ELECTROLYTE COMPOSITION OF BONE AND THE PENETRATION OF RADIOSODIUM AND DEUTERIUM OXIDE INTO DOG AND HUMAN BONE [1, 2]

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INTRODUCTION

Although bone is the primary rigid supporting tissue of the body, the fact that its individual inorganic components exchange constantly with like substances in the serum has been established beyond cavil (1–5). Since previous estimates indicate that approximately 30 per cent of the body sodium and 8 per cent of the body water is contained in bone (4, 6, 7), the role of this tissue with regard to these substances in metabolic emergencies, may prove to be of considerable importance.

This communication will present data on the chemical composition of bone and the exchangeability of bone sodium and water. While adequate information on the calcium content of human and dog bone is available, similar data on sodium, chloride, and water are scarce. For this reason the first section is devoted to a presentation of analyses for calcium, sodium, chloride, and water in a variety of dog and human bones.

The second section presents observations on the penetration of radiosodium (Na^{24} and Na^{22}) and D_2O into bone. The implications of these experiments are discussed in the terms of: *a*) Bone structure; *b*) the place of bone in the fluid and electrolyte anatomy of the body; and *c*) the physiologic role of bone sodium and water.

I. CHEMICAL COMPOSITION OF BONE

A. Materials and Methods

Grossly normal bones from five adult humans, without clinical evidence of bone disease, were obtained at postmortem, within 3 to 12 hours after death.[6] These samples together with their muscle attachments were stored in sealed vessels at −40° C. At the time of analysis, the bones were stripped of all adherent tissue, including the periosteum. They were then either immediately wrapped in several layers of aluminum foil, crushed with a heavy mallet, and the bone fragments transferred to tared, dry weighing bottles, or intact samples were placed directly into tared, dry weighing bottles. Bone samples were obtained from healthy, mongrel adult dogs under intravenous nembutal anesthesia, or within one hour of sacrifice. These specimens were stripped of their periosteal coverings, immediately wrapped in multiple layers of aluminum foil and stored in sealed, dry vessels at −40° C.

At the time of analysis the samples were either crushed with a mallet and the fragments transferred to sealed, dry, tared weighing bottles, or the intact specimen was transferred directly to such vessels. The particular methods employed in storage and handling of specimens were intended to minimize undetected losses of water. Water contents were estimated by heating to constant weight in an oven at 105° C. This was achieved in 48 hours. Neutral bone fat was extracted by refluxing of the dried samples with a 50 per cent by volume mixture of alcohol-ether in a Soxhlet's apparatus for 24 hours at 37° C. Approximately 20 ml. of solvent per gram of bone was used. The samples were then oven dried at 105° C. for 24 hours. The difference in weight of the dried samples before and after the extraction procedure was taken to be the neutral fat content of the samples.

Solution of the fat-free dried bones was achieved by HNO_3 digestion on a steam bath for 6 to 24 hours in platinum crucibles. Approximately 10 mEq. of acid per gram of dry bone was used and the strength of the acid was varied so that no more than 40 ml. of solvent was required for any one sample. The digest was filtered, the

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residue washed several times with hot distilled water and the combined filtrate and washings made up to 50 ml. with distilled water. Separate aliquots of the nitric acid extract were then taken for sodium, calcium, and chloride analyses. The sodium content was determined with a lithium internal standard Barclay flame photometer (8).

Since calcium and sodium ions have emission spectral lines in close proximity, the raw sodium values are in error in proportion to the amount of calcium present in the extract. To correct for this error, a series of 12 standard solutions containing calcium and sodium in amounts comparable to those present in the bone extracts were made up and analyzed photometrically. The data so obtained are summarized in Table I. In practice, the sodium figures were corrected by multiplying the number of mEq. of calcium present per liter of extract by 0.009 and subtracting this from the photometer analysis for sodium, expressed in mEq. per liter. The reproducibility of this method is within 4 per cent and the estimated accuracy is ± 6 per cent.

