Calibration of Diffractometer Measurement for Fluorine Determination of Fossil Bones from Sangiran
—A Reappraisal of X-ray Diffractiometric Application to the Fluorine Dating—*

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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), spectro-photometry (Glover & Phillips, 1965), thorium nitrate titration (Shimoda & Kita-mura, 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 comsumption 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

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* 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, $1°$ 2θ/min; time constant, 10 sec; chart-drive rate, 20 mm/degree.

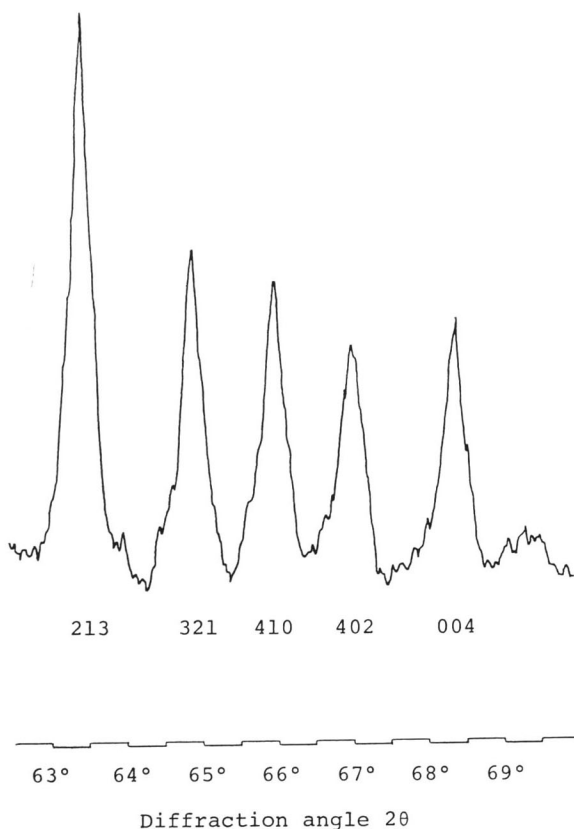On the diagram obtained (see Fig. 1), the distance of 2θ is measured between (004)

213   321   410   402   004

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

63°   64°   65°   66°   67°   68°   69°

Diffraction angle 2θ

and (410), and between (004) and (321), using a glass ruler graduated in 0.1 mm with the aid of a 15× 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		FeKα, 2θ(004)–2θ(410)			FeKα, 2θ(004)–2θ(321)			CuKα 2θ(004)–2θ(410)	CuKα 2θ(004)–2θ(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†	Ang. sepn. calc.†† (degree)	Ang. sepn. calc.†† (degree)
1.	2.68	7.98	2.16_0	2.48	7.39	3.26_0	2.53	7.51	1.60_1	2.41_0
2.	2.52	7.59	2.16_5	2.51	7.46	3.27_0	2.46	7.32	1.59_8	2.41_7
3.	2.49	7.55	2.17_4	2.46	7.31	3.27_5	2.43	7.23	1.60_5	2.42_1
4.	2.27	6.36	2.21_4	2.23	6.63	3.30_6	2.23	6.63	1.63_4	2.44_4
5.	2.12	6.01	2.24_5	2.05	6.11	3.33_7	2.03	6.04	1.65_7	2.46_7
6.	2.10	6.44	2.25_2	2.01	5.99	3.31_1	2.20	6.54	1.66_3	2.44_8
7.	2.06	6.46	2.17_0	2.43	7.22	3.29_6	2.29	6.82	1.60_0	2.43_7
8.+	1.68	4.80	2.29_4	1.77	5.28	3.36_8	1.83	5.45	1.69_4	2.49_0
9.+	1.61	4.48	2.34_6	1.47	4.40	3.40_4	1.62	4.83	1.73_2	2.51_4
10.	1.49	4.78	2.38_0	1.23	3.67	3.44_4	1.34	3.99	1.76_4	2.54_7
11.	1.37	4.11	2.37_0	1.34	3.99	3.44_4	1.34	3.99	1.75_0	2.54_6
12.	1.35	3.94	2.34_0	1.46	4.35	3.43_0	1.43	4.26	1.73_4	2.53_6
13.	1.34	4.17	2.37_7	1.30	3.87	3.45_0	1.30	3.88	1.75_5	2.55_1
14.	1.07	3.19	2.39_8	1.21	3.60	3.47_1	1.16	3.48	1.76_7	2.56_7
15.	1.05	3.10	2.43_6	0.96	2.88	3.50_6	0.94	2.81	1.79_0	2.59_3
16.	1.02	3.07	2.42_4	1.03	3.08	3.46_4	1.21	3.61	1.79_0	2.56_1
17.	0.956	2.67	2.41_0	1.06	3.16	3.50_5	0.94	2.82	1.78_6	2.59_1
18.	0.936	2.97	2.43_4	0.97	2.91	3.49_9	0.98	2.94	1.79_7	2.58_7
19.	0.936	2.82	2.44_2	0.93	2.78	3.50_5	0.94	2.82	1.80_3	2.59_2
20.	0.916	2.71	2.43_6	0.96	2.88	3.50_7	0.93	2.79	1.79_9	2.59_3
21.	0.911	2.79	2.43_8	0.95	2.84	3.52_5	0.81	2.44	1.80_0	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			

