1 m.eq. and dilution, estimated from Na, $100\left(\frac{148.6-136.1}{136.1}\right)=9.2$ per cent. From the serum SO_4 and the quantity of SO_4 given, corrected for excreted SO_4 and endogenous SO_4, the estimated volume of the interstitial fluids at the end of the injection is $\frac{266-(76.9-0.8)}{20.3-0.5}=9.6$ kgm., which would mean that before the injection it was, by the calculations above, from Cl 9.6/105.7 = 9.1, from SO_4 and Na 9.6/109.1 = 8.9 kgm. This would mean a gain of 0.5 to 0.8 kgm. of water. The fluid injected minus the urine excreted amounted to 500 — 260 = 240 cc. If extrarenal losses of water are neglected, then, by this method of calculation at least (0.5 to 0.8) — 0.2 = 0.3 to 0.6 kgm. of water must have been derived from the cellular fluids. The interstitial fluid volume estimated from thiocyanate is, in this instance, far larger than that calculated from the distribution of sulfate and of a more plausible magnitude. The explanation for the difference probably lies in the fact that time was not given for the complete diffusion of sulfate, while thiocyanate, which had been administered the night before, was evenly distributed through the body fluids. Thiocyanate, therefore, should be a more suitable criterion of both the original volume and the change of volume of the interstitial fluids. From thiocyanate the interstitial fluids should have gained 1.0 — 0.2 = 0.8 kgm. of water from the cells, becoming diluted to the extent of about 8 per cent.

If it be assumed that the body of the subject, who weighed 55 kgm., contained altogether 70 per cent by weight of water, the total body water was 38.5 kgm., and the cellular water (from KCNS) 38.5 — 13.1 = 25.4 kgm. Of this, $\frac{100\times 0.8}{25.4}$ or about 3 per cent was transferred to the extracellular fluids.

If osmotic equilibrium between cells and interstitial fluid was maintained in spite of the salt

which was injected and if it may be assumed that with the exception of the inorganic salts and proteins there are no osmotically important solutes to which the cellular membranes are not permeable, it follows: (1) that the effective osmotic pressure in intracellular and extracellular fluids is related to the concentration of salt in these fluids and (2) that a change in the concentration of salt in one medium must be adjusted by an exchange of water or salt such that at equilibrium the concentrations of salt in the two media will again be equal. if it was 0.24 kgm., $160.4W + 182 = 166.8\ (W + 0.24)$ and $W = 22.2$ kgm. Both these figures are far below the theoretical estimate, $0.7 \times$ body weight $= 38.5$ kgm. This is to be expected, however, if the distribution of SO_4 has not been completed.

The calculations for Experiment 14 have been given in full to illustrate the procedures employed. The results of similar calculations for Experiments 13 and 12 are presented in Table V. The results of Experiment 13 are in all respects com-

TABLE V

Approximate calculations of exchange of water in the body after intravenous injection of sodium sulfate

	Experiment		
	12	13	14
Expansion of interstitial fluid from [Cl], *per cent*	0	5.8	5.7
Expansion of interstitial fluid from [CNS], *per cent*	2.0	5.5	7.6
Expansion of interstitial fluid from endogenous [Na], *per cent*	3.9	6.5	9.2
Final endogenous [Na] from [Cl], *m.eq.*	135.9	140.7	140.6
Final endogenous [Na] from [CNS], *m.eq.*	133.0	141.0	138.2
Final endogenous [Na] from [SO_4], *m.eq.*	130.5	139.7	136.1
Total volume of body fluids, *kgm.*:			
as (0.7 × body weight)	49.7	41.3	38.5
as $W = \frac{B_e - [B]_s''E}{[B]_s - [B]_s'}$ if E = 0	28.3‡		28.4
as $W = \frac{B_e - [B]_s''E}{[B]_s - [B]_s'}$ if E = 0.24		32.9†	22.2
Increment of interstitial fluid:			
from cells, *kgm.*	<0.4‡	0.7	0.8
as per cent of cell water *	<1.4‡	2.8	3.1

* Cell water = (0.7 body weight) − (interstitial fluid from CNS).