Calcium contents were determined by a modification of the Kramer-Tisdall method (9, 10). From known standard solutions this method is accurate to within 5 per cent.

Chloride contents were determined by the Volhard method as modified by Wilson and Ball (11). The fat-free bone samples were digested in nitric acid for at least 24 hours and the titrations were carried out in an ice bath in order to achieve a sharper end point.

TABLE I

The effect of calcium on the analysis of sodium by the flame photometer

Calcium concentration	Actual sodium concentration	Observed sodium concentration	Correction factor*
mEq./L.	*mEq./L.*	*mEq./L.*	$\times 10^{-3}$
88	2.9	3.9	1.14
202	0.0	1.9	0.94
202	11.0	12.7	0.84
220	7.5	9.3	0.82
293	14.3	17.1	0.96
294	0.0	2.4	0.82
294	11.0	13.5	0.85
352	14.3	16.8	0.71
366	0.0	3.6	0.98
366	11.0	14.2	0.87
440	0.0	4.3	0.98
440	11.0	14.7	0.84
		Mean:	0.90

* The correction factor is obtained by dividing the difference between the actual and observed sodium concentrations by the known calcium concentration.

B. *Results*

The chemical data are summarized in Table II. Each value listed is a mean figure of the number analyzed. For any one type of bone, the variance from the mean is ± 10 per cent, except for the water analyses where it is ± 25 per cent. Sixteen human and twelve dog samples were analyzed. Since the fat contents were quite variable (0.0 to 19.5 per cent by weight) all data have been expressed on a fat-free basis. The wide variations in water content among the various types of bone makes it reasonable to reduce the data to a fat-free dry weight base as others have done (12–15). It is apparent that there is a close similarity in chemical composition between dog and human bones when expressed in these terms.

1. Bone water: The cortical specimens (femur, skull and humerus) are relatively dry and consist of 13 to 22 per cent water. On the other hand, cancellous bone (rib, ilium and vertebra) has higher water contents, varying from 32 to 52 per cent. In general, there is a rough parallelism between water and chloride content. This supports the view that most of the bone water is associated with the cement substance (16) and free extracellular water. These data agree fairly well with previous estimates (7, 17) of bone water content. Only a very approximate estimate of the relative amount of body water in bone is possible because of the variability in water content among different bones. Accepting a figure of 60 per cent of the body weight being water (18) and taking whole bone as comprising about 16 per cent of the body weight (19), one arrives at a value of 4 to 8 per cent of body water being in bone. It should be emphasized, however, that the water content of bone decreases markedly with increasing age falling as low as 10 per cent in senile, cortical bone (20).

2. Bone sodium: When expressed as mEq. per kilogram of dry, fat-free bone, the sodium content of all samples (both in humans and dogs) is fairly uniform (Table II). The maximum difference found was a value of 191 mEq. per kilogram in a human rib as compared to 282 mEq. per kilogram in a sample of dog skull (cortical plate). The mean of all human samples is 234 mEq. of sodium per kilogram of fat-free bone solids, with a range of 215 to 273. The mean in dog bones is 229 mEq. per kilogram, with a range of 216 to 239.

These data agree approximately with those given by Forbes and Perley (21) for human bone, and our calcium/sodium ratios agree well with the

