bank. The March and June cores were cut into 3-cm sections, and the November core was cut into 2.5-cm sections. During the last 10 years, pore-water and solid-phase species (2), including sulfur species, have remained consistent at this site and agree to better than ±10% (frequently ±5%) for samples at the same depth and for the same season. This consistency is borne out in successful diagenetic modeling for sediments and their pore waters at this salt marsh site (2).
18. Tetrathionate reduces to thiosulfate at the mercury electrode at $E_{1/2} = -0.32$ V in seawater matrices. Cyclic voltammetry on the November sample (surface to 2.5 cm) confirmed the formation of thiosulfate from tetrathionate.
19. R. P. Swarzenbach, W. Giger, C. Schaffner, O. Wanner, *Environ. Sci. Technol.* **19**, 322 (1985).
20. K. Mopper, in *Organic Marine Chemistry*, M. Sohn, Ed. (American Chemical Society Symposium Series, vol. 305, Washington, DC, in press).
21. J. P. Casey, Ed., *Pulp and Paper; Chemistry and Technology* (Wiley, New York, 1980).
22. J. W. H. Dacey and B. L. Howes, *Science* **224**, 487 (1984).
23. Although $S_2O_3^{2-}$ concentrations are in some instances smaller than thiol concentrations, $S_2O_3^{2-}$ levels may not build up and may only approach a steady-state level. This has been noted in a Massachusetts salt marsh (*15*).
24. A. B. Roy and P. A. Trudinger, *The Biochemistry of Inorganic Compounds of Sulfur* (Cambridge Univ. Press, London, 1970).
25. H. L. Ehrlich, *Geomicrobiology* (Dekker, New York, 1981).
26. D. K. Nordstrom, in *Acid Sulfate Weathering*, J. A. Kittrick *et al.*, Eds. (Soil Science Society of America, Madison, WI, 1982), pp. 37–56.
27. M. Goldhaber, *Am. J. Sci.* **283**, 193 (1983).
28. This research was supported by grants to G.W.L. from the National Science Foundation, Division of Biotic Systems and Resources (DEB82-16376), and the Marine Chemistry Program (OCE85-17138), and the Petroleum Research Foundation; and grants to T.M.C. from the Marine Chemistry Program of the National Science Foundation (OCE84-11064 and OCE85-41757). We thank L. Cifuentes, G. Cutter, A. Giblin, R. Howarth, C. Lord, and J. Morse for comments on earlier drafts.

2 October 1985; accepted 25 February 1986

# New Dates on Northern Yukon Artifacts: Holocene Not Upper Pleistocene

D. E. NELSON, RICHARD E. MORLAN, J. S. VOGEL, J. R. SOUTHON, C. R. HARINGTON

**New radiocarbon dates on four artifacts that were thought to provide evidence for human occupation of the Yukon Territory during the upper Pleistocene indicate that all four are of late Holocene age. The original radiocarbon age obtained for one artifact (the so-called "Old Crow flesher") was in error by almost 26,000 years.**

THE STUDY OF THE TIME AND CIRcumstances of the human colonization of the New World has preoccupied archeologists for more than a century. The earliest universally acknowledged North American sites are those that were occupied by people who made distinctive fluted stone projectile points approximately 11,500 years ago and who are usually given the name Clovis, after a locality in New Mexico. Although many sites and study areas have been presented as providing evidence for pre-Clovis human occupation in both North and South America (*1*), the validity of this evidence is not accepted by all investigators (*2*). Reexamination of one such body of evidence shows that four artifacts from the Old Crow locality in the northern Yukon Territory, Canada, which were previously thought to be of late Pleistocene age, were in fact from the late Holocene.

These artifacts were originally obtained from localities scattered along the Old Crow River (*3*, *4*), which meanders across the intermontane Old Crow Basin. In late Wisconsinan time, the river dissected the basin, exposing more than 30 m of Pleistocene sediment (*5*). This action exhumed hundreds of thousands of fossil bones from the Pleistocene deposits, and these were subsequently redeposited in and on the terraces and gravel bars that developed during the Holocene (*6*).

In 1966, a caribou (*Rangifer tarandus*) tibia that had been fashioned into a fleshing tool was found in one of these Holocene terrace deposits at locality 14N. This artifact is similar in form and material to tools made in late prehistoric and historic time for removing the flesh from skins used for clothing, shelter, and other purposes. In addition to this flesher, mammoth limb bones were found that had been fractured and flaked, presumably by humans, in a manner reminiscent of the production of stone tools. The man-made flesher and the flaked mammoth bone were immediately radiocarbon dated (*3*) to determine whether they were of the same age. If so, there would be strong evidence for a human culture of considerable antiquity that used mammoth bone for tools.

Carbon extracted from the inorganic, or apatite fraction (*7*) of the flesher and two of the fractured mammoth bones gave radiocarbon ages of 27,000 years before present (BP), 25,750 BP, and 29,100 BP, respectively (*3*). These dates were in general agreement with dates on carbon from the organic, or collagen fraction (*7*) of a mammoth femur (22,600 BP) and a bison humerus (33,800 BP) (*3*). The rather broad time range represented by the dates was explained by the observation that locality 14N is a Holocene terrace deposit into which the Pleistocene fossils had been transported by fluvial processes. The concordance between the early date for the flesher and those for the flaked mammoth bones suggested that an early North American culture existed in the Yukon in Pleistocene times and that the flesher represented a tool type that had been in use for a long time.

