[RADIOCARBON, VOL. 35, NO. 3, 1993, P. 387–391]

AN IMPROVED METHOD FOR RADIOCARBON DATING FOSSIL BONES

KH. A. ARSLANOV

Geographical Research Institute, St. Petersburg State University, Sredniy Prospect 41
St. Petersburg 199004 Russia

and

YU. S. SVEZHENTSEV

Institute of Material Culture of the Russian Academy of Sciences, Dvorts-ovaya Nabezhnaya 18
St. Petersburg 199000 Russia

ABSTRACT. We demonstrate here that the use of either the acid-alkali or Longin method alone does not fully extract contaminants from bone-collagen fractions. We present our methods and results of extracting bone collagen that involve successive use of both of these methods.

INTRODUCTION

Radiocarbon dating of fossil bones, including, for example, mammoth teeth and antler, is of great interest because such material is the only organic matter remaining in Quaternary sediments and Paleolithic sites. It is well known that the organic matter of bones, comprising 20% of body mass, consists mainly (80–90%) of very thin collagen fibers, closely connected with hydroxyapatite crystals, $3Ca_3(PO_4)_2 \cdot Ca(OH)_2$ + carbonic acid salts, which represent most of the non-organic component of bone. Because the crystals are very small (200 × 400 × (15–30) Å), the surface area of bone is rather large, >100m^2 g^{-1} (Engström *et al.* 1958).

However, the large surface area and porous structure of bone make it an excellent medium for sorption and precipitation of dissolved and colloidal particles of humic material, which, in groundwater, derives from modern soils and lacustrine-bog deposits. Such mixing of modern with ancient deposits often reduces the ^{14}C age of fossil bones. Saprophytes and bacteria that assimilate CO_2 from the air can be another source of bone contamination.

We discuss the problems of dating fossil bones using only organic components. We share the widespread, experimentally proven view that the carbonate fraction of bones, including the fraction connected with apatite, is unsuitable for ^{14}C dating because of contamination by groundwater carbonates and atmospheric CO_2 resulting from adsorption, recrystallization and isotopic exchange (Hassan, Termine and Haynes 1977).

The acid-alkali method (HCl–NaOH) of collagen extraction and purification from humic contaminants was developed in the late 1960s (Berger and Libby 1966; Haynes 1967; Vogel and Waterbolk 1967; Arslanov and Gromova 1970). Longin's (1971) method of collagen extraction from pulverized bone by 8% HCl and subsequent purification by dissolution (pH = 3, T = 90°C) also became accepted.

METHODS AND RESULTS

The use of collagen for ^{14}C dating, after humus removal by NaOH extraction or by Longin's method of humus precipitation, is based on the questionable assumption that all humic impurities are removed completely from the collagen. Because part of the humic matter in fossil bones might occur as compounds hardly related to the mineral component of the sample, or as insoluble

complexes with fulvic acids, some highly polymerized and nearly insoluble compounds might not be extracted from the collagen by NaOH treatment at room temperature. On the other hand, extracting collagen by Longin's method might not transfer all the humic matter to the residue; a less polymerized and more soluble portion could be dissolved in the hot, slightly acidic solution and transferred in solution together with gelatin. Thus, contamination of extracted substances by humic matter is possible in both cases. The reliability of these methods has been checked by dating young fossil bones using different fractions of organic matter and by parallel dating of collagen-charcoal and gelatin-charcoal pairs (Haynes 1967; Longin 1971).

Minute carbon contamination of relatively young fossil bones changes their age only slightly and is difficult to detect. However, contamination by young carbon strongly affects the ^{14}C dates of Pleistocene fossil bones; collagen content decreases with age and humic impurities accumulate in the porous bone structure. To evaluate contamination by young carbon, we collected bone samples from Late Pleistocene geological deposits and Paleolithic sites.

