

Calibration of Diffractometer Measurement for Fluorine Determination of Fossil Bones from Sangiran

—A Reappraisal of X-ray Diffractometric Application
to the Fluorine Dating—*

By

Shuji MATSU'URA

Department of Anthropology, National Science Museum, Tokyo

Introduction

Diagenetic chemical changes during fossilization sometimes form a geochronometer for phosphatic skeletal material. Fluoride ions taken up by buried bone (or tooth) from sedimentary matrices and ground water, accumulate over a period of time at the cost of hydroxyl ions in the apatite crystals. Thus the fluorine content of fossil bone can be a useful guide to relative age. The fluorine dating method (OAKLEY, 1951) has received recognition of palaeontologists, especially of palaeo-anthropologists, and has been used to assist the development of the chronology of prehistoric human remains (OAKLEY & MONTAGU, 1949; OAKLEY, 1980; TANABE, 1962; MATSU'URA, 1982; and others).

Fluorine in bone substance for dating has been generally determined by means of chemical analysis; for example, standard series method (TANABE, 1962), spectrophotometry (GLOVER & PHILLIPS, 1965), thorium nitrate titration (SHIMODA & KITAMURA, 1978), and electrometry (MATSU'URA, 1981). Besides, semi-quantitative estimation of fluorine content using X-ray diffraction technique was applied in some cases (e.g. OAKLEY & HOWELLS, 1961; MOLLESON & OAKLEY, 1966). This physical analysis presents the advantage of saving consumption of sample.

STÜHLER (1938) first noted a slight shift in the position of the trio of lines, (321), (410) and (402) toward the line (004) on the X-ray diffraction diagram according to the enrichment of fluorine in bone. This appearance is due to the fact that the substitution of F^- for OH^- in the hexagonal apatite structure causes a decrease in the unit cell parameter a_0 with little change of c_0 . NIGGLI *et al.* (1953) have introduced the powder method of Debye-Scherrer to the relative dating of fossil bones. They employed the distance between lines for (004) parallel to the a -axis and for (410) parallel to the c -axis, as a measure of fluorine level; and showed that the distance reduces with geological age. Other examples of application were given by BAUD (1957, and

* Short notes on the results have been presented at the 36th Joint Meeting of the Anthropological Society of Nippon and the Japanese Society of Ethnology, Tokyo, 1982; and part of this work is reported in MATSU'URA (1983).

others), who could correlate the axial ratio c/a to the quantity of fluorine. The X-ray diffraction approach, on the other hand, was heavily criticized by McCONNELL (1962). However his critical remarks are based much on some extreme or unusual cases. Meanwhile TANABE & WATANABE (1968), using a diffractometer, found a rectilinear relation ($r = -0.99$) between the angular separation of (004)–(321) and chemically assayed fluorine content for prehistoric bones from Japan.

This report is to demonstrate the applicability of the X-ray method to fossil bones from the Sangiran area in Java, which has yielded many remains of early man; this is also devoted to a realization of the significance of the method to the fluorine dating.

Material

Nineteen bones and two tusks were taken for this study from a collection of fossil vertebrate remains, excavated by the fieldwork team of the Indonesia–Japan Joint Research Project CTA-41 (1977–79). Of the 21 specimens, 9 were derived from the Kabuh formation in the Sangiran area, Central Java; and the rest, from the Pucangan formation in the same area. For the stratigraphical contexts and ages of fossil-bearing beds in Sangiran, readers are referred to Indonesia–Japan Research Cooperation Programme—CTA-41 (1979), Indonesia–Japan Joint Research Team (1979), and MATSU'URA (1982). All the fossil specimens used here may be assigned to Lower Pleistocene (MATSU'URA, 1982, 1983).

Experimental

Sample Preparation

A small section of compact tissue, cut from each fossil bone specimen, was scraped to remove surface dirt, and then ground in an agate mortar to a homogeneous powder. Ultrasonic cleansing in deionized water and drying before pulverization were used if required.

