

The Mineral Content of Normal Human Bone

James W. Agna, ... , Harvey C. Knowles Jr., Gabriele Alverson

J Clin Invest. 1958;[37\(10\)](#):1357-1361. <https://doi.org/10.1172/JCI103725>.

Research Article

Find the latest version:

<https://jci.me/103725/pdf>



THE MINERAL CONTENT OF NORMAL HUMAN BONE¹

By JAMES W. AGNA,² HARVEY C. KNOWLES, JR., AND GABRIELE ALVERSON

(From the Metabolism Laboratory, Department of Medicine, University of Cincinnati College of Medicine, and the Veterans Administration Hospital, Cincinnati, Ohio)

(Submitted for publication February 14, 1958; accepted June 12, 1958)

Evidence has been forthcoming from animal experimentation that the skeleton may gain or lose electrolytes under conditions of abnormal electrolyte metabolism (1). A complete evaluation of the significance of these findings in man depends upon knowledge of the composition of normal human bone. However, information regarding human bone is sparse. In 1894, Gabriel, in a very detailed study of animal bone, included two representative samples from an unstated number of specimens of humerus of man (2). Klement, in 1936, also conducted a comprehensive investigation of mineral content, but his material was limited to three samples of skull, and two each of pelvis and femur (3). In recent years, further reports of human bone composition have appeared (4-12). However, these latter observations have not been wholly satisfactory, in that the number of samples analyzed were few and the determinations were limited to two or three minerals. Furthermore, a majority of the subjects in these reports from whom bone specimens were obtained either died with disease, or specific statements concerning the condition of the subjects prior to death were not given. The largest study currently reported is that given in abstract form by Pellegrino and Farber (13). These workers analyzed tibial bone of 15 normal subjects for Ca, P, Na and K.

The present study is concerned with the composition of bone obtained at autopsy from 16 normal adults who died suddenly. A comparative analysis of skull, rib and ilium, for water, calcium, phosphorus, carbonate, nitrogen, chloride, potassium and sodium is given.

METHODS

Thirty-seven bone specimens (skull, rib, ilium) were obtained from 16 human subjects who came to autopsy 2 to 12 hours post mortem. The subjects died suddenly and were believed to be in normal health prior to the fatal episode. The causes of death included electrocution, subarachnoid hemorrhage, stab and gunshot wounds, strangling, and head injury. No abnormal conditions other than those causing death were disclosed at necropsy. There was no evidence of bone disorder. The mean age of the subjects was 35 years (range, 23 to 62).

Samples of bone were obtained with a saw from the occipital area of the skull, the iliac crest, and the anterior third of the ribs lateral to the sternal cartilage. The bone was stripped of muscle attachments and periosteum and broken into fragments with a mallet. Marrow was removed as completely as possible by scraping.

The specimens were dried at 105° C. for 96 hours to determine the water content. It is possible that further water loss would have occurred with heating to higher temperatures (14). The dried material was then reduced to a powder by passing it through a Wiley mill, using a 20 mesh. Fat was extracted from the powdered sample in a Soxhlet apparatus with reflux for six hours each with ethyl ether and petroleum ether. Two hundred fifty to 300 mg. aliquots of the fat-free material were then dry-ashed in platinum crucibles overnight in a muffle furnace at 525° C. The ash was dissolved in 1.5 ml. of 3 N HCl. This solution was then subjected to column separation as recommended by Forbes and D'Ambruso (15).

For preparation of the columns, dried sulfonated polystyrene resin (Dowex 50 × 12, 50 to 100 mesh) was mixed with a small amount of water for convenience of handling and introduced to a height of 45 cm. in burettes 1.2 cm. in diameter and 60 cm. in height. The columns were washed with 300 ml. of 0.7 N HCl prior to infusion of the sample. The bone ash was transferred to the resin bed in a volume of approximately 10 ml. and elution with 0.7 N HCl at a flow rate of 2.0 ± 0.5 ml. per minute was carried out. The first 100 ml. of the eluate was used for P analysis according to Fiske and Subbarow (16). The 100 to 300 ml. fraction was utilized for Na analysis. The elutriant was then changed to 5 N HCl, and the 300 to 500 ml. fraction collected for determination of Ca and K. The latter two elution fractions were evaporated over a steam bath and made to volume for flame spectrometric determination of Na, Ca and K, using a Weichselbaum-Varney photometer. The column

¹ Supported by Grant H 1445 from the National Institutes of Health, United States Public Health Service.