We investigated the solubility of bone organic matter as a function of the concentration of HCl and NaOH solutions. This enabled us to determine the best conditions of collagen extraction by HCl treatment of bones and subsequent removal of humic contaminants by NaOH treatment. We used modern cow bones and fossil bones that contained humic impurities. We added pulverized (≤1 mm) sample splits to HCl solutions of different concentrations and let them stand at room temperature for 24 h. Collagen was separated by centrifugation and washed until it showed a negative reaction for Ca^{2+} ($(NH_4)_2C_2O_4$ solution test). We then determined the carbon content of the samples. Then, dried collagen (3 g) was treated with different concentrations of NaOH solution for 24 h at room temperature. The collagen was washed following removal from the alkali extract until it became neutral; then we dried it in vacuum and determined its carbon content. We also analyzed the acid and alkali solutions for organic carbon content. Table 1 shows the results.

TABLE 1. Organic Components of Bones Dissolved at Room Temperature for Different Concentrations of HCl and NaOH*

Sample	Concentration of HCl or NaOH (N)	Weight of insoluble collagen (g)	Quantity of carbon in collagen (g)	Weight of carbon in soluble fraction (g)	Part of organic carbon in soluble fraction (%)
		HCl			
Modern bone	0.25**	16.96	5.99	0.65	9.8
(50 g)	0.5	13.40	6.12	0.62	9.2
	1	11.20	5.90	0.78	11.7
	2	10.11	5.36	1.34	20.0
Old bone	0.5	17.40	4.65	0.63	11.9
	1	15.74	4.51	0.80	15.1
	2	14.41	4.11	1.16	22.0
		NaOH			
Modern bone	0.1	2.974	1.567	0.0824	3.45
(3 g collagen)	0.25	2.608	1.374	0.1742	11.25
	0.5	2.475	1.304	0.2414	15.62
	1.0	2.122	2.118	0.6724	37.56
Old bone	0.1	2.7746	0.796	0.1225	13.33
(3 g collagen)	0.25	2.5070	0.7195	0.1723	19.33
	0.5	1.7601	0.505	0.5281	51.12
	1.0	1.0705	0.3072	0.7858	71.89

*Treatment time = 24 h
**Treatment time = 48 h

https://doi.org/10.1017/S0033822200060392 Published online by Cambridge University Press

The data in Table 1 show that the amount of dissolved collagen increases when HCl concentration exceeds 0.5 N. Thus, we recommend using a 0.5 N HCl solution for dissolving the fossil bone non-organic component, and 1.0 N HCl for well-preserved, compact bones and teeth. L. D. Sulergitskiy (personal communication) established that collagen yield increases considerably if HCl treatment is performed at low temperature (in a refrigerator, for example). Table 1 also shows that collagen solubility increases greatly when NaOH concentration is >0.1 N. For this reason, humic matter should be extracted with a ≤0.1N NaOH solution (Arslanov and Gromova 1970).

We assumed that the best conditions for humus extraction can be achieved by purifying collagen *via* extraction of soluble humus impurities with 0.1 N NaOH at room temperature, then precipitating nearly insoluble impurities from the collagen extraction using Longin's method (Arslanov, Svezhentsev and Markov 1981). As a control, we dated a large number of ancient fossil bone samples, using these two methods and our own.

The bones were mechanically cleaned and washed, then pulverized and treated at low temperature (4–6°C) by 2–3 fresh solutions of 0.5–1.0 N HCl for a few days (depending on preservation condition) until mineral components dissolved completely. We washed the collagen obtained in distilled water until no Ca^{2+} was detectable. We then treated the collagen with 0.1 N NaOH at room temperature for 24 h, and washed it again in distilled water until neutral. We treated the collagen with a weak HCl solution (pH = 3) at 80°–90°C for 6–8 h. Finally, we separated the humic acid residue from the gelatin solution by centrifugation, and the solution was evaporated. Benzene was synthesized from the dried gelatin by burning in a "bomb" or by dry pyrolysis, using the standard methods (Arslanov, Svezhentsev and Markov 1981). Table 2 shows the results.