† In this experiment E is derived from the change of body weight (0.22 kgm.) which was measured. K excretion was 3.9 m.eq.

‡ Urine volume was not measured.

If total base of serum may serve as a rough approximation of the osmolar concentration of salt,

$$[B]_s'W + B_e = [B]_s''(W + E)$$

in which $[B]_s'$, $[B]_s''$ and B_e represent respectively the concentrations of base in serum before and after the injection and the increment of exogenous base, W the initial body water and E the increment of exogenous water (20). In Experiment 14, E was not measured, but its limits can be approximately defined. It can hardly have exceeded 0.24 kgm., the difference between the fluid injected and the urine excreted. If it was 0, $160.4W$ [2] $+ 182$ [2] $= 166.8W$, and $W = 28.4$ kgm.; parable to those of Experiment 14. Except that chloride remained unaltered in Experiment 12, the changes in the serum are similar to those in the other experiments. The calculated changes in the volumes of the various body fluids are, however, far smaller. This may be due in part to the larger size of the subject, but can probably be attributed chiefly to the fact that equilibrium has been less perfectly established. In this experiment the fluid was injected more rapidly than in the other two and the second blood sample was withdrawn earlier, only 30 minutes after the beginning of the injection. This view finds support in the fact that serum proteins were more diluted (22 per cent) in Experiment 12 than in the other two although the volume of the blood, judging from the size of the subject, must have been

[2] $[B]_s'$ and $[B]_s''$ are here expressed in m.eq. per liter of water, estimated by the equation, serum water = serum volume (100 − protein concentration). $B_e = Na_e + K_e$, the potassium excreted amounting to 3.7 m.eq.

larger. It is unfortunate that neither oxygen capacity nor cell volume was measured.

The data can be tested in a somewhat different manner. If the volume of interstitial fluid, F, from CNS is correct, the total amount of Cl in the body before the injection should be (assuming Cl distribution to be approximately equal in serum and extravascular spaces) $[Cl]_s' F'$, and at the end $[Cl]_s'' F''$, and

$$[Cl]_s' F' = [Cl]_s'' F'' + \Delta Cl,$$

Δ Cl being the balance of Cl during the experiment. This permits the calculation of $[Cl]_s''$. The same method may be applied to the analysis of the figures for base and for SO_4. The results of such calculations give in Experiment 14: $[Cl]_s''$ calculated 96.8, found 99.2; $[B]_s''$ calculated 151.3, found 155.9; $[SO_4]_s''$ calculated 13.9, found 20.3; for Experiment 13: $[Cl]_s''$ calculated 92.6, found 93.1; $[B]_s''$ calculated 152.4, found 154.5; $[SO_4]_s''$ calculated 11.7, found 15.3; for Experiment 12: $[Cl]_s''$ calculated, 93.3, found 96.2; $[B]_s''$ calculated, 143.5, found 144.5; $[SO_4]_s''$ calculated 11.8, found 14.5. In all experiments SO_4 gives the worst agreement, which might be expected if equilibrium had not been attained, since $[Cl]_s$ is affected only by dilution, $[SO_4]_s$ only by diffusion, and $[Na]_s$ by both.

Experiments 9, 10 and 11 are incomplete in so many respects that they can not be treated in the same detail. As far as they go, they agree with the more complete experiments.

When the interchanges in the components of the blood are compared with the shifts of water between tissues and interstitial fluids, the former are found to be far more satisfactory. In Experiment 14 the blood cells gave up 7 per cent of their water when the concentration of base in the serum rose 5 per cent; in Experiment 13 the agreement is still more exact, 4 per cent of water issuing from the cells in response to a 4 per cent increment of base. For the same increase of base the estimated loss of water from the tissue cells are only 4 per cent in Experiment 14, 3 per cent in Experiment 13. Again, however, it must be emphasized that equilibrium with the tissue cells was probably not established. Equilibrium within the blood must have been attained, because adjustments which were incomplete when the blood was withdrawn, were free to proceed to equilibrium *in vitro*.