TABLE II

Bone composition *

No. analyzed	Sample	Water content Gm./Kg.	Calcium content mEq./Kg. Wet wt.	Calcium content mEq./Kg. Dry wt.	Sodium content mEq./Kg. Wet wt.	Sodium content mEq./Kg. Dry wt.	Chloride content mEq./Kg. Wet wt.	Chloride content mEq./Kg. Dry wt.	Excess† sodium mEq./Kg. Wet wt.	Excess† sodium mEq./Kg. Dry wt.	Sodium‡ per chloride / Total sodium content Ratio	Calcium / Excess Na Molar ratio
					A. Humans							
4	Ilium (Cortex and medulla)	393	7,330	12,080	135	222	28	46	99	164	0.27	37
5	Femur (Cortex)	134	11,300	13,020	214	246	15	17	195	224	0.09	29
4	Rib (Cortex and medulla)	366	8,250	12,940	136	215	22	35	108	171	0.21	38
2	Skull (Cortex)	174	11,220	13,600	227	273	19	23	202	244	0.11	28
1	Vertebra (Cortex and medulla)	522	5,160	10,800	103	215	10	23	89	186	0.14	29
	Mean:			12,490		234		29		198	0.16	32.2
					B. Dogs							
2	Humerus (Cortex)	184	7,940	9,690	195	239	15	18	175	215	0.10	23
2	Femur (Cortex)	218	9,610	12,290	170	216	18	23	148	187	0.14	33
4	Skull (Cortex)	205	10,600	13,130	182	233	14	17	165	211	0.10	31
4	Rib (Cortex and medulla)	319	7,830	11,500	155	229	11	16	136	202	0.09	29
	Mean:			11,650		229		19		204	0.11	29.0

* All values are based on fat-free bone.
† Sodium content in excess of that associated with chloride, assuming chloride to be extracellular.
‡ The ratio of extracellular sodium (based on chloride correction and a Donnan Factor of 0.96) to the total sodium content per Kg. of dry fat-free bone.

molar ratio of 32.5 for dog bone given by Harrison (22). On the other hand, Shohl's (6) figure for human bone as containing 79 mEq. of sodium per kilogram of whole fat-free bone is about 50 per cent lower than ours, while Harrison, Darrow and Yannet's (23) figure for dog bone of 142 mEq. of sodium (corrected for extracellular sodium by chloride content) per kilogram of fat-free bone solids is about 25 per cent lower than our corresponding figures for excess sodium [7] in dog bone (Table II—200 mEq. of excess sodium per kilogram of fat-free dry bone). These differences may be resolved, according to Bergstrom (24) on the basis that these earlier analyses involved a considerable coprecipitation of sodium with calcium and hence the lower sodium values. Other investigators (25) assayed dog femur for sodium content by flame photometry after precipitation of calcium with ammonium oxalate. They did not detect significant coprecipitation of sodium with calcium and their figure of 246 mEq. of sodium per kilogram of wet weight is about 45 per cent higher than our corresponding figure of 170 mEq. per kilogram of wet weight of femur (Table II). On the other hand, Davies, Kornberg, and Wilson (26) using Na^{24} as a tracer did detect 20 per cent coprecipitation of sodium with calcium and they reported the sodium content of human rib at 239 mEq. per kilogram and of human femur at 300 mEq. per kilogram of marrow free, whole bone (containing fat).

In terms of total body composition for the human, and on the basis that 16 per cent of the body weight is bone (19), that bone is approximately 30 per cent water and 10 per cent fat, and with a mean sodium content of 230 mEq. per kilogram of dry, fat-free solids, the estimated bone sodium would be about 23 mEq. per kilogram of body weight. In other words, in a 70 Kg. human, the

[7] "Excess sodium" is the sodium content in excess of that amount associated with chloride. Bone chloride is assumed to be free in the extracellular water.

total bone sodium content comes to 1,500 to 2,000 mEq. Similarly in the dog, with the skeletal weight comprising about 25 to 30 per cent of body weight (23) and a mean sodium content of approximately 160 mEq. per kilogram of whole bone, the estimated sodium in bone would be 40 to 50 mEq. per kilogram of body weight.

Present evidence indicates that the excess bone sodium lies in the cement substance among the hydroxyapatite crystals and adsorbed on to the surface of these crystals (16, 27).