Fluorine and Phosphate Analyses

Fluorine in fossil bone was determined by the fluoride electrode method, and phosphate was determined by acid-base titration after precipitation as ammonium phosphomolybdate. Details of the procedures and the reproducibilities are described in MATSU'URA (1982).

X-ray Diffraction

The procedure described below is founded on TANABE & WATANABE (1968). The pulverized bone sample is ignited for 6 h at 800°C to gain a distinct diffraction pattern, and set in a Philips PW1349 X-ray diffractometer. Operational conditions are:

Mn-filtered Fe radiation at 40 kV–20 mA; traverse speed, $1^\circ 2\theta/\text{min}$; time constant, 10 sec; chart-drive rate, 20 mm/degree.

On the diagram obtained (see Fig. 1), the distance of 2θ is measured between (004)

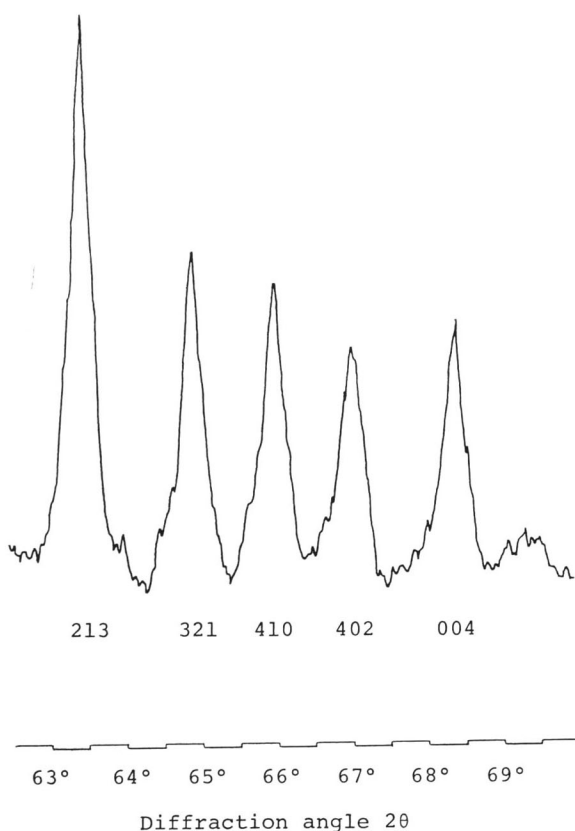


Fig. 1. X-ray diffraction pattern of a fossil bone from Sangiran. For analytical conditions, see text.

and (410), and between (004) and (321), using a glass ruler graduated in 0.1 mm with the aid of a $15\times$ magnifier. Note that the diffraction angle 2θ is appointed at the middle of the line intersecting the peak at half-maximum intensity. The two measured distances are then converted into the angular separations of 2θ by carrying out on each chart an *in situ* calibration for relating the former to the latter (usually, the distance of marks for 65° and 68° was used as the reference; see Fig. 1).

The reproducibility of measurements of 2θ separation was about 0.02° (S.D.) either for (004)–(410) or for (004)–(321).

Results and Discussion

The resulting data for X-ray analysis on the Sangiran specimens are tabulated in Table 1, together with those for chemical determinations. In the study reported here, measurements of separation of diffraction angles were made between (004) and (410), and between (004) and (321). The pair of peaks, (004) and (402), also varies

Table 1. X-ray diffraction data on compact bone specimens from Sangiran with chemical analyses.