² Clinical Investigator, Veterans Administration Hospital, Cincinnati, Ohio. This work commenced during a United States Public Health Service Postdoctoral Research Fellowship, 1955-1957.

TABLE I
The composition of normal skull, rib and ilium*

No.	H ₂ O (wet bone)	Fat (dry bone)	Per kilogram dry, fat-free bone							
			H ₂ O	Ca	P	CO ₂	N	Cl	K	Na
			Gm.	Eq.	Gm.	Eq.	Gm.	mEq.	mEq.	mEq.
Skull	(10)	17.6 3.7	229 63	12.5 0.27	111.1 3.7	1.70 0.06	48.1 4.5	28.3 6.1	16.4 6.8	291 6.8
Rib	(14)	21.6 6.4	319 143	11.6 0.47	104.5 4.7	1.63 0.10	53.4 3.1	35.3 9.0	34.0 11.6	268 8.3
Ilium	(13)	25.8 4.0	448 139	11.4 0.64	100.5 5.1	1.61 0.08	56.0 3.9	41.1 8.6	38.9 9.4	269 10.8

* Values given are mean and standard deviation.

was washed with 300 ml. of 0.7 N HCl after each run. Repouring of the column was usually necessary after two runs. New resin was employed after six runs. Resin separation of ions was found to be essential because of spectral interference of P and Ca in determination of Na, an effect noted also by others (17-19). In contrast, no mutual interfering effects of Ca and K were found. Average recovery values from synthetic mixtures passed through the resin columns were: P, 98.6 per cent; Ca, 99.9 per cent; Na, 99.1 per cent; and K, 101.6 per cent.

Aliquots of fat-free powder were analyzed for chloride in duplicate according to Cheek and West (20), for carbonate in triplicate according to Bergstrom (21), and for total nitrogen in duplicate by the micro-Kjeldahl method.

Analysis of fat-free powder in duplicate agreed within 2 per cent for Na, 4 per cent for K, 3 per cent for Ca, 2 per cent for P, 3 per cent for Cl, and 2 per cent for nitrogen. Triplicate analyses of carbonate were made, since the differences between individual aliquots occasionally ranged to 6 per cent. Analyses of data were made according to Snedecor (22).

It is possible that postmortem changes of pH or of some other nature might alter bone composition. How-

ever, it is believed that failure of the circulation would preclude significant removal of any substances from the areas analyzed.

RESULTS

In Table I are given the mean concentrations of water and minerals expressed per kilogram of fat-free solids in skull, rib and ilium. In comparison to ilium, skull was characterized by significantly greater amounts of Ca, P, CO₂ and Na, and lesser amounts of H₂O, N, Cl and K ($p < 0.02$). The concentrations in rib ranged between those of skull and ilium. These findings are probably related to the compact nature of skull as contrasted to the cancellous nature of ilium, with the specimens of rib occupying intermediate positions in density. Because the bones compared were not always obtained from the same subjects, a check of the findings was made with variance analysis in order to eliminate the effects of patient variance on the means of bones (Table II). In six subjects from whom all three bones were obtained, the same differences between skull and ilium were found except in the case of carbonate, where the concentration was only probably decreased below that of skull ($p < 0.05$). In the same analysis, no differences in concentrations of minerals between patients were found except for those of P and Cl.

The data obtained are in general agreement with those published by others. The amounts of water found are similar to those reported by Edelman, James, Baden and Moore when the large variance is considered (11). The concentrations of Ca, P and CO₂ found agree closely with those reported elsewhere (5, 9-11, 13). Despite the differences in Ca and P concentrations between bones, the molar Ca to P ratios were 1.76, 1.72

TABLE II

The variance ratios (F) of means of subjects and bones*

	Subjects	Bones
Ca	1.04	9.22†
P	11.5†	113†
CO ₂ ‡	1.12	4.37§
Na	2.14	23.7†
N	1.87	39.7†
H ₂ O	0.91	18.2†
K	1.19	21.2†
Cl	6.30†	23.1†

* Degrees of freedom include 5 for subjects, 2 for bones, 10 for interaction, and 18 for analytical error.