Discussion and Conclusions

The data in Table 2 show that the oldest measured age is achieved by extracting the collagen fraction by the method that we recommend here. The fraction extracted using Longin's method has the youngest age. The age discrepancy of these fractions is caused by incomplete removal of younger humic substances. Thus, the experimental results observed earlier, as well as new data presented here, demonstrate incomplete removal of humic substances by a hot, weak HCl solution (Longin's method) or by a 0.1 N NaOH solution (Arslanov, Svezhentsev and Markov 1981). This illustrates that a single method, especially Longin's, should not be used alone in dating old samples. Recently, Gurfinkel (1987) compared different methods of collagen purification and concluded that the acid-alkali method or the two-stage combined method is preferable to Longin's method. Our current procedure (Arslanov, Svezhentsev and Markov 1981) allows us to remove separate soluble and nearly insoluble forms of humic matter, and to obtain more reliable ages. Hakansson (1976) first used the two-stage combined method for ^{14}C dating fossil bones, but did not provide any experimental data.

We compared the combined treatment method to the technique of extracting collagen with EDTA (Olsson *et al.* 1974). Table 3 presents ^{14}C dates measured from samples prepared using each method (Arslanov and Svezhentsev 1983). The data clearly indicate that samples treated by the combined method yield older ages.

From these results, we cannot recommend the EDTA method for dating ancient bones; further, the EDTA procedure is laborious. We recommend the combined method with which we can remove more completely exogenous carbon contamination and obtain more reliable dates. The method may be used for dating by conventional β-counting or by accelerator mass spectrometry.

TABLE 2. ^{14}C Dates on Bones Obtained by Different Chemical Treatments

Site	Material	^{14}C ages (yr BP)			^{14}C ages for charcoal or other material
		Longin method	HCl – NaOH method	Combination method	
Golubaja I, Krasnojarsk region	Bone	12,380 ± 140 (LE-1101A)	12,900 ± 150 (LE-1101B)	13,650 ± 180 (LE-1101C)	13,050 ± 120 (LE-1101D) charred bone
Gagarino, Lipeckanian region	Mammoth tooth	17,930 ± 100 (LE-1432A)	20,150 ± 300 (LE-1432B)	20,620 ± 300 (LE-1432C)	21,800 ± 300 (GIN-1872) charred bone
Kostenki XI, Voronezh region, Layer Ia	Bone	14,610 ± 120 (LE-1637)	16,040 ± 120 (LE-1704A)	17,310 ± 200 (LE-1704B)	19,900 ± 350 (GrN-2532) charred bone
Kostenki XII, Layer Ia	Bone	28,700 ± 400 (LE-1428A)	30,240 ± 400 (LE-1428B)	31,150 ± 400 (LE-1428C)	32,700 ± 700 (GIN-7758) charcoal
Kostenki XXI, lower Layer III	Bone	19,100 ± 150 (LE-1437A)	20,250 ± 100 (LE-1437B)	22,900 ± 150 (LE-1437C)	22,270 ± 150 (GrN-7363) charcoal
Jidinovo, northern bank of Sudost River, Brjansk region, Dugout II	Bone	15,790 ± 320 (LE-3301)	--	17,100 ± 310 (LE-3302A)	17,800 ± 810 (LE-3302B) charred bone
Anetovka II, bank of Bakarsha River, Nikolaevsk region	Bone	18,000 ± 150 (LE-2424A)	18,260 ± 1650 (LE-2424B)	19, 170 ± 120 (LE-2424C)	
Sagaidak I, 1st terrace of South Bug River, Nikolaevsk region	Mammoth tooth	20,300 ± 200 (LE-1602A)	--	21,240 ± 200 (LE-1602B)	
Ui-I, Tuva Autonomous Republic	Bone	16,760 ± 120 (LE-3358)	17,520 ± 130 (LE-3359A)	19,280 ± 200 (LE-3359B)	
Maininskaya, Layer 4, Tuva Autonomous Republic	Bone	12,910 ± 100 (LE-2133A)	12,980 ± 130 (LE-2133B)	13,690 ± 390 (LE-2133C)	
Kurtak-4, upper layer, depth 5 m, terrace, Yenisei Krasnojarsk region	Bone	23,470 ± 200 (LE-3351)	23,800 ± 900 (LE-3357)	24,890 ± 670 (LE-2833)	

