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Feasibility of Dating Archaeological Bones from Japan Using the Aspartic Acid Racemization in Peptides

By

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Introduction

Of the chemical methods of dating fossil bones F-U-N (fluorine, uranium, nitrogen) analyses have played a main part (see *e.g.* OAKLEY, 1969). The accumulation of fluorine and uranium, and the depletion of nitrogen (*i.e.* the degradation of collagen) with the lapse of time provide criteria for estimating the relative ages of a series of bones which have experienced the same environmental conditions (OAKLEY, 1951, 1958, 1974; BERGMAN & KARSTEN, 1952; TANABE, 1967; SHIMODA, 1977; MATSU'URA, 1978; and others).

In addition to these accumulative or degradative reactions, a non-degradative reaction termed racemization, in which the L-amino acids are reversibly converted into the corresponding D-amino acids, received attention (HARE & MITTERER, 1967; HARE & ABELSON, 1968). This reaction proceeds in geological environments at such a slow rate as to form a geochronometer, and it has been proved in the last several years that the extent of racemization of amino acids provides valuable information on the absolute chronology of various fossil materials (BADA & SCHROEDER, 1975; SCHROEDER & BADA, 1976; MILLER, *et al.*, 1979). In bone, aspartic acid has been the subject of extensive investigations, and the D/L ratio of this amino acid has been used to estimate ages of human skeletal remains (BADA & PROTSCH, 1973; BADA, SCHROEDER, PROTSCH & BERGER, 1974; BADA, SCHROEDER & CARTER, 1974; BISCHOFF & CHILDERS, 1979; IKE, *et al.*, 1979). These estimates are, however, based on the degree of racemization determined for total remnant aspartic acid which may be present in various forms (protein, peptide and free amino acid).

Earlier works by BADA (1972) and by DUNGWORTH, *et al.* (1974) have shown respectively that the non-dialysable material in a heated modern sample has a lower racemization rate (isoleucine) than the total organic matrix, and that free amino acids in fossil bone are more highly racemized than are bound (HCl-insoluble) amino acids. Likewise, recently MATSU'URA & UETA (1980) discussed a possible fluctuation of aspartic acid racemization age for a given bone specimen as caused by the variability of the form in which the amino acid exists, and suggested:

(a) The degree of racemization in the total amino acid fraction could be dependent on the dynamics of collagen decomposition, or on the ratio of partially

degradated to pure collagen. (This dependency was also argued by HEDGES & WALLACE, 1978.)

(b) The aspartic acid racemization for the HCl-insoluble fraction appears to be a basis to give reliable age estimates for older fossil bones.

(c) A measurement of the extent of racemization on the HCl-soluble fraction (largely of peptides with collagenous origin), to which little attention has hitherto been paid, should have chronological significance particularly for younger fossils.

This report gives an assessment of the above suggestion (c).

Materials

Listed in Table 1 are the localities and descriptions of the bone specimens used.

The Kidosaku shellmounds have been assigned to the Horinouchi pottery phase of beginning Late Jōmon on the basis of their association with a number of potsherds exclusively of the Horinouchi pottery style (mainly, Horinouchi I style) (GŌDA & KURIMOTO, 1979). There are no reasonable doubts in that the animal bones, KS–1 to 4, are of this age.

Arayashiki-Site A is located on the outskirts of the shellmound and on a gentle slope. This site apparently had undergone post-dipositional disturbances, *e.g.* cultivation or reclamation, and the shell-layer containing artifacts and animal bones is ascribed to drifting and reaccumulation (NAKAYAMA & MORI, 1976). The potsherds found in Site A spanned the time from the Kurohama pottery phase of middle Early Jōmon to the Angyō (I & II) pottery phase of ending Late Jōmon (MORI & NAKAYAMA, 1976), roughly from 5,500 to 3,100 years ago. However, it was inferred by the results of uranium analysis using fission track techniques (MATSU'URA, 1976) that one specimen (AR–12) used in this study was probably a younger intrusion to the site whereas the other two (AR–11 & AR–16) were referred to Jōmon Period.