3. Bone calcium: There is a surprising constancy of calcium content in most bones regardless of type or species origin. A figure of 12,000 mEq. per kilogram of fat-free bone solids approximates all of the values given in Table II. These data agree closely with the reported calcium analyses for human rib (12–14) and for bones from cats, rabbits and dogs (22, 28). Cattle femur cortex is more heavily calcified, having about 16,000 mEq. of calcium per kilogram of dry fat-free tissue (15). It seems fair to say that the calcium value of 5,250 mEq. per kilogram of wet bone for human bone listed by Shohl (6) is in error by a factor of two. Robinson (16) summarizes the evidence as to the disposition of calcium in bone as follows: *a*) It forms a basic unit of the hydroxyapatite crystals, the formula for which is given as $Ca_{10}\ P_6\ O_{26}\ H_2$; and *b*) it lies along with other cations (sodium and magnesium) in the amorphous cement substance.

II. EXCHANGE STUDIES WITH RADIOSODIUM AND DEUTERIUM OXIDE

A. Materials and Methods

Seven adult mongrel dogs were studied. One hundred microcuries of Na^{24} was injected intravenously and samples of bone and blood obtained under nembutal anesthesia over a period of 2 to 50 hours after injection. The time of sampling and the type of bone removed is given in Table III. In one human subject 100 microcuries of Na^{24} was administered intravenously. A segment of rib and a simultaneous blood sample were secured twenty six hours after the injection. Only 5 per cent glucose was used to rinse operative fields.

About 2 to 3 Gm. of periosteum-free whole bone was placed in 20 ml. of 0.75 N nitric acid in a platinum crucible and heated until complete solution obtained; the acid was evaporated off leaving a charred ash. The bone ash was extracted with 20 ml. of warm distilled water. The extract was filtered through pre-washed glass wool and the volume of the filtrate reduced to about 2 ml. by gentle heat. Separate aliquots of the filtrate were taken for radioactive assay and sodium analysis.

In order to carry out a more extended study of bone sodium exchange, three mongrel, adult dogs were injected with 150 microcuries of Na^{22} ($t\frac{1}{2} = 3.0$ years) and serial bone and blood biopsies obtained under aseptic conditions over a one-month period as listed in Table IV. To keep the serum specific activity constant during this period, the dogs were fed on a diet of sodium-poor, reconstituted milk (Lonolac), boiled rice and sugar. The sodium intake on this diet was estimated as approximately 2 mEq. per day. The bone samples obtained from dogs No. 28 and No. 78 were treated as outlined above for the Na^{24} experiments. The samples obtained from Dog No. 15 were handled so as to permit analyses for calcium, sodium, and chloride contents. These samples were dried and defatted as described in Section I. After redrying, the bone solids were digested in nitric acid, the solution filtered and made up to 25 ml. of distilled water. Calcium, sodium, and chloride analyses were carried out on a 15 ml. aliquot of the bone digests. To the remaining aliquot excess solid ammonium oxalate was added (*i.e.*, 0.4 to 1.2 Gm.) and the calcium precipitated. The solution was neutralized with ammonia water, centrifuged and the supernatant evaporated to dryness. This was then extracted with 2 to 3 ml. of warm distilled water. Aliquots of this solution were used for radioactive assay and sodium analyses.

All of the blood samples were centrifuged after clotting had taken place and recentrifuged until clear cell-free serum was obtained. Aliquots were then taken for radioactive assay and sodium analyses.

Half ml. aliquots of serum and bone extracts were plated in duplicate and dried under infra-red lamps. The radio-

TABLE III

Uptake of radiosodium (Na^{24}) by bone in dogs and a human

Subject	Equilibration time	Tissue	Bone S.A.* / Serum S.A.
	hours		*ratio*
Dog No. 54	2	Skull (Cortex)	0.26
Dog No. 47	3½	Rib (Cortex + medulla)	0.42
Dog No. 36	3½	Rib (Cortex + medulla)	0.37
Dog No. 36	8	Rib (Cortex + medulla)	0.52
Dog No. 37	24	Rib (Cortex + medulla)	0.53
Dog No. 49	24	Skull (Cortex)	0.36
Dog No. 49	24	Femur (Cortex)	0.52
Dog No. 59	49	Rib (Cortex + medulla)	0.58
Dog No. 67	50	Rib (Cortex + medulla)	0.56
L. S.	26	Rib (Cortex + medulla)	0.35

* The ratio of bone sodium specific activity to serum sodium specific activity.

