FLUORINE CONTENT OF HUMAN BONES (1)

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Mr. Murotabe of the Tokyo University Anthropology Department, who had been endeavoring to apply chemical analyses to the studies of anthropology, reported in 1945 on the calcium and phosphorus content of human bone from the shellmound of Homi. The chemical composition of the human bone is generally as follows: approximately 40% organic substances (after dehydration), and 60% inorganic constituents, of which approximately 85% is calcium phosphate and the remainder chiefly calcium carbonate. Taken as a whole, the past studies have revealed the following proportions of inorganic constituents:

SiO_2 less than 1%	CO_2 around 5%	Cl around 0.1%
CaO around 52%	$(Fe, Al)_2O_3$ less than 3%	P_2O_5 around 42%
Na_2O around 1%	MgO less than 2%	$CaO:P_2O_5 = 1:0.8$
F around 0.1%	K_2O around 0.1%	

Carnot (1891-2) pointed out that the relationship between the content of F and P can be used as an indicator to deduce the age of the fossilized bone. When the respective F:P ratios of the fossilized bones and apatite are compared, it will be observed that the older the geological age of the fossilized bone, the greater the ratio as shown in Table I.

Bemmelen (1897) expressed the opinion that the enrichment of fossilized bone with F is due either to the conversion of $CaCO_3$ to CaF caused by the alkali fluoride in the subterranean water or to the adsorption of F on P. A matter of interest to the archaeologist is Sidersky's discovery (1934) that the ratio of F:P in the bones of animals increases in proportion to the number of years after death (e.g., F is 0.52% of P in present-day bones but increases to 8.85% in bones from primary geological age) and that consequently this ratio may be used in estimating the age of the bones. Boyle et al. (1939) also conducted some studies on changes in the content of organic substances, carbonate salts of alkali earth metals and F during the aging process.

We were very fortunate in being able to conduct some studies with human bones of known geologic age to see whether the facts mentioned above in connection with fossilized bone can be verified for bones about 4000 years old. The bones were given us by the Tokyo University Anthropology Department.

Experimental Procedure

From among the gravimetric, titration and colorimetric methods available for the determination of small amounts of F, we chose the titration by thorium nitrate using sodium alizarin sulfonate as indicator. This method requires

TABLE I

Ratio of F/P O in fossilized bone to F/P_2O_5 in apatite (Carnot)

Paleozoic				= 1.07 ~ 0.9
Tertiary	$\frac{1.90}{30.24}$	:	0.0892	= 0.70
	$\frac{2.05}{36.81}$	:	0.0892	= 0.63
	$\frac{1.95}{33.24}$	:	0.0892	= 0.65
	$\frac{1.83}{35.20}$	:	0.0892	= 0.58
Quaternary	$\frac{1.06}{33.83}$	:	0.0892	= 0.35
Unfossilized bone	$\frac{0.205}{40.28}$	:	0.0892	= 0.06

TABLE II

F Content of Human Bones (Tibiae)

Sample No.	Location	Period	Loss of wt. Upon Heating	F Content	$\frac{F}{P_2O_5}$ x 100 *
1	Kokufu shell mound	Early Jomon	12.85	0.11	0.27
2	Todoroki " "	" "	25.71	0.26	0.62
3	Yatsukuri " "	Late Jomon	17.55	0.25	0.62
4	Yoyama " "	" "	21.82	0.27	0.65
5	Tsukumo " "	Final Jomon	11.05	0.21	0.52
6	Inariyama " "	" "	14.06	0.22	0.52
7	Okitsu - cave	Tomb Period	19.04	0.24	(0.54)
8	Tagagun - hole	Late Tomb Period	30.77	0.21	0.48
9	Kanekoyama - hole	" " "	24.11	0.22	(0.50)
10		1949	37.53	0.07	(0.15)

* The P content was determined by M. Ashikaga of the Chemistry Department and is as yet unpublished by him. P content is 40-44% and the value declines with age. Nos. 7, 9, 10 are incomplete and were based on P_2O_5 content of 44%.

the removal of interferring substances prior to F determination. For this purpose distillation of hydrofluosilicic acid as described by Willard and Winter (1933) is suitable. The method has been widely applied and improved by many later workers. We chose the method employed by Okuno (1941), who investigated the F content of hot spring water, and by McClure (1939), who investigated the F content of bone ashes.

The following distillation apparatus is employed. Distillation flask B has a capacity of 100 ml. and is covered with asbestos to preserve heat. A thermometer is hung in the flask by a platinum wire to measure the temperature of the distilling liquid. Flask A contains water with a small amount of NaOH and is used to generate the steam. The amount of steam going to B is controlled by a pinchcock D. To heat flask B, electricity rather than the burner is preferred to minimize the fluctuation in the temperature. The temperature is kept at 135-140° C so that 150 cc. of distillate comes over in 1 to 1½ hours.