† Significant at the 1 per cent level.

‡ In regard to CO₂, triplicate analyses were made, and the degrees of freedom for error were 36.

§ Significant at the 5 per cent level.

TABLE III

*Human bone sodium—mean concentration (mEq. per kilogram of dry bone) and number of samples **

Author	Reference	Skeleton	Skull	Rib	Ilium	Femur	Humerus	Tibia	Vertebr
Gabriel, 1894†	(2)						251 (2)		
Klement, 1936†	(3)		159 (3)		131 (2) (pelvis)	140 (2)			
Davies, Kornberg and Wilson, 1952‡	(8)			239 (10)		300 (6)			
Edelman, James, Baden and Moore, 1954§	(11)		273 (2)	215 (4)	222 (4)	246 (5)			215 (1)
Forbes and Lewis, 1956§	(12)	309 (2)							
Pellegrino and Farber, 1958‡	(13)							248 (15)	
Present study§			291 (10)	268 (14)	269 (13)				

* Numbers in parentheses indicate numbers of samples.

† Analysis of glycol-ashed bone. Values calculated per dry bone assuming an organic phase of 25 per cent by weight (23).

‡ No statement concerning fat extraction.

§ Values expressed per fat-free dry bone.

and 1.76 for skull, rib and ilium, respectively. The N determined approximates the amounts obtained by others (5–7). The finding of a decreased N content in skull is in keeping with the observations of Baker, Butterworth and Langley who reported that compact bone had less N than cancellous bone (5). The mean chloride concentrations obtained, ranging from 28.3 to 41.1 mEq., are equivalent to those of Edelman and co-workers (11). Concerning the K content of human bone, the only information found for comparison was in the older work of Gabriel (2), Klement (3), and in the recent report of Pellegrino and Farber (13). When the values obtained by the first two authors are recalculated, assuming an organic content of 25 per cent (23) to express the contents per kilogram of dry bone, mean concentrations of 50 and 13 mEq. are obtained, respectively. The concentration of 10 mEq. found by Pellegrino and Farber in tibia is in keeping with the amount herein obtained from skull (13).

The concentrations of Na reported have been varied (Table III). Differences in sites analyzed and in methods employed are the likely sources for these variations. The higher concentration of sodium in skull than in rib or ilium is consistent with the observations of Edelman and associates, who found similar changes in grossly normal bone obtained from five adults (11). However, the actual sodium concentrations obtained by these investigators are somewhat lower than the pres-

ent results. From the data in Table III it appears that the sodium concentration is higher in compact bone than in cancellous bone.

The data were further examined in efforts to clarify the relative locations in whole bone of H₂O, Cl, K and Na. If bone is considered to be composed of two major solid phases, crystalline and organic, then the Ca and N concentrations of the total fat-free solids should serve respectively as reference bases for these phases. There is no evidence that either element exists as a significant component of the other phase. In agreement with this concept is the fact that the N and Ca concentrations expressed per fat-free solids in the current study were significantly inversely related ($p < 0.02$). Accordingly, the relations of water, Cl, K and Na to these references were studied.

In Table IV are given the common regressions obtained by combining the individual regressions of the three bones. Currently it is held that a large amount of water is associated with the hydration shell, and that Cl ions penetrate this shell (24). In the present study it is apparent that the water content was significantly related positively to N and negatively to Ca. This finding would be compatible with the concept that in the bones studied, the water determined at 105° C. existed mainly as cellular and interstitial water rather than as water of the hydration shell demonstrated to be present in synthetic apatites and veal bone by Neuman, Toribara and Mulryan (25).