TABLE IV

*Uptake of radiosodium (Na^{22}) by bone in dogs**

Equilibration time	Body weight	Serum sodium	Tissue	Bone S.A. / Serum S.A.	Corrected† Bone S.A. / Serum S.A.
days	*Kg.*	*mEq./L.*		*ratio*	*ratio*
			Dog No. 15		
1	13.2	148	Rib	0.45	0.41
7	12.8	149	Rib	0.49	0.46
14	12.7	149	Rib	0.55	0.52
21	12.8	145	Rib	0.58	0.47
28		145	Rib	0.56	0.51
28		145	Skull (cortex)	0.49	0.48
28		145	Femur (cortex)	0.53	0.47
			Dog No. 28		
1	15.0	147	Rib	0.27	
3	15.5	146	Rib	0.49	
8	15.0	147	Rib	0.45	
15	15.0	147	Rib	0.49	
29	14.5	142	Rib	0.49	
29	14.5	142	Skull (cortex)	0.43	
			Dog No. 78		
1	20.8	148	Rib	0.46	
8	20.0	146	Rib	0.44	
17	18.6	144	Rib	0.48	
24	18.6	149	Rib	0.37	
24	18.6	149	Skull (cortex)	0.35	
30	17.8	145	Rib	0.43	

* These animals were maintained on a low salt (rice, salt poor milk and sugar) diet throughout the period of study beginning on the day preceding injection.

† Corrected for free extracellular sodium, assumed to be completely exchangeable, and based on the chloride content of these samples, the serum chloride and a Donnan Factor of 0.96.

activity was measured with a thin, mica end-window G-M tube (Tracerlab No. TGCl) and an autoscaler, with a fixed geometry for all samples. Counting efficiency was about 25 per cent. A minimum of 5,000 counts were collected on each planchet. All counts were corrected for coincidence loss (0.4 per cent per 1,000 counts per min.), background and radioactive decay.[8] A correction for self-absorption was applied to the serum samples where the dried solids caused measurable diminution in the counting rates. For the Na^{22} samples it was about 20 per cent. The bone extracts other than those of Dog No. 15 contained a very small amount of solid, and self-absorption was neglected. For the self-absorption by the bone solids in the digests from Dog No. 15 a standard curve, constructed by measuring the absorption caused by known weights of bone ash on standard Na^{22} plates, was applied. Sodium analyses in serum and bone extracts were made with an internal-standard flame photometer (Barclay) (8). The bone extracts contained small, but variable amounts of calcium and except for the first few experiments, this was removed by precipitation with ammonium oxalate.

Four mongrel adult dogs were injected with 99.8 per cent deuterium oxide for the purpose of estimating bone water exchange. Samples of blood and bone were drawn at the times indicated in Table V. The periosteum was stripped from the bone prior to resection in a dry field. The bone biopsies were immediately wrapped in several layers of aluminum foil to avoid atmospheric loss of heavy water and both serum and bone samples were stored at −25° C. until analyzed. Bone water was collected by vacuum distillation for 8 to 12 hours after crushing the aluminum covered samples. The details of the methods for D_2O analyses with the mass spectrometer (bone water), and with the falling drop apparatus (serum) have been published previously (31, 32).

B. Calculation of the Exchangeable Fraction

The ratio of the specific activity in a given tissue to that in serum will give the fraction of the element exchanged provided that the specific activity in the serum is constant or nearly so during the period of equilibration. This can be stated as follows:

a) $$\text{F.E.} = \frac{a}{A},$$

where F.E. = fraction exchanged,

a = amount of the original substance replaced by isotope from serum, and

A = Total amount of the parent substance in the tissues.