	Chemically analysed			FeK α , 2 θ (004)-2 θ (410)			FeK α , 2 θ (004)-2 θ (321)			CuK α 2 θ (004)-2 θ (410)			CuK α 2 θ (004)-2 θ (321)		
	F (%)	100F/P ₂ O ₅ *	Angular separation (degree)	F (%) estimated†	100F/P ₂ O ₅ estimated†	Angular separation (degree)	F (%) estimated†	100F/P ₂ O ₅ estimated†	Angular separation (degree)	F (%) estimated†	100F/P ₂ O ₅ estimated†	Angular separation (degree)	F (%) estimated†	100F/P ₂ O ₅ estimated†	Angular separation (degree)
1.	2.68	7.98	2.16 ₉	2.48	7.39	3.26 ₆	2.53	7.51	3.26 ₆	2.53	7.51	1.60 ₁	2.41 ₀	2.41 ₀	2.41 ₀
2.	2.52	7.59	2.16 ₆	2.51	7.46	3.27 ₀	2.46	7.32	3.27 ₀	2.46	7.32	1.59 ₈	2.41 ₇	2.41 ₇	2.41 ₇
3.	2.49	7.55	2.17 ₄	2.46	7.31	3.27 ₅	2.43	7.23	3.27 ₅	2.43	7.23	1.60 ₅	2.42 ₁	2.42 ₁	2.42 ₁
4.	2.27	6.36	2.21 ₄	2.23	6.63	3.30 ₆	2.23	6.63	3.30 ₆	2.23	6.63	1.63 ₄	2.44 ₄	2.44 ₄	2.44 ₄
5.	2.12	6.01	2.24 ₅	2.05	6.11	3.33 ₇	2.03	6.04	3.33 ₇	2.03	6.04	1.65 ₇	2.46 ₇	2.46 ₇	2.46 ₇
6.	2.10	6.44	2.25 ₂	2.01	5.99	3.31 ₁	2.20	6.54	3.31 ₁	2.20	6.54	1.66 ₃	2.44 ₈	2.44 ₈	2.44 ₈
7.	2.06	6.46	2.17 ₉	2.43	7.22	3.29 ₈	2.29	6.82	3.29 ₈	2.29	6.82	1.60 ₉	2.43 ₇	2.43 ₇	2.43 ₇
8. ⁺	1.68	4.80	2.29 ₄	1.77	5.28	3.36 ₈	1.83	5.45	3.36 ₈	1.83	5.45	1.69 ₄	2.49 ₀	2.49 ₀	2.49 ₀
9. ⁺	1.61	4.48	2.34 ₆	1.47	4.40	3.40 ₀	1.62	4.83	3.40 ₀	1.62	4.83	1.73 ₂	2.51 ₄	2.51 ₄	2.51 ₄
10.	1.49	4.78	2.38 ₀	1.23	3.67	3.44 ₄	1.34	3.99	3.44 ₄	1.34	3.99	1.76 ₄	2.54 ₇	2.54 ₇	2.54 ₇
11.	1.37	4.11	2.37 ₀	1.34	3.99	3.44 ₄	1.34	3.99	3.44 ₄	1.34	3.99	1.75 ₀	2.54 ₈	2.54 ₈	2.54 ₈
12.	1.35	3.94	2.34 ₉	1.46	4.35	3.43 ₀	1.43	4.26	3.43 ₀	1.43	4.26	1.73 ₄	2.53 ₆	2.53 ₆	2.53 ₆
13.	1.34	4.17	2.37 ₇	1.30	3.87	3.45 ₀	1.30	3.88	3.45 ₀	1.30	3.88	1.75 ₅	2.55 ₁	2.55 ₁	2.55 ₁
14.	1.07	3.19	2.39 ₃	1.21	3.60	3.47 ₁	1.16	3.48	3.47 ₁	1.16	3.48	1.76 ₇	2.56 ₇	2.56 ₇	2.56 ₇
15.	1.05	3.10	2.43 ₆	0.96	2.88	3.50 ₆	0.94	2.81	3.50 ₆	0.94	2.81	1.79 ₈	2.59 ₃	2.59 ₃	2.59 ₃
16.	1.02	3.07	2.42 ₄	1.03	3.08	3.46 ₄	1.21	3.61	3.46 ₄	1.21	3.61	1.79 ₀	2.56 ₁	2.56 ₁	2.56 ₁
17.	0.956	2.67	2.41 ₀	1.06	3.16	3.50 ₅	0.94	2.82	3.50 ₅	0.94	2.82	1.78 ₆	2.59 ₁	2.59 ₁	2.59 ₁
18.	0.936	2.97	2.43 ₄	0.97	2.91	3.49 ₉	0.98	2.94	3.49 ₉	0.98	2.94	1.79 ₇	2.58 ₇	2.58 ₇	2.58 ₇
19.	0.936	2.82	2.44 ₂	0.93	2.78	3.50 ₅	0.94	2.82	3.50 ₅	0.94	2.82	1.80 ₃	2.59 ₂	2.59 ₂	2.59 ₂
20.	0.916	2.71	2.43 ₆	0.96	2.88	3.50 ₇	0.93	2.79	3.50 ₇	0.93	2.79	1.79 ₉	2.59 ₈	2.59 ₈	2.59 ₈
21.	0.911	2.79	2.43 ₈	0.95	2.84	3.52 ₅	0.81	2.44	3.52 ₅	0.81	2.44	1.80 ₀	2.60 ₆	2.60 ₆	2.60 ₆
Correl. coeff. between															
i) F-Ang. sepn.				r = -0.977			r = -0.984						r = -0.977		r = -0.984
ii) 100F/P ₂ O ₅ -Ang. sepn.				r = -0.972			r = -0.980						r = -0.972		r = -0.980
Standard error of estimate				0.13	0.43		0.11	0.37							