TABLE IV
The covariance ratios and significances of common regression slopes (three bones)*

Regression	Slope	F	t
H ₂ O-N	177	1.78	3.62†
K-N	14.7	2.60	4.01†
Cl-N	13.6	1.18	4.70†
Na-N	0.76	0.77	0.18
CO ₃ -N	-0.13	0.66	4.03†
H ₂ O-Ca	-147	0.40	4.06†
K-Ca	-12.7	0.20	4.81†
Cl-Ca	-5.84	1.66	2.16‡
Na-Ca	6.17	0.44	2.08‡
CO ₃ -Ca	2.37	0.89	2.41§

* Degrees of freedom include 2 and 31 for the F ratio, and 33 for the t test.

† Significant at the 1 per cent level.

‡ Significant at the 5 per cent level.

§ Significant at the 2 per cent level.

In similar fashion, it is shown that Cl and K were positively related to the organic phase. However, a similar relation between water, Cl, K and N could also be obtained even if water, Cl and K were predominantly present in the hydration shell. In this situation the aging of crystals, with increase in size and displacement of water, would of necessity lead to an increased Ca to N ratio and a decrease in chloride and K. In addition, it is also possible that change in proportion of the organic phase may in some way affect the amount of water and ions associated with the hydration shell and crystalline phase. Finally, the possibility of course cannot be overlooked that water, Cl and K are located in bone in such a fashion that they would not be related to either organic or crystalline phase.

Recently Neuman and Neuman (24) have questioned the advisability of applying the calculations for interstitial water and excess sodium of bone which Harrison, Darrow and Yannet first proposed (26). Perhaps in bone which not only contains apatite but an organic phase intimately related to the apatite, the concept of a chloride space has physiologic meaning. In the present study, the mean derived chloride spaces³ in skull and rib were 239 and 297 Gm. of water per Kg. of fat-free solids, respectively. These values do not differ significantly from the mean water contents, determined by drying, of 229 and 319 Gm.

³ Derivation of the chloride space was made assuming a plasma Cl concentration of 102 mEq. per L., 90 per cent water, and a Donnan factor of 0.95.

In contrast, the mean water content of ilium of 448 Gm. was significantly greater than that of the 345 Gm. derived from the chloride space. These calculations would suggest that in rib and skull the water was mainly interstitial, whereas in ilium there were additional components of cellular or hydration shell water.

It is of interest that the ratios of water, Cl and K to N were lower in skull than in rib and ilium. It may be that failure to remove all marrow in rib and ilium during the initial processing accounts in part for this discrepancy. Direct analysis of marrow revealed higher ratios of water, Cl and K to N than those found in whole bone.

Although the concentrations of Na and Ca in skull were significantly greater than those of rib and ilium (Table I), the ratios of Na to Ca (mM to M) were remarkably constant, being 46.4, 46.3 and 47.5, respectively. These constant ratios support the concept that Na is predominantly related to the crystalline phase (17). However, the common regression of Na on Ca was only probably significant ($p < 0.05$). It is possible that sufficient Na was present in the organic phase to invalidate a more significant relation of Na to Ca.

SUMMARY

Analyses of normal human skull, rib and ilium were made for concentrations of water, Ca, P, CO₃, N, Cl, K and Na. Skull contained significantly greater amounts of Ca, P, CO₃ and Na, and lesser amounts of water, N, Cl and K than did ilium. The composition of rib was intermediate between skull and ilium. The concentrations of water, Cl and K were related in quantity to the amount of organic phase present. Sodium appeared to be related to the crystalline phase.

ACKNOWLEDGMENT

The authors wish to acknowledge helpful advice from Drs. Gilbert B. Forbes and William Bergstrom in connection with methods.

REFERENCES

1. Bergstrom, W. H. The skeleton as an electrolyte reservoir. *Metabolism* 1956, 5, 433.
2. Gabriel, S. Chemische Untersuchungen über die Mineralstoffe der Knochen und Zähne. *Z. physiol. Chem.* 1894, 18, 257.