Since the tracer concentration in the serum is constant the number of tracer particles N* in the tissue is given by:

b) $$N^* = (\text{S.A.})_s \times a$$

Where $(\text{S.A.})_s$ is the serum specific activity. The tracer concentration in the tissue is:

c) $$(\text{S.A.})_t = \frac{(\text{S.A.})_s \times a}{A}$$

Therefore:

d) $$\text{F.E.} = \frac{a}{A} = \frac{(\text{S.A.})_t}{(\text{S.A.})_s}$$

While equating the ratios of the specific activities in tissue and serum to the fraction exchanged is not rigorously precise, it is sufficiently accurate for the purposes of this study.

C. Results

Equilibrium of distribution for radiosodium requires about 12 to 24 hours (21, 33, 34). Tissues other than bone (*i.e.*, skin, skeletal muscle, brain, kidney, liver, heart, stomach, and erythrocytes) in dog and man show a ratio of tissue to serum specific

[8] Na^{24} is a $\beta-$, γ emitter with a half-life of 15.04 hours (29) and Na^{22} is a $\beta+$, γ emitter with a half-life of 3.0 years (30). The purity of two of the Na^{24} shipments and the three Na^{22} shipments was tested by prolonged observation of their separate rates of decay. In all instances there were no significant differences from the expected values of 15.04 hours and three years, respectively.

TABLE V

Bone composition—dog No. 15 *
(Maintained on a low sodium [rice] diet)

Sample	Equilibration time	Sodium content	Calcium content	Chloride content	Excess sodium†
	days	*mEq./Kg.*	*mEq./Kg.*	*mEq./Kg.*	*mEq./Kg.*
Rib	1	198	11,200	10.2	185
Rib	7	196	11,500	8.2	185
Rib	14	207	11,520	12.5‡	191
Rib	21	204	11,890	32.4	163
Rib	28	191	11,700	13.5	174
Skull	28	202	11,936	2.3	199
Femur	28	188	11,940	18.6	164

* All values are expressed per Kg. of dry fat-free bone.
† Corrected for free extracellular sodium in association with chloride and a Donnan Factor of 0.96.
‡ An assumed value.

activity of 1.00 in 24 hours (33). The data on the results of Na^{24} uptake in bone are listed in Table III. The bone to serum specific activity ratios for rib, skull, and femur indicate that from 35 to 58 per cent of bone sodium has exchanged in 3 to 24 hours. In general, these data agree reasonably well with similar studies in dogs and humans by other investigators (21, 25). It should be noted, however, that Davies, Kornberg, and Wilson (34) reported only 25 per cent exchange of sodium in marrow-free human ribs.

One of two interpretations might be offered to explain the findings of incomplete exchange. Exchange of bone sodium may continue at a slow rate and require a few days or even weeks to reach completion or else there may be a phase of bone sodium which is excluded from equilibration with serum sodium and no further exchange would occur. In order to discriminate between these two possibilities the more extended study using Na^{22} and summarized in Table IV was undertaken. To satisfy the condition of a constant serum specific activity during the one-month period of study, these animals were fed on a low sodium ($\simeq$ 2 mEq. per day) diet. The specific activity of the serum fell 13 per cent, 13 per cent, and 3 per cent in the three animals, respectively, at the end of the one-month period of study. The results indicate that approximately 45 per cent of bone sodium is readily exchangeable and that the remainder shows no tendency to exchange over a period of four weeks —this, in the face of a low sodium diet of the kind which probably produces extracellular sodium depletion of a significant degree (35–37). In Dog No. 15 an estimate of chloride-free (excess) bone sodium exchange was made (Table IV). The corrected exchangeable sodium is only reduced by some 10 per cent on the assumption that all of