Table 1. Bones for amino acid analysis.

Code	Taxon	Bone type	Note
1. Kidosaku shellmounds, Chiba City; 140°10′29″E, 35°33′04″N			
KS–1	*Cervus nippon* T.	metacarpus	from Kidosaku shellmound II
KS–2	—	metatarsus	—
KS–3	—	metacarpus	from Kidosaku shellmound V
KS–4	—	metatarsus	—
2. Arayashiki shellmound, Chiba City; 140°08′55″E, 35°37′05″N			
AR–11	*Equus caballus* L.	metatarsus	from Exc. Site A; Reg. No. 1*
AR–12	*Equus caballus* L.?	tibia?	— ; Reg. No. 2
AR–16	*Cervus nippon* T.	humerus	— ; Reg. No. 5

* Registered number in Table & Fig. in MATSU'URA, 1976.

Experimental

Isolation of amino acids

A small piece (~0.2 g) of transverse section of *Substantia compacta* was cut from each bone specimen with a fret saw. The outer layers were scraped away and then the bone pieces were carefully cleaned by ultrasonication in 0.1 M HCl, ether, double-distilled water and methanol. After being dried under reduced pressure, the bone samples were pulverized in an agate mortar and dissolved in 1 M HCl. Amino acid fractions were isolated using the procedure shown in Fig. 1 unless otherwise mentioned in the text.

It should be noted that the amino acids in Sp fraction are present almost entirely in peptide form because free amino acids comprise a small and negligible component of fossil bones, which would be due to removal of free amino acids by ground water leaching and/or by the ultrasonic cleansing (SCHROEDER & BADA, 1976; MATSU'URA & UETA, 1980).

Derivatization of amino acid isolates

To the dried amino acid extract was added 10 ml of 5% HCl/Methanol. After 1 hour at room temperature the mixture was stored at −20°C for periodic analysis.

An aliquot of each mixture was evaporated to dryness in a screwcap vial with

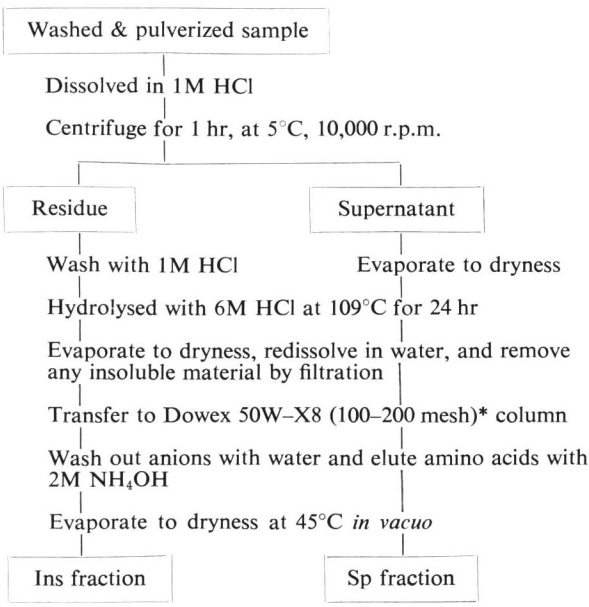

Fig. 1. Fractionation of remnant amino acids in fossil bone. Ins, insoluble (protein); Sp, soluble peptide-free amino acid.

* Prewashed sequentially with 2M NH_4OH, water, 4M HCl, water, 2M NaOH, water, 4M HCl and finally water.

a nitrogen stream. Two ml 5% HCl/Isopropanol was added to the residue and the vial was sealed tightly with a Teflon-lined cap. The transesterification was effected at 100°C for 3 hours.

After removal of the excess HCl/Isopropanol, 100 μl of cooled dichloromethane and 60 μl trifluoroacetic anhydride (−20°C) was added, and then the vial was capped. The reaction mixture was stirred for 30 seconds and allowed to react for 1 hour at room temperature. The solvent was carefully evaporated under a gentle stream of nitrogen. The resulting N-trifluoroacetyl (TFA) amino acid isopropyl esters were dissolved in chloroform and subjected to the enantiomeric analysis by gas-liquid chromatography.

