

# ON THE CALCIUM AND PHOSPHORUS CONTENT OF HUMAN BONE

## FROM THE STONE AGE SHELLMOUND OF HOMI<sup>(1)</sup>

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In order to prepare myself for the chemical analysis of human bones from the Stone Age, I carried out determinations of Ca and P for scattered pieces of human bones obtained in 1941 from the first district of the Homi Shellmound in Fukue-machi, Ohmi-gun, Mikawa Province. Before analysis, a bone is washed cursorily, placed in a weighing bottle of known weight, dried in an electric oven at about  $110^{\circ}$ , placed in a dessicator until cool, and repeatedly weighed on an analytical balance to the 4th decimal place until a constant weight is obtained. The weight of the bone is the total minus the weight of the bottle. Next the bone is placed in a crucible and heated with a burner to destructively carbonize the organic substances. When the odor of organic substances is no longer detectable, the lid is put on the crucible, and the bone once more heated thoroughly. When ashing is complete, the sample is cooled and drying and weighing repeated until the weight of the ash reaches a constant value. The difference thus obtained between the original weight of bone and the weight of the ash is taken to be the weight of organic substances and  $\text{CO}_2$ . The ash is then placed in a beaker to which dilute HCl has been added, heated gently, and the undissolved part filtered off. The filtrate is placed in a volumetric flask, diluted to a certain level and analyzed. The undissolved portion is dried, weighed and taken to be the soil adhering to the bone. Strictly speaking, since some components of the soil are soluble in acid, a correction should be applied after the soil has been analyzed for its proportions of soluble and insoluble constituents. There are also some insoluble components of the bone, but in this experiment all matter insoluble in acid was taken to be soil. When one subtracts the weight of the soil from that of the bone and ash, the actual values of the latter are obtained.

The solution in the volumetric flask is mixed very thoroughly and an aliquot is taken for analyzing the P, Ca, and other components of the bone. Errors are minimized by the experimenter's running through the entire procedure once again.

### The Method for Determining Calcium Content

Ordinarily Ca is determined gravimetrically, but since this procedure is time consuming, and the last step (oxidation of Ca oxalate to CaO and weighing) is difficult, I used oxidative titration with  $\text{KMnO}_4$ . A sample solution is placed in a beaker, a little amount of iron chloride solution added and alkalinized to phenolphthalein with aqueous  $\text{NH}_3$  when white precipitate appears. The solution is then acidified to methyl red with HAc (to pale yellowish red color) so that some precipitate is redissolved and filtered. The precipitates which were formed with the addition of aqueous  $\text{NH}_3$  included  $\text{FePO}_4$  and  $\text{CaPO}_4$ . The HAc dissolves everything except  $\text{FePO}_4$ .

TABLE I

Exp. No.		Wt. of Bone (g)	Ash (g)	Loss of wt. on combustion (g)	CaO (g)	P <sub>2</sub> O <sub>5</sub> (g)	P <sub>2</sub> O <sub>5</sub> /CaO
1	(Rib)	0.6581	0.5949	0.0632	0.3102	0.2367 (in 1.3608 g ash)	0.76
2	(Skull)	4.2661-**	3.7810-*	0.4751	0.7473	0.5550	0.74
3	(Lower Limb)	0.4507	0.3950	0.0552	--	--	--
4	" "	0.5806	0.5352	0.0454	0.2779	0.2020	0.73
5	" "	0.7540	0.7050	0.0490	0.3564	0.2738	0.77
6	" "	1.3811	1.2455	0.1356	0.6551	0.4913	0.75
7	" "	0.4377	0.3979	0.0398	0.2013	0.1582	0.79

Exp. No.	Percentage distribution of the bone weight		Percentage distribution of the ash	
	Ash	Loss of weight	CaO	P <sub>2</sub> O <sub>5</sub>
1	90.4	9.6	52.1	39.7
2	(88.9) **	(11.1)	54.9	40.5
3	88.0	12.0	--	--
4	92.3	7.7	51.9	37.7
5	93.6	6.4	50.6	38.8
6	90.4	9.6	52.6	39.4
7	90.9	9.1	50.6	39.8

\*\* Calculated without subtracting the soil weight.

\*\* Weight of soil.

The weights of bones and ash shown here are those after the insoluble portions were subtracted as being soil.

After filtering and washing, the precipitate is dissolved in dilute HCl, the precipitation with aqueous  $\text{NH}_3$  and HAc, filtration and washing are repeated and the filtrates are all pooled. The filtrate is treated with a small amount of aqueous  $\text{NH}_3$  until the solution is alkalized to a pale yellow color. Saturated solution of ammonium oxalate is added in excess to precipitate the white Ca oxalate, heated on water bath for 3 hours, cooled and filtered after decantation. The precipitate is washed with water containing ammonium oxalate, washed into the original beaker, and dissolved by means of dropwise addition of concentrated HCl and reprecipitated. This last step serves to separate the Mg. The reprecipitation is performed as follows: The concentrated HCl is first diluted with  $\text{H}_2\text{O}$ , a small amount of  $\text{NH}_4$  oxalate is added, the mixture is heated, an excess of aqueous  $\text{NH}_3$  is added, and the mixture again heated almost to the boiling point. It is now allowed to cool and then filtered after decantation. The washing is not accomplished with  $\text{NH}_4$  oxalate this time, about a 1% solution of  $\text{NH}_3$  being used instead. (Saturated  $\text{NH}_4$  oxalate is said to be good for washing. Water dissolves it to some extent.) Then the thoroughly washed precipitate is washed back into the beaker. The precipitate adhering to the filter paper is washed off with dilute  $\text{HNO}_3$ . The large amount of dilute  $\text{HNO}_3$  is added to the beaker and diluted to 200 cc. with  $\text{H}_2\text{O}$ , heated to about 700 and titrated with  $\text{KMnO}_4$  until the pale pink color persists.