TABLE VI

The uptake of D_2O by dog bone *

Rib (cortex + marrow)			Radius and femur (cortex)		
Dog *number*	Equilibration time *hours*	t/s† *ratio*	Dog *number*	Equilibration time *hours*	t/s† *ratio*
105	0.6	0.94	105	0.8	0.84 (Radius)
452	1.2	0.87	105	1.3	0.88 (Femur)
15	1.2	0.92	452	2.0	0.95 (Radius)
452	1.4	0.95	452	3.9	0.96 (Femur)
105	2.1	0.89	15	8 days	1.03 (Femur)
148	3.7	0.84			
15	21.0	1.06			
15	7 days	1.00			
15	8 days	1.00			
15	14 days	1.00			

* Rib samples were analyzed as cortex plus marrow. Radius and femur were solely cortical samples.
† t/s is the ratio of the concentration of D_2O in bone water to the concentration of D_2O in a simultaneously drawn serum water and both expressed as volume per cent D_2O.

the bone chloride is freely circulating in extracellular fluid and that its associated sodium is freely exchangeable. This suggests that most of the exchangeable sodium may be associated with the solid phase of bone. Table V lists the chemical composition of rib, skull, and femur in Dog No. 15 during its maintainance on the low sodium regimen. These data do not show clear-cut evidence of bone dissolution or of loss of bone sodium to the extracellular "pool," since sodium content, calcium content, and bone sodium in excess of chloride did not show variations outside of the limits of combined analytic and sampling errors during the period of study.

In contrast to bone sodium, the data on bone water exchange, detailed in Table VI, indicate complete exchangeability after 2 to 4 hours in rib, radius, and femur. For tissues other than bone, water exchange is complete in from minutes to two hours (38). The technique of collecting bone water by vacuum distillation surely does not sample the water bound to bone crystals since very high temperatures are required to release this water (39). However, water of crystallization constitutes a negligible fraction of total bone water (7).

DISCUSSION

A. Bone composition

Harrison, Darrow, and Yannet (23) recognized the unique character of bone sodium pointing out that most of it exists in excess of chloride (*i.e.*, excess sodium) and that its large magnitude precludes it from osmotic equilibrium with freely circulating extracellular fluid. Hendricks and Hill (27) reasoning on the basis of the differential solubility of bone sodium, concluded that sodium is located on the crystal lattice surface. Our data showing a fixed exchange of about 35 to 45 per cent for excess bone sodium indicate that somewhat more than 50 per cent of the sodium associated with the solid phase of bone is excluded from direct contact with serum sodium. By contrast all or almost all bone water is completely exchangeable supporting the view that excess bone sodium exists in a separate phase, *i.e.*, in association with the inorganic crystal structure.

B. Metabolism of bone sodium and water

In view of the relatively large amounts of sodium and water in bone, the question of the metabolism of these substances in disease states may be of some importance. The estimate that there is about 1600 mEq. of bone sodium (approximately 1400 mEq. of excess sodium) and 2 to 4 liters of bone water in a 70 Kg. adult is admittedly a crude one but it suffices to indicate the magnitude of these stores.

Until recently, direct evidence of displacement of sodium from bone to circulating extracellular fluid has been lacking. Bergstrom (40) observed that in rats acute sodium and potassium depletion resulted in a mobilization of both of these cations from bone. Other investigators (25) could not demonstrate any significant changes in bone sodium content in adrenalectomized dogs although the rate of bone sodium exchange using Na^{24} as a tracer appeared to be depressed in the adrenal insufficient animals as compared to the controls. At the present time, additional evidence that bone acts as a reservoir for sodium and water in acute disturbances in animals other than rats is needed. In chronic disease states this question may become complicated by the effect of bone dissolution and the obligatory release of sodium and water secondary to such a process. In the interpretation of exchange data in bone, isoionic or isomolar exchange is meaningful only over relatively short periods, *i.e.*, of the order of weeks. Since bone is constantly being formed and destroyed, all substances must eventually reach "equilibrium" with serum.