Gas-liquid chromatographic analysis

A Shimadzu Model GC–6A gas chromatograph equipped with an attachment for capillary column and a flame ionization detector, was used. Separation of enantiomeric derivatives of amino acids was achieved on i) a 50 m×0.25 mm i.d. glass capillary coated with N-lauroyl-L-valine *tert*-butylamide (FEIBUSH, 1971; BEITLER & FEIBUSH, 1976) or ii) a 30 m×0.28 mm i.d. glass capillary coated with N,N′-[2,4-(6-ethoxy-s-triazine)diyl]-bis-(L-valyl-L-valine isopropyl ester) (ŌI, 1978; MATSU'URA & UETA, 1980). Operational conditions were as follows:

for column i), temperature 125°C or 130°C isothermal, injector and detector 200°C, carrier gas He at a pressure of 13 p.s.i.;

for column ii), temperature 130°C or 135°C isothermal, injector and detector 200°C, He pressure 7 p.s.i.

The ratios of D to L-amino acid enantiomers were found from the measurements of their peak areas. The identification of the peaks for alanine and aspartic acid enantiomers was confirmed by the use of an LKB 9000 gas chromatograph-mass spectrometer.

Results and Discussion

Figs. 2 and 3 display chromatograms obtained for the Ins fraction in AR–16 and for the Sp fraction in KS–4, respectively. Table 2 gives the analytical results of D/L aspartic acid ratios for the animal bones from Arayashiki-Site A. The low D/L ratios for both the Ins and the Sp fractions in AR–12 support the provisional conclusion by MATSU'URA (1976) that this bone may be a later (younger) intrusion.

As Table 2 shows, the amino acids in peptide form have higher D/L Asp ratios than those bound in protein; this result is consistent with that observed in foraminifera (BADA & SCHROEDER, 1972), in shell (AKIYAMA, 1975) or in fossil bone (MATSU'URA & UETA, 1980). For the dating of younger fossil bones as is the case with this study, the Sp fraction seems to be effective because of its higher degree of racemization, while in using the Ins fraction any small error involved in the precision of the D/L determination (2–5%) would produce a relatively large error in a calculated age.

The interconversion of aspartic acid enantiomers is a reversible first-order reaction, represented by:

$$\mathrm{L} \underset{k}{\overset{k}{\rightleftharpoons}} \mathrm{D}$$

where L and D represent the concentrations of L- and D-aspartic acids respectively, and k is the reaction rate constant. Let the initial L-Asp concentration be L_0 units of concentration and the initial D-Asp concentration be very small ($\mathrm{D}_0 \fallingdotseq 0$). The concentration of D-Asp at time t is thus to be $(\mathrm{L}_0 - \mathrm{L})$ units of concentration.

The rate of reaction (disappearance of L-Asp) at time t is given by

$$-\frac{d\mathrm{L}}{dt} = k\mathrm{L} - k(\mathrm{L}_0 - \mathrm{L}) ,$$

which yields the integrated expression as a function of time (t):

$$-\ln\left\{\frac{\mathrm{L}-\mathrm{D}}{2}\right\} = 2kt + \text{constant} . \qquad \text{(i)}$$

When $t=0$, this equation reads

$$\text{constant} = -\ln\left\{\frac{\mathrm{L}_0 - \mathrm{D}_0}{2}\right\}$$

$$= -\ln\left\{\frac{\mathrm{L}+\mathrm{D}}{2}\right\} .$$

Therefore, equation (i) may be rearranged as

$$\ln\left\{\frac{1+\mathrm{D/L}}{1-\mathrm{D/L}}\right\} = 2kt . \qquad \text{(ii)}$$

However, the logarithmic term in equation (ii) should be corrected for the small amount of racemization that takes place during sample preparation procedures (acid hydrolysis step). Then, the racemization age equation is written as

$$\ln\left\{\frac{1+\mathrm{D/L}}{1-\mathrm{D/L}}\right\}_t - \text{constant} = 2kt . \qquad \text{(iii)}$$

The value of this constant can be found from a) the analysis of a modern bone carried through the same procedures as the fossil bone specimens (BADA & PROTSCH, 1973) or b) hydrolysing a fossil bone (amino acid fraction involved) for a set of different periods of time. The latter procedure is to be recommended since some amounts

Table 2. Aspartic acid racemization in bone samples from the Arayashiki shellmound.