1/10 N  $\text{KMnO}_4$  is used. The  $\text{KMnO}_4$  solution is standardized with 1/10 N oxalic acid or Na Oxalate. When, for example, 10 ml. 1/10 oxalic acid takes up a cc. of  $\text{KMnO}_4$

$$T = 10/a \text{ (T:Titer).}$$

When a titration end point is reached with 1 cc. 1/10 N  $\text{KMnO}_4$ , the amount of Ca is calculated as follows:

$$\begin{aligned} 1 \text{ ml. } 1/10 \text{ N } \text{KMnO}_4 &= 0.002 \text{ g Ca} \\ &= 0.0028 \text{ g CaO} \end{aligned}$$

$$\therefore 1 \text{ ml. of } \text{KMnO}_4 \text{ with } T = 10/a \text{ is equivalent to } 0.0028 \times 10/a \text{ grm. of CaO.}$$

### The Determination of Phosphorus Content

P also is generally determined by the gravimetric method, but here again I used alkalimetric titration. The procedure consists of adding a small amount of  $\text{FeCl}_3$  to remove a large amount of Ca, followed by precipitation with aqueous  $\text{NH}_3$  and filtration, thereby removing Ca into the filtrate. The precipitate is dissolved with dilute HCl, then alkalized with  $\text{NH}_3$ , acidified with  $\text{HNO}_3$ , and heated momentarily; a large amount of  $\text{NH}_4$  molybdate, and a small amount of  $\text{NH}_4\text{NO}_3$  are then added, and precipitation is caused by thorough shaking. While precipitation is still incomplete, a small amount of  $\text{HNO}_3$  is added. Shaking greatly accelerates precipitation. The precipitation is allowed to continue overnight. The precipitate is filtered after decanting, washed with a dilute solution of saltpeter, and, when the washing solution becomes

neutralized, is transferred to a flask along with the filter paper and shaken well with added H<sub>2</sub>O. Next, an excess of 1/10 N NaOH is added until red to phenolphthalein to dissolve the precipitate, and then 1/10 N HNO<sub>3</sub> is added until the red phenolphthalein color fades. If the amount of 1/10 N HNO<sub>3</sub> is a cc., and amount of 1/10 N HNO<sub>3</sub> is b cc., the actual amount of base used is (a-b)cc. 1 cc. of N/10 NaOH is equivalent to 0.000135 g P = 0.000309 g P<sub>2</sub>O<sub>5</sub>.

$$\begin{aligned} \therefore \text{P in the sample} &= (a-b) \times 0.000135 \text{ g P} \\ &= (a-b) \times 0.000309 \text{ g P}_2\text{O}_5. \end{aligned}$$

The actual data are shown below:

A sample of rib bone originally weighed 0.6649 g.; after ashing, it weighed 0.6017 g.; the insoluble part weighed 0.0068 g. If the weight of soil is subtracted, the weight of the bone becomes 0.6581 g., and that of the ash, 0.5949 g. The loss of weight upon combustion was 0.0632 g., which was equal to 9.6% of the weight of the rib bone, and was very small. Next, 100 cc. of the acid-soluble part of the ash was collected in a volumetric flask, 5 cc. being taken for Ca determination. Amount of KMnO<sub>4</sub> required was 4.1 cc. This KMnO<sub>4</sub> had a T value of 5.0/3.7.

$$\begin{aligned} \therefore \text{CaO in 5 ml. portion} &= 0.0028 \text{ g} \times 4.1 \times 5.0/3.7 = 0.0155 \text{ g} \\ \therefore \text{CaO in the whole solution} &= 0.3102 \text{ or } 52.14\%. \end{aligned}$$

Another portion of 5 cc. was used for P determination. 40 cc. of N/10 NaOH and 1.7 cc. of N/10 HNO<sub>3</sub> were required.

$$\begin{aligned} \therefore \text{In 5 cc. solution P}_2\text{O}_5 &= 0.00309 \text{ g} \times (40-1.7) = 0.0119 \text{ g} \\ \therefore \text{In whole solution} &0.2367 \text{ g or } 39.74\% \text{ P}_2\text{O}_5. \end{aligned}$$

The ratio of P<sub>2</sub>O<sub>5</sub> to CaO is 0.76:1.

The samples used in this experiment were 1 rib, 1 skull, 5 lower limbs, making a total of 7 pieces of bone. See Table I for results.

According to the data from K. Kuroda's analysis on human bones taken from Unggi, Hangyong-pukto, Korea as reported in the Journal of the Anthropological Society Vol. 50, No. 1 (1935), the organic substance constitutes 10.57% of the total, and CaO constitutes 25.29% of the soluble ash. The former percentage is not much different from that in Homi, but the CaO content is about half of that in Homi. The bone is usually 85% CaPO<sub>4</sub>, the remainder consisting of CaCO<sub>3</sub> and other substances. Consequently it is hard to believe that the value for CaO was 25.29% as reported. Were this the true proportion, the other components would remain undetermined.

Fresh bone consists of approximately 40% (after dehydration) of organic substances, according to Recklinghausen et al. The human bones from Homi are very deficient in organic components. Perhaps this

deficiency is a result of the natural decomposition of organic matter which has been buried a long time.

The Ca and P contents of the Homi bones seem to vary according to sex, age, type of bones, and different parts of the same bone, but it is said that after maturity, age no longer determines the amounts of these minerals. I intend to look into this matter in the future.

Many thanks are due to Mr. Y. Matsumura of the Biochemistry Department, who helped in setting up this experiment.

#### NOTE

- (1) This article was originally published in Zinriugaku Zassi (Journal of the Anthropological Society of Japan) 59:1-5 (1944).