On the whole the data are compatible with the hypothesis that Cl, Na, SO_4 and CNS are effectively excluded from the cells of the body, and that when the concentration of sodium in the body is increased, uniformity of osmotic pressure between cells and interstitial fluids is restored by transfer of water from the former to the latter.

COMMENT

Denis (7) claimed that the kidney selectively retained the SO_4 ion when Na_2SO_4 was injected. This opinion was based on analyses of serum only. It is clear from the experiments reported above that Na and SO_4 are excreted at the same speed, a thing which Greenwald's (13) experiments on dogs had already shown. Serum determinations alone will induce to error if one forgets that a dilution of serum may mask completely an increase of serum Na, and leave an increase of SO_4 practically unaffected. For example, if, to 1000 cc. of serum, containing initially 130 m.eq. of Na and 1 m.eq. of SO_4, is added 13 m.eq. of Na_2SO_4 in 100 cc. of water, the Na concentration remains $\frac{130 + 13 \text{ m.eq.}}{1000 + 100 \text{ cc.}} = 130$ m.eq. per liter, whereas the SO_4 concentration becomes $\frac{1 + 13 \text{ m.eq.}}{1000 + 100 \text{ cc.}} = 12.7$ m.eq. per liter.

Neglect of this consideration in Experiments 13 and 14, in which the apparent increment of SO_4 was almost three times that of total base, would lead to a completely erroneous interpretation. When a substance in hypertonic solution is introduced into the parenteral spaces, the osmotic disturbance it creates tends to be compensated immediately: if the substance can diffuse evenly into the total body fluids, including cell water, a new equilibrium will be reached in this manner; if, as for Na_2SO_4 its diffusion is limited to the extracellular fluids, a new equilibrium will be reached only through an outpour of water from the cells. When Denis and von Meysenbug (10) thought that NaCl and Na_2SO_4 injections in dogs had an acidifying effect because they observed a sharp drop of CO_2 and a slight drop of pH, the solutions they used were so large and concentrated that the apparent acidosis was probably mostly

an effect of dilution. Similar, but lesser reductions of CO_2 are seen in the experiments of Table IV. As Peters (24) has pointed out, if it *seems* that when sodium is injected it diffuses into the total body water, it is because the organism tends to distribute the increase in osmolar concentration equally in all body fluids by increasing the water content of the extracellular fluid which contains the sodium, and decreasing correspondingly the water content of the cells.

A word should be added about the position of the SO_4 and the CNS ions in the lyotropic series (12, 28). It is known that SO_4 permeates membranes most slowly, CNS most rapidly, so that one could expect to observe a considerable difference in the speed of diffusion of these ions in the body. Actually, the difference, though demonstrable, is not considerable.

On the whole, the impression gained is that sodium sulfate parenterally introduced behaves in a fashion which is probably common to most electrolytes, diffusing into the interstitial fluid only and creating thus osmotic disturbances which have to be readjusted through transfers of water. At the same time it is excreted by the kidney at a rate proportional to its concentration in the body.

SUMMARY

The effect of sodium sulfate intravenously injected in hypertonic solutions was tested in man. Subjects were healthy men and women and patients with chronic nephritis. Doses injected ranged from 1.3 to 20 grams, and were in most cases accompanied by sodium thiocyanate. In some cases, potassium thiocyanate was given perorally twelve hours before.

A few preliminary data are given on the distribution of endogenous sulfate between serum and transudates.

It was found that the sodium ion and the sulfate ion were excreted at the same rate, and that the amount of salt injected could probably be recovered totally in urine. The rate of sulfate excretion appeared to be simply proportional to the concentration of sulfate in serum.

The figures obtained for the diffusion of sulfate suggest that, like thiocyanate, it is distributed only in the interstitial fluid. The larger doses of sulfate were found to cause usually a dilution of interstitial fluid and a shrinkage of red corpuscles, these phenomena being interpreted as a readjustment of the osmotic equilibrium, necessitated by the fact that neither sulfate nor sodium can penetrate cells.

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