Sample	D/L Asp	
	Ins fraction	Sp fraction
AR-11 (horse)	0.094	0.198
AR-12 (horse?)	0.074	0.164
AR-16 (deer)	0.093	0.219

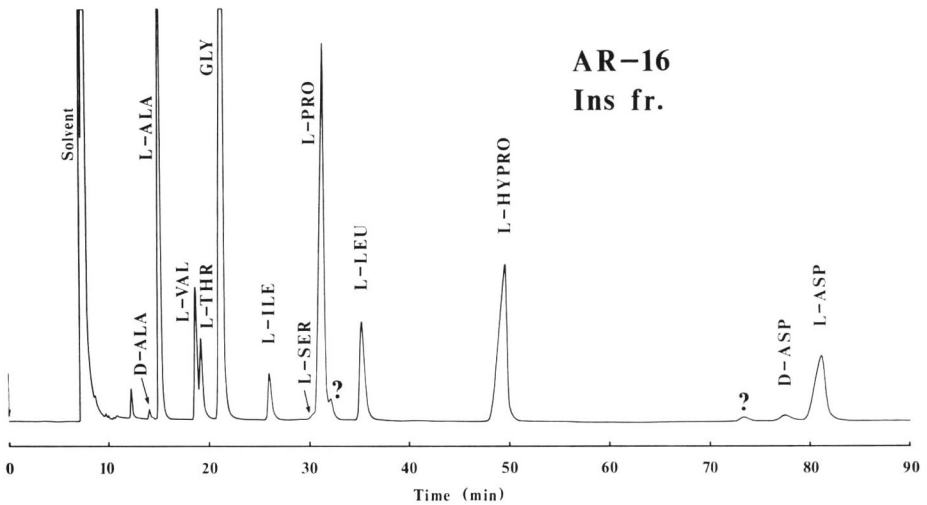

Fig. 2. Gas chromatogram of N-TFA isopropyl esters of amino acids in the Ins fraction isolated from the sample AR–16 from the Arayashiki shellmound. Chromatography: 50 m×0.25 mm i.d. column coated with N-lauroyl-L-valine *tert*-butylamide; temperature, 125°C isothermal; He pressure, 13 p.s.i.; FID detector.