* P$_2$O$_5$ 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$_2$O$_5$=(F%/P$_2$O$_5$%)×100
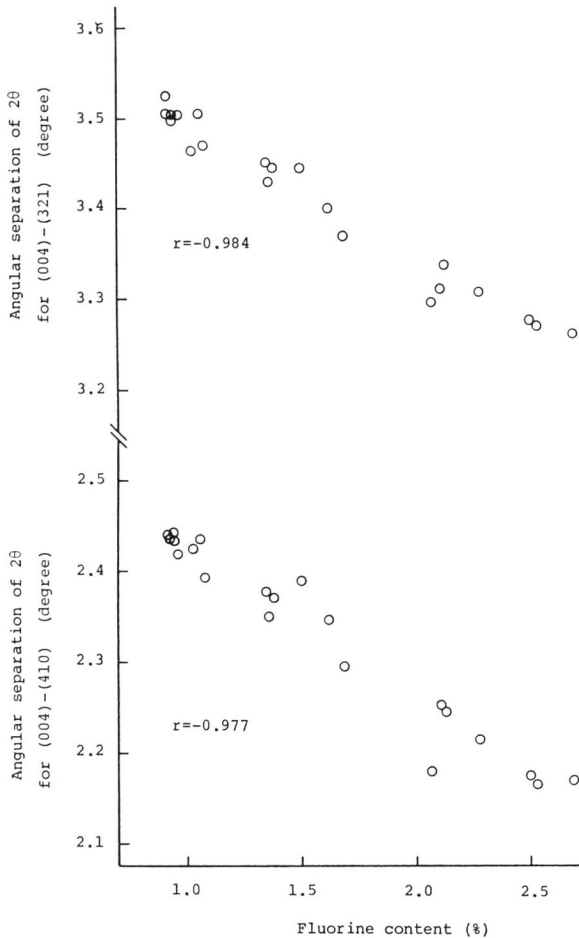
† For regression equations, see text.

†† Calculated from the FeKα diffraction angle for (004) and from the FeKα angular separation of 2θ for (004)–(410) or (004)–(321), using FeKα=1.9373Å and CuKα=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$=2\theta$ separation concerned (degree) (the same hereinafter)