* P₂O₅ content gives a measure of the apatite present in fossil bone. Fluorine/phosphate ratio shows the degree of fluorination of bone and is used to eliminate the problem of contamination by foreign mineral matter. $100F/P_2O_5 = (F\%/P_2O_5\%) \times 100$

† For regression equations, see text.

†† Calculated from the FeK α diffraction angle for (004) and from the FeK α angular separation of 2 θ for (004)-(410) or (004)-(321), using FeK α =1.9373Å and CuK α =1.5418Å.

+ Tusk

in spacing, but has not been dealt with here because the change is to be smaller than those of the former two pairs.

Fig. 2 illustrates that both of the measured angular separations correlate very well to the fluorine content of compact bone samples (including two elephantid tusks); and Fig. 3, to the fluorine/phosphate ratio. The regression equations are:

- 1) of fluorine content on angular separation of 2θ (004)– 2θ (410)
wt. %F = $-5.706 \times AS + 14.86$ ($r = -0.977$)
where AS = 2θ separation concerned (degree) (the same hereinafter)
- 2) of fluorine content on angular separation of 2θ (004)– 2θ (321)
wt. %F = $-6.464 \times AS + 23.60$ ($r = -0.984$)

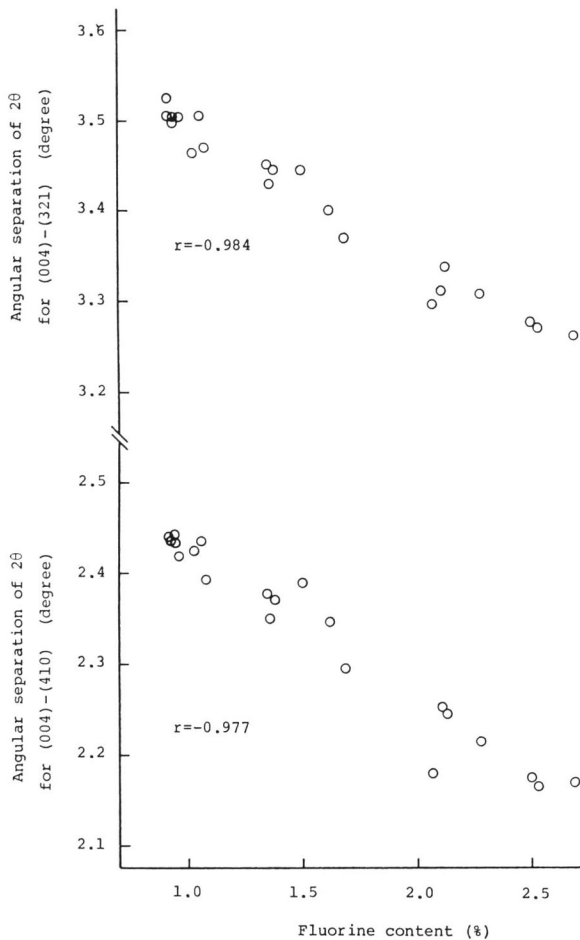


Fig. 2. Plots of the angular separations of $2\theta(004)$ – $2\theta(321)$ and $2\theta(004)$ – $2\theta(410)$ for iron $K\alpha$ radiation versus the fluorine content of compact bone specimens from Sangiran (data given in Table 1).