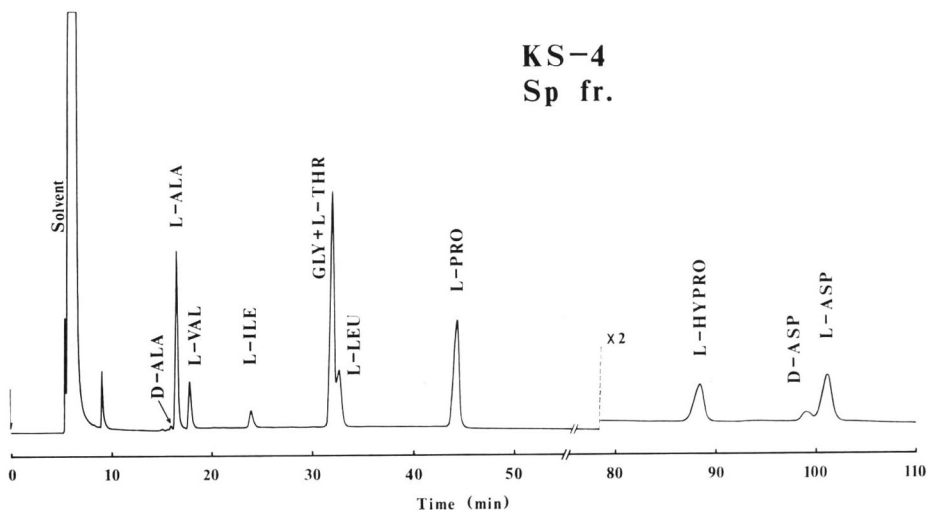

Fig. 3. Gas chromatogram of N-TFA isopropyl esters of amino acids in the Sp fraction recovered from the sample KS–4 from the Kidosaku shellmounds. Chromatography: 30 m×0.28 mm i.d. column coated with N,N′-[2,4-(6-ethoxy-s-triazine)diyl]-bis-(L-valyl-L-valine isopropyl ester); temperature, 130°C isothermal; He pressure, 7 p.s.i.; FID detector.

of noncollagen proteins, which will be broken down rapidly with increasing time of burial, are present in modern bone (Ho, 1965; Hedges & Wallace, 1978; and others).

Fig. 4 represents the racemization kinetics of aspartic acid in Sp fraction in 6 M HCl at 109°C. The constant term in the aspartic acid racemization age equation (iii) can be determined from Fig. 4 by reading off the value of

$$\ln\left\{\frac{1+\mathrm{D/L}}{1-\mathrm{D/L}}\right\}_{t=24\mathrm{hr}} - \ln\left\{\frac{1+\mathrm{D/L}}{1-\mathrm{D/L}}\right\}_{t=0}$$

for each set. This gives 0.135 and 0.145 for AR-16 and AR-11 respectively. However, the extrapolation of the result in Fig. 4 to $t=0$ must be viewed with some caution, since the net racemization kinetics for the Sp fraction during hydrolysis step is a combination of those for the peptides and for the free amino acids formed by hydrolysis. Nevertheless, this inaccuracy involved may generate a negligible error in calculating the racemization age for Sp fraction with setting the constant term in equation (iii) to be 0.14.

A value of k for a fossil bone from a particular site is determined by measuring the extent of racemization in a bone of known age from that site; this step was designated as 'calibration' procedure (Bada & Protsch, 1973). It has been suggested by Bada & Helfman (1975), Bada & Protsch (1973), Bada & Shou (1980) and others that once an *in situ* k value has been determined by the 'calibration' procedure, it

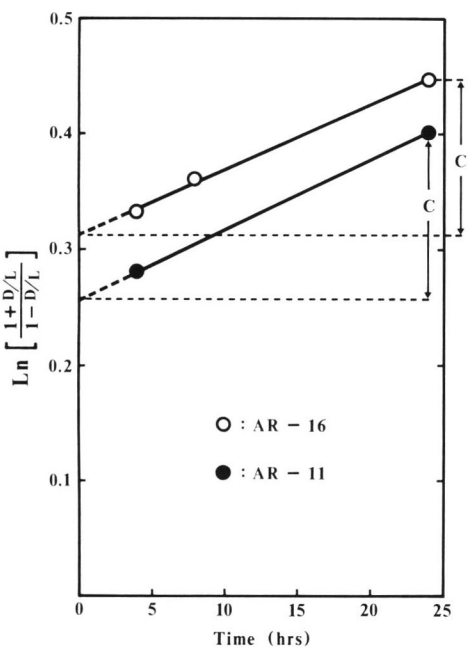

Fig. 4. Racemization kinetics of aspartic acid in Sp fractions (see Fig. 1) under hydrolytic conditions (6M HCl, 109°C). C: constant for the racemization age equation (iii), see text.

Table 3. Aspartic acid racemization in Sp fractions of deer bones from the Kidosaku shellmounds.