Before distillation, about 20 cc. of HNO_3, a few glass beads and a small amount of $AgNO_3$ (enough to catch all chlorides in the sample) are added to flask B and blank distillation is carried out. The volatile impurities in HNO_3 which emerge as a result of the initial abrupt rise of temperature are discarded and then 150 cc. of distillate is collected. Then, a good portion of the HNO_3 is taken up with a pipette and the weighted amount of the sample powder is added. The HNO_3 in the pipette is used to wash down the powder. Distillation is continued until 150 cc. is collected. This step is followed by the distillation of a known amount of purified NaF until 150 cc. has been collected. The receiver C contains 20 cc. of distilled water alkalized to phenolphthalein with NaOH.

The slightly alkali distillates are placed in a platinum evaporating dish and heated to dryness on a steam bath. The residue is dissolved in 10 ml. of 50% alcohol, after which HCl (1:50) is added dropwise to remove the red phenolphthalein color. 0.1 cc. of 0.05% solution of sodium alizarin solfonate is then added; if this indicator turns orange more HCl is added until a yellow color appears. 1 cc. of mono $ClHOA_c$-NaOH buffer (pH 3.5) is added and the solution titrated with a standard thorium nitrate until the color changes from yellow to pale pink. Incidentally, the titration of the blank is carried out beforehand to correct the amount of thorium nitrate consumed. The blank also serves as a standard for the end point. The correction for the recovery is made from the data on distillation of the known amount of F.

The procedure may be elucidated by the actual experimental data. The distillate on 0.5813 gm. of sample was dried and taken up with 10 cc. 50% alcohol. It required 1.62 cc. of thorium nitrate for titration. The blank took 0.03 cc. (1 drop). Therefore the actual amount required is 1.59 cc. The distillate from 1 cc. of standard NaF (F 1.82 mg.) required 4.45 cc. The standard thorium nitrate solution has a concentration of 2.9024 gm. $Th(NO_3)_4$ $4H_2O$ per 1 and requires 2.51 cc. to titrate against 1 mg. of F. Therefore, the recovery of F is 97% and the F content in the sample is 0.11%.

A drop of thorium NO_3 is 0.03 cc. which is equivalent to 0.01 mg. of F. When the sample is 0.1 g. accuracy of more than 0.01% is not attainable.

Results and Discussions

The results of F analysis are shown in Table II. The values we obtained for the F content are within the range of F. J. McClure's (1939) of about 0.01-0.5% for bone ash, and of Carnot's (1891-2) 0.17-0.3% for fresh bones (human, sea-cow, cow, elephant).

The samples are all tibiae and the list is in chronological order, with the oldest being at the top. Table II indicates that the F content is lowest in recent bones and higher in all others, with the exception of the oldest Kokufu-shellmound bones, the proportion of F in the other 8 samples varying from 0.21 to 0.27%. If one were to push the data a little, one might say that there is a tendency for Nos. 2, 3, 4 to be somewhat higher in F content than those after No. 5. This difference is more clearly brought out in the last column where the ratio of F content to P content is shown. The variation in F:P ratio may in part be accounted for by the tendency for the P content of older bones to be proportionally lower.

Although at first glance it seems that the findings of Carnot (1891-2), Bemmelen (1897) and Sidersky (1934) can be verified with the samples dealt with here, but definite conclusions cannot be drawn since the number of samples was too small. The opinion of the authors is that the determination of the age of bones by measuring the F content is not practical when the differences in age are as small as those involved in our experiment.

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NOTE

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BIBLIOGRAPHY

Bayle, E., L. Amy, M. Rondeau du Noyer

1939 Bull. soc. chim., 6, p. 1011.
1939 Chem. Abst., 33, p. 6757.

Bemmelen, J. M. van

1897 Z. anorg. allgem. Chem., 15, p. 90.

Carnot, Ad.

1891-2 Comt. rend., 114, p. 1189; 115, p. 243, p. 337.

Hisateru Okuno

1941 Journ. Chem. Soc. Japan, 62, p. 234.

McClure, F. J.

1939 Ind. Eng. Chem., Anal. Ed. 11, p. 171.

Sidersky, D.

1934 Bull. assoc. chim. sucr. dist., 51, p. 514.
1935 Chem. Abst., 29, p. 1366.

Giichi Tanabe

1944 J. Anthrop. Soc. Japan.
1945 J. Anthrop. 59, p. 1.

Willard, H. H., O. B. Winter

1933 Ind. Eng. Chem., Anal. Ed. 5, p. 7.