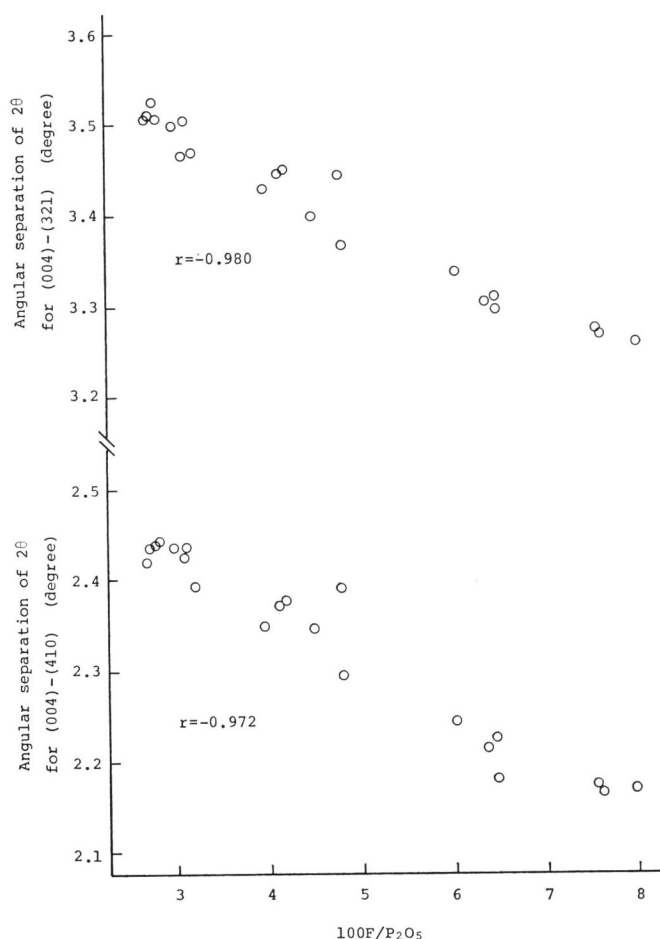


Fig. 3. Plots of the angular separations of $2\theta(004)-2\theta(321)$ and $2\theta(004)-2\theta(410)$ for iron $K\alpha$ radiation versus the $(F\%/P_2O_5\%)\times 100$ ratio of compact bone specimens from Sangiran (data given in Table 1).

- 3) of fluorine/phosphate ratio on angular separation of $2\theta(004)-2\theta(410)$
 $100F/P_2O_5 = -16.91 \times AS + 44.07$ ($r = -0.972$)
- 4) of fluorine/phosphate ratio on angular separation of $2\theta(004)-2\theta(321)$
 $100F/P_2O_5 = -19.14 \times AS + 69.91$ ($r = -0.980$)

The standard error of estimate for each equation is given in Table 1.

The above calibration lines are to hold for compact bones from the Kabuh and Pucangan formations of Sangiran, and presumably of other Pleistocene outcrops in the Kendeng Hills in Java (see Indonesia-Japan Research Cooperation Programme—CTA-41, 1979). They would be of practical utility when limited amounts of sample

available demand the least loss of it, since the X-ray diffraction procedure involves no dissolution of material with the calcination step be acknowledged.

Apart from fluorine, increasing content of carbonate in the apatite molecule yields shortening of the a -axis dimension and lengthening of the c -axis, which also result in a decrease in the angular separations concerned in this investigation. The inorganic phase of bone, fresh or fossil, contains a minor quantity of carbonate, chiefly in the form of $\text{CO}_3\text{OH}^{3-}$ which is likely to exchange for the PO_4^{3-} ions in the crystals (see LEGEROS *et al.*, 1967; BROPHY & NASH, 1968; MATSUBARA, 1980). Although it is quite possible that the carbonate ions are present in a separate phase such as CaCO_3 , the high correlations found in Figs. 2 & 3 seem to suggest that the CO_2 content of the bone apatites in these fossil samples is virtually the same.