Sample	D/L Asp	Rate constant (k) yr^{-1}
KS–1	0.199	3.41×10^{-5}
KS–2	0.220	3.98×10^{-5}
KS–3	0.205	3.57×10^{-5}
KS–4	0.214	3.82×10^{-5}
mean	0.210	3.70×10^{-5}
s.d.	0.009	0.25×10^{-5}
*s.e./x**	0.021	0.034

* Standard error of mean divided by the mean.

can be used to racemization-date other bones from the general area with the same temperature of the 'calibration' site, because temperature is the major factor affecting amino acid racemization whereas other factors have inconsiderable effects on racemization rates. The Kidosaku site, which was employed for 'calibration' of the racemization rate, is located in the immediate vicinity of Arayashiki (only about 8 km distant), and the mean annual temperatures of the two sites are virtually identical.

Table 3 includes the D/L aspartic acid ratios for the Sp fractions in the bone specimens from Kidosaku. The high degree of inter-specimen consistency of the D/L ratios indicates that the site 'calibration' procedure has good reproducibility. Radiocarbon dates for the cultural phase of Horinouchi I pottery style are available, being 3,940$\pm$105 yr BP (N–1429) and 3,780$\pm$150 yr BP (N–59). Substitution of 3,860 yr (mean of these two ^{14}C-dates) for t, and the other relevant date in equation (iii) gives the k values listed in Table 3. Preliminary analyses of main components of amino acids isolates suggested that the Sp fractions in KS–1 to 4 are of collagenous origin, and that those in AR–11, 12 & 16 are less collagenous in composition. This was in agreement with the finding that the Sp fractions in bones from Arayashiki yield anomalously high D/L ratios for alanine ($\sim$0.14) probably due to bacterial activity, compared with the low D/L-Ala ratios for the Sp fractions of KS–1$\sim$4 ($\sim$0.03) and for the Ins fractions of AR–11, 12 & 16 ($<$0.02). Still, this effect would have no direct bearing on the validity of aspartic acid racemization-deduced ages (see KESSELS & DUNGWORTH, 1980).

Substituting 3.70×10^{-5} for k along with the D/L-Asp ratios for the Sp fractions in Arayashiki samples into equation (iii), we have the aspartic acid-derived ages:

3,530 yr BP for AR–11
2,580 yr BP for AR–12
4,120 yr BP for AR–16.

Of particular interest is the date for the AR–11 horse remain, which is to be assigned to Late Jōmon period. Archaeological evidences (*e.g.* HAYASHIDA, 1956; YOSHIKURA, 1975) suggested that ancient horses of Japan, classified as 'small sized (HAYASHIDA, 1956)', came into appearance in Late Jōmon. As the horse remain of

AR–11 is of small size (MATSU'URA, 1976), this specimen would be an additional evidence in this connection.

The specimen of AR–12 can be tentatively attributed to an intrusion of the beginning of Early Yayoi period. However, it was suggested by MATSU'URA & UETA (1980) that continuous decomposition of collagen is supposed to take place in fossil bone, reflected by the increasing proportion of aspartic acid in peptide to protein form in longer-buried bones, and that the resulting effect of this introduction of the collagen-breakdown product (less racemized) into the Sp fraction may cause a deviation from first order kinetics for the Sp fraction in geological environments. This deviation would induce the lower (younger) age estimate for a bone with an age much older than that of the calibration sample, and *vice versa*. The racemization-deduced age for AR–12, hence, is to be interpreted as a maximum estimate.

Conclusions

The instances presented here lead me to maintain that the extent of aspartic acid racemization in peptides extracted from a fossil bone could give a useful clue for estimating the age of the specimen. This method may have potential practicability to date bones from Holocene or Upper Pleistocene deposits in Japan with a suitable calibration sample used, since the magnitude of the half-life ($T_{1/2}=\ln 2/2k$, or the time required for the D/L ratio to reach 1/3, in the case of amino acid with a single asymmetric carbon) for aspartic acid racemization in Sp fraction would have an order of 10^3 to 10^4 years which is expected from MATSU'URA & UETA (1980) and this work.

The peptide racemization in bone would also provide a promising approach to pick out any later intrusion or burial of skeletal remains into an archaeological deposit; this approach will rule out an unexpected bias and misleading conclusions to the study of, for example, microevolutional changes in a particular population or origins of domestic animals.