2) of fluorine content on angular separation of 2θ (004)–2θ (321)
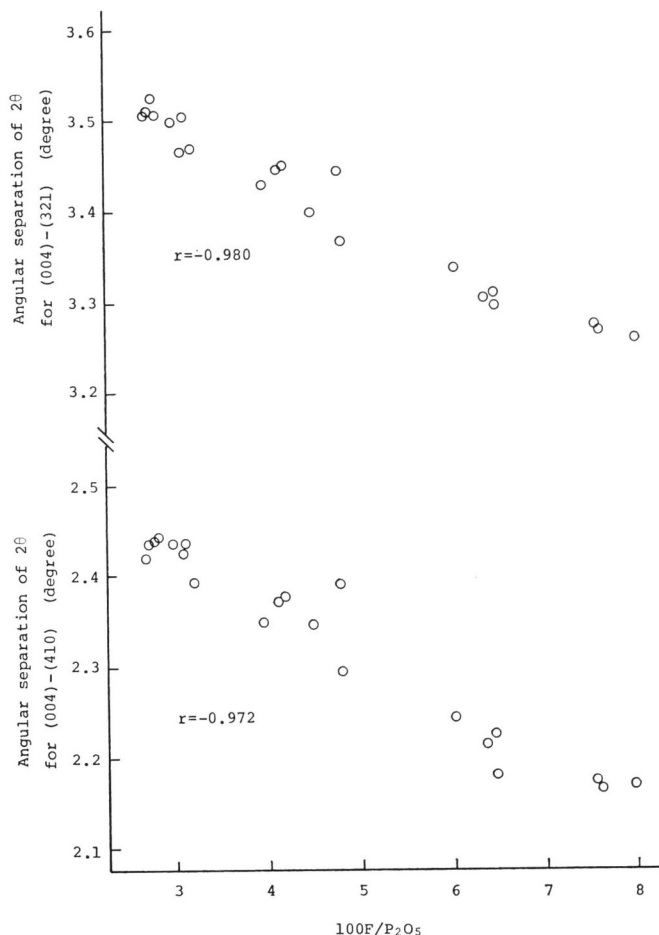
wt. %F$=-6.464\times AS+23.60$ (r$=-0.984$)

Fig. 2. Plots of the angular separations of $2\theta(004)$–$2\theta(321)$ and $2\theta(004)$–$2\theta(410)$ for iron Kα 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 2θ (004)–2θ (410)
   $$100F/P_2O_5 = -16.91 \times AS + 44.07 \quad (r = -0.972)$$
4) of fluorine/phosphate ratio on angular separation of 2θ (004)–2θ (321)
   $$100F/P_2O_5 = -19.14 \times AS + 69.91 \quad (r = -0.980)$$

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 CO_3OH^{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 Kα radiation have been so converted as to be comparable with the latter which were
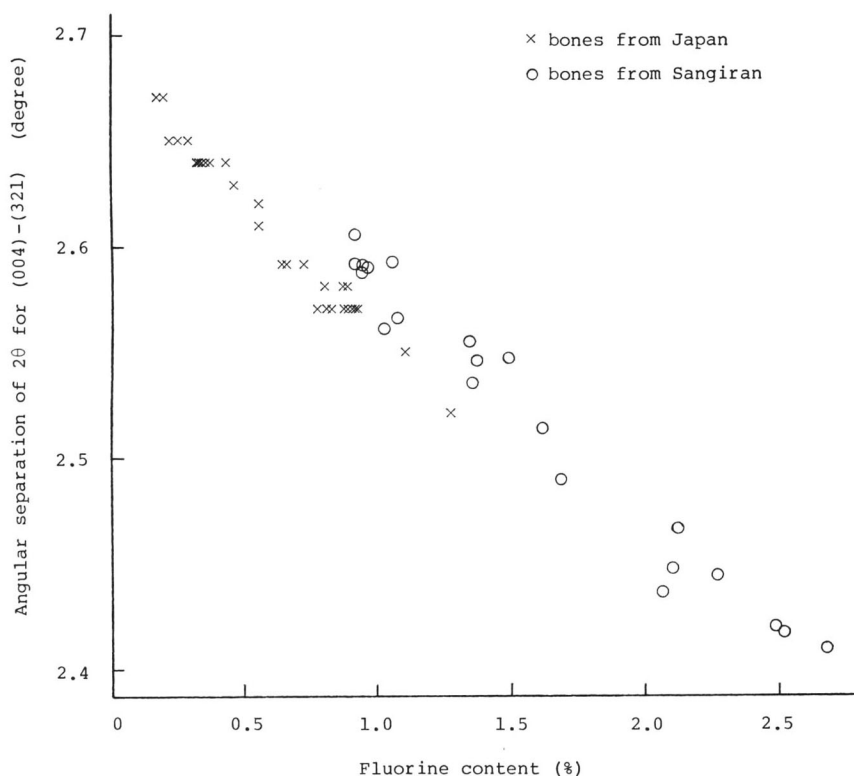

Fig. 4.   Correlation between the angular separation of $2\theta(004)$–$2\theta(321)$ for copper Kα radiation and the fluorine content of compact bone specimens.   ×, data from Tanabe & Watanabe (1968); ○, this study (see Table 1).

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

    for prehistoric bones from Japan

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

        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

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

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 "Semiquantitative 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 recrystalization 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

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