Now the diffractometer measurements of the spacing between (004)–(321) on the Sangiran specimens are brought together in Fig. 4 with those on the Japanese specimens presented by TANABE & WATANABE (1968). The former data obtained with iron $K\alpha$ radiation have been so converted as to be comparable with the latter which were

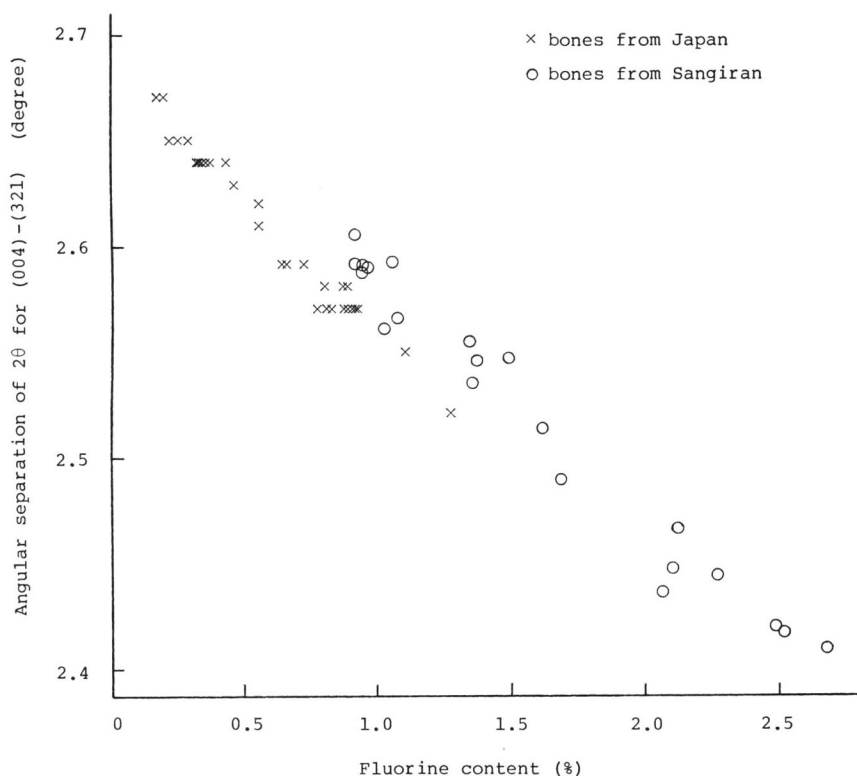


Fig. 4. Correlation between the angular separation of $2\theta(004)-2\theta(321)$ for copper $K\alpha$ radiation and the fluorine content of compact bone specimens. $\times$, data from TANABE & WATANABE (1968); $\circ$, this study (see Table 1).

obtained with copper $K\alpha$ radiation (see the last column and footnote of Table 1). The 2θ (004)– 2θ (321) separation (AS) for Cu $K\alpha$ radiation is related to the fluorine content by the equations:

for prehistoric bones from Japan

$$\text{wt. \%F} = -7.362 \times \text{AS} + 19.79 \quad (r = -0.986) \quad (\text{i})$$

which is derived from data given in TANABE & WATANABE (1968, Table 1) [for '(140)' in that article, read '(321)']

for fossil bones from Sangiran, Java

$$\text{wt. \%F} = -8.716 \times \text{AS} + 23.53 \quad (r = -0.984) \quad (\text{ii})$$

As will be seen from Fig. 4, the separation of the diffraction angles involved that corresponds to a given fluorine content is a little smaller for bones from Japan than for those from Sangiran; or, the fluorine content corresponding to a given angular separation is lower in bones from Japan. The problem of introduction of extraneous mineral grains or soil particles into buried bones may not take part in that bias, since the samples X-ray-analysed in the study by TANABE & WATANABE (1968) as well as in this study are of compact textures. There appear to be the following two possible explanations to account for Fig. 4.