Experiments and applications to elucidate the validity of these arguments would need to be conducted on a much larger scale.

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Literature Cited

AKIYAMA, M., 1975. Racemization of isoleucine and a possible application to geology. *Earth Sci.*, **29**: 161–165. (In Japanese with English abstract)

BADA, J. L., 1972. The dating of fossil bones using the racemization of isoleucine. *Earth Planet. Sci. Lett.*, **15**: 223–231.

BADA, J. L. & P. M. HELFMAN, 1975. Amino acid racemization dating of fossil bones. *World Archaeol.*, **7**: 160–173.

BADA, J. L. & R. PROTSCH, 1973. Racemization reaction of aspartic acid and its use in dating fossil bones. *Proc. natn. Acad. Sci., U.S.A.*, **70**: 1331–1334.

BADA, J. L. & R. A. SCHROEDER, 1972. Racemization of isoleucine in calcareous marine sediments: kinetics and mechanism. *Earth Planet. Sci. Lett.*, **15**: 1–11.

BADA, J. L. & R. A. SCHROEDER, 1975. Amino acid racemization reactions and their geochemical implications. *Naturwissenschaften*, **62**: 71–79.

BADA, J. L., R. A. SCHROEDER & G. F. CARTER, 1974. New evidence for the antiquity of man in North America deduced from aspartic acid racemization. *Science*, **184**: 791–793.

BADA, J. L., R. A. SCHROEDER, R. PROTSCH & R. BERGER, 1974. Concordance of collagen-based radiocarbon and aspartic-acid racemization ages. *Proc. natn. Acad. Sci., U.S.A.*, **71**: 914–917.

BADA, J. L. & M.-Y. SHOU, 1980. Kinetics and mechanisms of amino acid racemization in aqueous solution and in bones. In: (P. E. HARE, T. C. HOERING & K. KING, JR., eds.) Biogeochemistry of Amino Acids. 235–255. New York, John Wiley & Sons.

BEITLER, U. & B. FEIBUSH, 1976. Interaction between asymmetric solutes and solvents—Diamides derived from L-valine as stationary phases in gas-liquid partition chromatography. *J. Chromatography*, **123**: 149–166.

BERGMAN, R. A. M. & P. KARSTEN, 1952. The fluorine content of Pithecanthropus and of other specimens from the Trinil fauna. *Proc. K. Ned. Akad. Wet., Amsterdam, ser. B*, **55**: 150–152.

BISCHOFF, J. L. & W. M. CHILDERS, 1979. Temperature calibration of amino acid racemization: age implications for the Yuha skeleton. *Earth Planet. Sci. Lett.*, **45**: 172–180.

DUNGWORTH, G., N. J. VINCKEN & A. W. SCHWARTZ, 1974. Compositions of fossil collagens: analysis by gas-liquid chromatography. *Comp. Biochem. Physiol.*, **47B**: 391–399.

FEIBUSH, B., 1971. Interaction between asymmetric solutes and solvents. N-Lauroyl-L-valyl-*t*-butylamide as stationary phase in gas liquid partition chromatography. *Chem. Commun., 1971*, 544–545.

GŌDA, R. & Y. KURIMOTO, 1979. Jōmon jidai no kaizuka to ibutsu (Shellmounds of Jōmon Period and the archaeological relics). In: Chiba Southeast New Town 7; Kidosaku Site, 2nd Survey. 38–357. Chiba, The Cultural Properties Center of Chiba Prefecture. (In Japanese)

HARE, P. E. & P. H. ABELSON, 1968. Racemization of amino acids in fossil shells. *Carnegie Inst. Washington, Yearb.*, **66**: 526–528.

HARE, P. E. & R. M. MITTERER, 1967. Nonprotein amino acids in fossil shells. *ibid.*, **65**: 362–364.

HAYASHIDA, S., 1956. Observations on the ancient horses of Japan. *J. Anthrop. Soc. Nippon*, **64**: 197–211. (In Japanese with English summary)

HEDGES, R. E. M. & C. J. A. WALLACE, 1978. The survival of biochemical information in archaeological bone. *J. Archaeol. Sci.*, **5**: 377–386.