TABLE 3. Comparison of Methods for Collagen Dating of Fossil Bones

Site	Material	^{14}C ages (BP)	
		Combined method	EDTA method
Kostenki XXI, depth 1.55–1.73 m	Bone	22,900 ± 150 (LE-1437C)	19,050 ± 130 (LE-1437D)
Dvuglaska Cave, Layer 6, Krasnojarsk region	Bone	>40,210 (LE-2346)	28,030 ± 290 (LE-2347)
Maininskaya Layer 3, Tuva Autonomous Republic	Bone	14,070 ± 150 (LE-2149A)	12,330 ± 150 (LE-2149B)
Kurtak-4 upper layer, depth 5m, terrace of Yenisei River, Krasnojarsk region	Bone	24,890 ± 670 (LE-2833)	24,170 ± 230 (LE-3351)
Junovo, near Junovo village, terrace of Yenisei River, Krasnojarsk region	Bone	36,500 ± 500 (LE-1435A)	35,000 ± 500 (LE-1435B)
Leski zone, South Bug River, Nikolaevsk region	Mammoth tooth	23,770 ± 750 (LE-2946A)	19,200 ± 200 (LE-2946B)

REFERENCES

Arslanov, Kh. A. and Gromova, L. I. 1970 The increased reliability of fossil bone age determination by radiocarbon dating. In *Astrophysical Phenomena and Radiocarbon.* Tbilisi, Tbilisi University Press: 67–74.

Arslanov, Kh. A. and Svezhentsev, Ju. S. 1983 The comparison of principal procedures for fossil bone dating by radiocarbon. In Shukolukov, Y. A., ed., *Conference Reports. Methods of Isotope Geology.* Moscow: 143–145.

Arslanov, Kh. A., Svezhentsev, Ju. S. and Markov, Ju. N. 1981 The improved procedure of fossil bone dating by radiocarbon method. In Kuzvits, Y. A., ed., *Isotope and Geochemical Methods in Biology, Geology and Archeology.* Tartu: 12–15.

Berger, R. and Libby, W. F. 1966 UCLA radiocarbon dates V. *Radiocarbon* 8: 467–497.

Engström, A., Bjornerstedt, R., Clemedson, C.-J. and Nelson, A. 1958 *Bone and Radiostrontium.* Stockholm, Almquist and Wiksell: 127 p.

Gurfinkel, D. M. 1987 Comparative study of the radiocarbon dating of different bone collagen preparations. *Radiocarbon* 29(1): 45–52.

Hakansson, S. 1976 University of Lund radiocarbon dates IX. *Radiocarbon* 18(3): 290–320.

Hassan, A. A., Termine, J. D. and Haynes, C. V., Jr. 1977 Mineralogical studies on bone apatite and their implications for radiocarbon dating. *Radiocarbon* 19(3): 364–374.

Haynes, C. V. 1967 Bone organic matter and radiocarbon dating. In *Radioactive Dating and Methods of Low-Level Counting.* Vienna, IAEA: 163.

Longin, R. 1971 New methods of collagen extraction for radiocarbon dating. *Nature* 230(5291): 241–242.

Olsson, I. U., El-Daoushy, A. F., El-Mageed, A. I. and Klasson, A. 1974 A comparison of different methods for pretreatment of bones. *Geologiska Föreningens i Stockholm Förhandlingar* 96(557): 171–181.

Vogel, J. C. and Waterbolk, H. T. 1967 Groningen radiocarbon dates VII. *Radiocarbon* 9: 107–155.