- a) Whereas the vertebrate remains of the Sangiran area had been buried in sediments of limnic and fluvial origin with tuff layers; those of Japan which are concerned with Equation i, in limestone cave-fissure deposits (32 of the 33 specimens) or shell-mound (the remaining one) where the levels of carbonates in circulation should be higher. Uptake, if any, of carbonate ions by bone apatite results in a diminution of the distance between 2θ (004)– 2θ (321) independently of fluoride ions, as mentioned before.
- b) The Sangiran specimens and the Japanese ones are inferred to be respectively of the Lower Pleistocene (see MATSU'URA, 1982, 1983) and of the late Middle Pleistocene to the mid-Holocene. In addition, the former had experienced tropical climates, while the latter, temperate climates. The more remaining of the bone organic matter (to act as a weight in the sample), as is supposed to be the case with the Japanese specimens, may cause a downward shifting of the fluorine content values on the abscissa of Fig. 4.

With all uncertainties as to fluorine assessment, the X-ray diffraction approach would yet provide chronological or stratigraphical information on bone materials preserved in similar matrix at the same site or region.

Criticisms from MCCONNELL (1962) on this physical approach must receive particular attention when we deal with bones having fossilized in unusual geochemical environments such as percolation of ground water rich in chloride, or we treat enamels in company with other bone substances. Still I at least agree with OAKLEY that "Semi-quantitative determination of fluorine [by the X-ray powder diffraction technique] is often adequate for relative dating when a long series of bones is available for comparison" (OAKLEY, 1963, p. 113).

It should be remarked here that this experiment has calibrated, in the same way as that by TANABE & WATANABE (1968), X-ray measurements using a diffractometer to fluorine percentages. The use of a powder-camera might need another calibration because of differences in resolution and manner for measuring the spacing of the diffraction lines (of powder-camera) or peaks (of diffractometer).

Another matter to be noted is that, as BAUD (1960) and MATSUBARA (1982, verbal communication) have pointed out, the recrystallization of bone apatites during calcination (see Experimental section) alters the axial dimensions. Therefore any application or comparison of calibration equations for X-ray analysis requires the uniformity of conditions of the calcination step.

In conclusion, although less precise and reproducible, the X-ray diffraction method for estimating the fluorine content saves sample material from decomposition for analysis, and can be usually a substitute for the chemical fluorine determination in relative-dating of a series of bone specimens. This method will have more practicability, after further studies have substantiated that it gives a convenient measure of the degree of fluorination in the bone apatites even to heavily contaminated samples.

Acknowledgements

I wish to express my appreciation to Dr. N. WATANABE, Professor Emeritus of Faculty of Science, University of Tokyo, for suggestions of this work and encouragement. I am also indebted to Dr. A. KATO and Dr. S. MATSUBARA of Department of Geology, National Science Museum, Tokyo, for helpful discussion and advice, and to Dr. H. SAKURA and Dr. B. YAMAGUCHI of Department of Anthropology of the same Museum, for reading and improving the manuscript.

This work was begun while I was involved in the Indonesia-Japan Joint Research Project CTA-41, co-directed by Dr. N. WATANABE and Dr. D. KADAR; and my particular thanks go to the Geological Research and Development Centre in Bandung, Java, for hospitality and many courtesies.

Literature Cited

- BAUD, C. A., 1957. Détection histochimique quantitative du Fluor dans les tissus osseux. *Acta Histochemica*, **4**: 117-121.
- BAUD, C. A., 1960. Dating of prehistoric bones by radiological and optical methods. *Viking Fund Publications in Anthropology*, **28**: 246-264.
- BROPHY, G. P. & J. T. NASH, 1968. Compositional, infrared, and X-ray analysis of fossil bone. *The American Mineralogist*, **53**: 445-454.
- GLOVER, M. J. & G. F. PHILLIPS, 1965. Chemical methods for the dating of fossils. *Journal of Applied Chemistry*, **15**: 570-576.
- Indonesia-Japan Joint Research Team, 1979. Progress report of the Indonesia-Japan joint research project on geology of human fossil bearing formations in Java (II); Stratigraphy and geological structure in the central part of the Sangiran dome. *Bulletin of the Geological Research and Development Centre*, No. 2: 55-61.