3. Klement, R. Der Carbonatgehalt der Anorganischen Knochensubstanz und ihre Synthese. *Ber. dtsh. chem. Ges.* 1936, **69**, 2232.
4. Mitchell, H. H., Hamilton, T. S., Steggerda, F. R., and Bean, H. W. The chemical composition of the adult human body and its bearing on the biochemistry of growth. *J. biol. Chem.* 1945, **158**, 625.
5. Baker, S. L., Butterworth, E. C., and Langley, F. A. The calcium and nitrogen content of human bone tissue cleaned by micro-dissection. *Biochem. J.* 1946, **40**, 391.
6. Vogt, J. H., and Tønsager, A. Investigations on the bone chemistry of man. II. Phosphatase, calcium, phosphorus and nitrogen in the substantia spongiosa cristae ilei. *Acta med. scand.* 1949, **135**, 231.
7. Rogers, H. J., Weidmann, S. M., and Parkinson, A. Studies on the skeletal tissues. The collagen content of bones from rabbits, oxen and humans. *Biochem. J.* 1952, **50**, 537.
8. Davies, R. E., Kornberg, H. L., and Wilson, G. M. The determination of sodium in bone (abstract). *Biochem. J.* 1952, **52**, xv.
9. Follis, R. H., Jr. The inorganic composition of the human rib with and without marrow elements. *J. biol. Chem.* 1952, **194**, 223.
10. Forbes, R. M., Cooper, A. R., and Mitchell, H. H. The composition of the adult human body as determined by chemical analysis. *J. biol. Chem.* 1953, **203**, 359.
11. Edelman, I. S., James, A. H., Baden, H., and Moore, F. D. Electrolyte composition of bone and the penetration of radiosodium and deuterium oxide into dog and human bone. *J. clin. Invest.* 1954, **33**, 122.
12. Forbes, G. B., and Lewis, A. M. Total sodium, potassium and chloride in adult man. *J. clin. Invest.* 1956, **35**, 596.
13. Pellegrino, E. D., and Farber, S. J. Human bone electrolytes in various disease states. *Clin. Res.* 1958, **6**, 259.
14. Dellamagne, W. J. Some recent facts about the properties of tricalcium phosphate and the composition of the bone salt. *Trans. Fourth Josiah Macy Jr. Conf.* 1952, p. 154.
15. Forbes, G. B., and D'Ambruso, M. Determination of sodium in bone with the aid of cation exchange chromatography. *J. biol. Chem.* 1955, **212**, 655.
16. Fiske, C. H., and Subbarow, Y. The colorimetric determination of phosphorus. *J. biol. Chem.* 1925, **66**, 375.
17. Stoll, W. R., and Neuman, W. F. The uptake of sodium and potassium ions by hydrated hydroxyapatite. *J. Amer. chem. Soc.* 1956, **78**, 1585.
18. Wallace, W. M., Holliday, M., Cushman, M., and Elkinton, J. R. The application of the internal standard flame photometer to the analysis of biologic material. *J. Lab. clin. Med.* 1951, **37**, 621.
19. Parks, T. D., Johnson, H. O., and Lykken, L. Errors in the use of a model 18 Perkins-Elmer flame photometer for the determination of alkali metals. *Analyt. Chem.* 1948, **20**, 822.
20. Cheek, D. B., and West, C. D. An appraisal of methods of tissue chloride analysis: The total carcass chloride, exchangeable chloride, potassium and water of the rat. *J. clin. Invest.* 1955, **34**, 1744.
21. Bergstrom, W. H. The relationship of sodium and potassium to carbonate in bone. *J. biol. Chem.* 1954, **206**, 711.
22. Snedecor, G. W. *Statistical Methods*, fifth ed. Ames, The Iowa State College Press, 1956.
23. Stack, M. V. The chemical nature of the organic matrix of bone, dentin, and enamel. *Ann. N. Y. Acad. Sci.* 1955, **60**, 585.
24. Neuman, W. F., and Neuman, M. W. Emerging concepts of the structure and metabolic functions of bone. *Amer. J. Med.* 1957, **22**, 123.
25. Neuman, W. F., Toribara, T. Y., and Mulryan, B. J. The surface chemistry of bone. VII. The hydration shell. *J. Amer. chem. Soc.* 1953, **75**, 4239.
26. Harrison, H. E., Darrow, D. C., and Yannet, H. The total electrolyte content of animals and its probable relation to the distribution of body water. *J. biol. Chem.* 1936, **113**, 515.