HO, T.-Y., 1965. The amino acid composition of bone and tooth proteins in late pleistocene mammals. *Proc. natn. Acad. Sci., U.S.A.*, **54**: 26–31.

IKE, D., J. L. BADA, P. M. MASTERS, G. KENNEDY & J. C. VOGEL, 1979. Aspartic acid racemization and radiocarbon dating of an early milling stone horizon burial in California. *Am. Antiq.*, **44**: 524–530.

KESSELS, H. J. & G. DUNGWORTH, 1980. Necessity of reporting amino acid compositions of fossil bones where racemization analyses are used for geochronological applications: in homogeneities of D/L amino acids in fossil bones. In: (P. E. HARE, T. C. HOERING & K. KING, JR., eds.) Biogeochemistry of Amino Acids: 527–541. New York, John Wiley & Sons.

MATSU'URA, S., 1976. The concentration and distribution of uranium in animal bones from the Arayashiki shellmound. In: Arayashiki Shellmound in Chiba City. 70–75. Chiba, The Cultural Properties Center of Chiba Prefecture. (In Japanese)

MATSU'URA, S., 1978. Uranium analysis of fossil bones using fission track techniques and its applications to archaeological science. *Quat. Res. (Japan)*, **17**: 95–104. (In Japanese with English abstract)

MATSU'URA, S. & N. UETA, 1980. Fraction dependent variation of aspartic acid racemization age of fossil bone. *Nature*, **286**: 883–884.

MILLER, G. H., J. T. HOLLIN & J. T. ANDREWS, 1979. Aminostratigraphy of UK pleistocene deposits. *Nature*, **281**: 539–543.

MORI, N. & Y. NAKAYAMA, 1976. Ibutsu (Archaeological relics). In: Arayashiki Shellmound in Chiba City. 27–49. Chiba, The Cultural Properties Center of Chiba City. (In Japanese)

NAKAYAMA, Y. & N. MORI, 1976. Chōsa no keika to gaiyō (Process of the investigation and an outline). *ibid.*, 15–22. (In Japanese)

OAKLEY, K. P., 1951. The fluorine-dating method. *Yearb. Phys. Anthropol.*, **5**: 44–52.

OAKLEY, K. P., 1958. Das Alter des Schädels von Rhünda. II; Application of fluorine, uranium and nitrogen analysis to the relative dating of the Rhünda skull. *N. Jahrb. Geol. Paläontol. Monaths.*, **3**: 130–136.

OAKLEY, K. P., 1969. Analytical method of dating bones. In: (D. BROTHWELL & E. HIGGS eds.) Science in Archaeology, 2nd ed. 35–45. London, Thames & Hudson.

OAKLEY, K. P., 1974. Revised dating of the Kanjera hominids. *J. Hum. Evol.*, **3**: 257–258.

ŌI, N., 1978. Optically active stationary phases. In: (T. TAKEUCHI & S. TSUGE eds.) Recent Developments in Gas Chromatography. *J. Jap. Chem., Spec. Issue*, **120**: 39–46. (In Japanese)

SCHROEDER, R. A. & J. L. BADA, 1976. A review of the geochemical applications of the amino acid racemization reaction. *Earth Sci. Rev.*, **12**: 347–391.

SHIMODA, N., 1977. On the form of the relative curve between the content of the manganese and the fluorine and the age of bones. *Hokkaido Kōkogaku (Archaeology in Hokkaidō)*, **13**: 1–12. (In Japanese with English abstract)

TANABE, G., 1967. Dating fossil bones by fluorine analysis. *Quat. Res. (Japan)*, **6**: 164–167. (In Japanese with English abstract)

YOSHIKURA, M., 1975. Tsukahara Kofun-gun Shutsudo no Bashi (Horse teeth from the ancient tombs in Tsukahara). *Reports on the Investigations of Cultural Properties in Kumamoto Prefecture*, **16**: 323–337. (In Japanese)