- Indonesia-Japan Research Cooperation Programme—CTA-41, 1979. Progress report of the Indonesia-Japan joint research project on geology of human fossil bearing formations in Java (I). *Bulletin of the Geological Research and Development Centre*, No. 1: 47–60.
- LEGEROS, R. Z., O. R. TRAUTZ, J. P. LEGEROS, E. KLEIN & W. P. SHIRRA, 1967. Apatite crystallites: Effects of carbonate on morphology. *Science*, **155**: 1409–1411.
- MCCONNELL, D., 1962. Dating of fossil bones by the fluorine method. *Science*, **136**: 241–244.
- MATSUBARA, S., 1980. Barite and carbonate-apatite constituting fossil dinosaur bone in sandstone from Berivotra, Madagascar. *Bulletin of the National Science Museum, Tokyo, Ser. C (Geology)*, **6**: 27–31.
- MATSU'URA, S., 1981. Determination of fluorine in fossil bone with an ion-selective electrode. *Bulletin of the National Science Museum, Tokyo, Ser. D (Anthropology)*, **7**: 7–16.
- MATSU'URA, S., 1982. A chronological framing for the Sangiran hominids; Fundamental study by the fluorine dating method. *Bulletin of the National Science Museum, Tokyo, Ser. D (Anthropology)*, **8**: 1–53.
- MATSU'URA, S., 1983 (1984?). A consideration of the stratigraphic positions of hominid finds from Sangiran by the fluorine method. To be published in a Special Publication of the Geological Research and Development Centre, Indonesia.
- MOLLESON, T. I. & K. P. OAKLEY, 1966. Relative antiquity of the Ubeidiya hominid. *Nature*, **209**: 1268.
- NIGGLI, E., C. J. OVERWEEL & I. M. VAN DER VLERK, 1953. An X-ray crystallographical application of the fluorine-dating method of fossil bones. *Proceedings. Koninklijke Nederlandsche Akademie van Wetenschappen, Amsterdam, ser. B*, **56**: 538–542.
- OAKLEY, K. P., 1951. The fluorine-dating method. *Yearbook of Physical Anthropology*, **5**: 44–52.
- OAKLEY, K. P., 1963. Fluorine, uranium, and nitrogen dating of bone. In PYDDOKE, E. (ed.) "The Scientist and Archaeology", J. M. Dent & Sons Ltd., London, 111–119.
- OAKLEY, K. P., 1980. Relative dating of the fossil hominids of Europe. *Bulletin of the British Museum (Natural History), Geology*, **34**: 1–63.
- OAKLEY, K. P. & W. W. HOWELLS, 1961. Age of the skeleton from the Lagow sand pit, Texas. *American Antiquity*, **26**: 543–545.
- OAKLEY, K. P. & M. F. A. MONTAGU, 1949. A reconsideration of the Galley Hill skeleton. *Bulletin of the British Museum (Natural History), Geology*, **1**: 25–46.
- SHIMODA, N. & T. KITAMURA, 1978. The relation between the fluorine content and the age of a bone dug up in foreign countries. *Hokkaido Kokogaku (Archaeology in Hokkaido)*, No. 14: 1–10. (In Japanese with English abstract)
- STÜHLER, R., 1938. Über den Feinbau des Knochens; Eine Röntgen-Feinstruktur-Untersuchung. *Fortschritte auf dem Gebiete des Röntgenstrahlen*, **57**: 231–264.
- TANABE, G., 1962. Fluorine contents of human bones found at Pleistocene deposits of Mikkabi. *Journal of the Anthropological Society of Nippon*, **70**: 41–45. (In Japanese with English abstract)
- TANABE, G., & N. WATANABE, 1968. Dating fossil bones from Japan by means of X-ray diffraction pattern. *Journal of the Faculty of Science, University of Tokyo, Sec. V*, **3**: 199–216.