C. Distribution of sodium and water in the body

By the use of tracers, total body constituents or their exchangeable fractions may be measured for water, sodium, potassium and chloride (18, 21, 41–47). It is appealing to combine these dilution techniques with some measure of the total extracellular phase so that a valid estimate of the mean intracellular constituents may be obtained. Were a dilution method for the extracellular phase to be available, it might be possible to express average figures for intracellular concentration. Such calculations have been carried out (48) but they are at best only approximations because there is no method currently available which will measure ac-

curately the extracellular phase under all conditions, both normal and pathological. Recent experience in these laboratories suggests that while the thiosulfate space (49) is a reproducible measurement in the normal individual, it is not well adapted to pathological situations. For these reasons, then, it is not possible to determine how much of the total exchangeable sodium is in the extracellular phase and how much is elsewhere, either in cells or bone, in the living patient. The data presented in this paper will permit estimate of an order of magnitude for the normal individual.

Estimates of mean intracellular water in man based on the difference between antipyrine dilution and bromide or inulin dilution have been reported (50). Deane (51) carried out a similar study in normal men using antipyrine, and sucrose as the measure of the extracellular water. Since there does not appear to be any data on the penetration of inulin or sucrose into bone water, these estimates may involve errors of from 2 to 4 liters of water as intracellular water, an uncertainty which remains to be resolved.

Similar studies on estimates of mean intracellular cation concentrations (sodium and potassium) based on total body water, inulin and sucrose dilution and Na^{24} and K^{42} exchangeable mass in dog (52) and man (53) have been reported. It hardly seems likely that the chloride-free bone sodium (approximately 200 mEq. per kilogram of fat-free solids) would be included as extracellular ions based on either inulin or sucrose dilution. Harrison, Darrow, and Yannet (23) pointed out that the excess bone sodium is osmotically inactive and relatively insoluble.

Similarly, it has been emphasized that true mean intracellular cation concentrations could not be expected from a combination of tracer methods because of the heterogeneity of the total extracellular space both in terms of its fluid and its solid constituents (48). Deane and Smith (53) report the mean intracellular sodium concentration to be 37.0 mEq. per liter of cell water in human subjects. If one corrects their data just for exchangeable excess sodium in bone alone and assuming this quantity to be mistakenly included as intracellular, one arrives at a mean intracellular sodium concentration of approximately 15 mEq. per liter, a figure which is more consistent with histochemical analyses (54).

In spite of the failure of multiple dilution methods in attempting to arrive at an absolute measure of intracellular substances, these methods should prove useful in the study of relative changes of body composition in the human subject (48).

SUMMARY

1. Sixteen human bone samples and twelve dog bone samples were analyzed for water, calcium, sodium and chloride contents. Human bone was found to contain 12,490 mEq. of calcium, 234 mEq. of sodium, 198 mEq. of excess sodium and 29 mEq. of chloride per kilogram of fat-free bone solids as a mean value. The water content of these samples varied from 134 Gm. to 522 Gm. per kilogram of wet, fat-free bone. In the dog mean values of 11,650 mEq. of calcium, 229 mEq. of sodium, 204 mEq. of excess sodium and 19 mEq. of chloride per kilogram of fat-free bone solids were obtained. The water content of these samples varied from 184 Gm. to 319 Gm. per kilogram of wet, fat-free tissue.

2. In seven dogs and one human subject it was found that approximately 45 per cent of bone sodium is exchangeable in about four hours and that no further exchange was evident over the next 20 hours. In three dogs on a low sodium diet bone sodium exchange did not increase from the initial level of about 45 per cent over a one-month period.

3. In four dogs bone water exchange was studied from 0.6 hour to 14 days after injection of D_2O. It was found that about four hours after injection bone water had exchanged completely with serum water. One hundred per cent exchange was noted for the next 14 days.

4. The implications of these data on the calculation of intracellular constituents from multiple dilution studies are discussed.

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