These dates, and in particular the one on the flesher, helped launch intensive multidisciplinary research in Old Crow Basin that continues to this day, and the flesher date is often cited as a cornerstone of arguments that favor pre-Clovis human occupation of the New World. Various arguments have also been advanced against these interpretations. It was suggested that the flesher could have been made recently on a bone that had already been fossilized, or that had been preserved by freezing (*8*). A much more serious possibility is that the age of the flesher is suspect as it was obtained on carbon extracted from the inorganic portion of the bone. Since the flesher was originally dated, research has shown that the inorganic bone fraction is susceptible to diagenetic exchange of carbon, and that the organic, proteinaceous carbon is preferable (*9*).

To test the original assumptions and conclusions about the Old Crow material, we took a new series of radiocarbon dates. The mammoth and bison bones from locality 14N had been entirely sacrificed for the original dates, but the man-modified working tip of the flesher (~21 g) was preserved so that it could be displayed. Only a small amount of this tip could be removed. The method of accelerator mass spectrometry (AMS) made it possible to obtain dates on bone samples of much less than 1 g.

The 36 bones chosen for dating formed three categories: (i) six bones were from known stratigraphic contexts, and three of these may have been modified by man when fresh; (ii) 26 of the bones (of which 24

D. E. Nelson, J. S. Vogel, J. R. Southon, Archeology Department, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6.
R. E. Morlan, National Museum of Man, Ottawa, Ontario, Canada K1A 0M8.
C. R. Harington, National Museum of Natural Sciences, Ottawa, Ontario, Canada K1A 0M8.

Table 1. Radiocarbon dates and $\delta^{13}C$ values for the four caribou antler and bone tools.

| Sample number | Artifact description | Laboratory number | $^{14}C$ age (years) | $\delta^{13}C$ value* (per mil) |
|---|---|---|---|---|
| NbV1-2:15 | Caribou antler billet | RIDDL-133 | 2930 ± 140 | −20.4 |
| MjV1-1:26c | Caribou antler wedge | RIDDL-140 | 1730 ± 100 | −18.1 |
| MiV1-1:1 | Caribou antler wedge | RIDDL-141 | 1880 ± 140 | −17.7 |
| M1V1-1:1 | Caribou tibia flesher | RIDDL-145 | 1350 ± 150 | −19.7 (−19.5) |

*The $\delta^{13}C$ values are given relative to the PBD standard. All measurements were on extracted protein, except for the second value (in parentheses) on the flesher. This was taken on a sample of the prepared graphite to ensure there had been no fractionation in the conversion process.

represent mammoths) are interpreted to have been artificially modified when fresh but were collected from modern river banks and bars; and (iii) four were functionally identifiable caribou antler and bone tools collected from modern river banks and bars. This last category included the flesher, a caribou antler billet (a hammer used for flaking stone), and two caribou antler wedges (*10*). The billet was collected at Old Crow River locality 29, and the wedges were found on the banks of the Porcupine River. Like the flesher, these antler tools were found among fossils of extinct Pleistocene animals, and the staining and apparent degree of permineralization on the specimens was so similar to that of the fossils that a similar age was suggested.

With the exception of the flesher, about 1 g of bone was removed from each of these specimens by drilling with a clean 4-mm metal bit. The drillings were collected on aluminum foil and stored in glass bottles. A small sample of the flesher tip was taken by using a clean miniature power saw to remove a piece about 18 by 6.5 by 2.5 mm from the area where the tool had been cut for the original radiocarbon sample. Approximately 0.3 g of bone was thus obtained. In all cases, there was a distinct odor of burnt bone during sampling, giving evidence of good protein preservation. Each sample was assigned an arbitrary number and processed without other identification.

The bone protein was extracted by a variation on the Longin technique (*11*). First, the samples were soaked in weak (0.25*N*) HCl to remove the inorganic portion of the bone as well as any acid-soluble protein degradation products. The filtrate was then refluxed in water at 80°C for 20 hours to convert the insoluble proteins to soluble gelatin. After filtering to remove any insoluble contaminants, the gelatin was obtained by evaporation of the water. A portion of this gelatin was burned to $CO_2$ by heating it with CuO for several hours at 900°C in a sealed quartz tube.

The AMS measurements were made at the Simon Fraser University facility at the Tandem Laboratory at McMaster University (*12*). Each $CO_2$ sample was catalytically converted to graphitic carbon on an iron powder substrate by the method of Vogel *et al.* (*13*). About 300 μg of the graphite from each sample was placed in the ion source of the accelerator, along with identically prepared graphite specimens made from the NBS radiocarbon standard Ox-1, from anthracite (to give the system background), and from known-age secondary standards. Each unknown sample was directly compared against the NBS primary standard a minimum of three times.

The stable isotope ratios ($\delta^{13}C$ values) of the extracted protein from the artifacts of category (iii) above were measured at the Quaternary Isotope Laboratory at the University of Washington. Because of the importance of the flesher, an aliquot of that graphite was also measured to test for isotopic fractionation in the graphitization process.

The 32 samples from the first two categories (the stratigraphically documented bones and the presumed artificially altered mammoth bones) had ages ranging from about 25,000 to greater than 47,000 radiocarbon years. These results fit well with geological data (*5*) and with radiocarbon dates previously obtained on other bones (*6*). Two are in good agreement with results obtained by another laboratory on the same bones. Only two of the 32 dates were in disagreement with earlier measurements.

However, the dates obtained for the flesher, the billet, and the two antler wedges (Table 1) show that these are Holocene, not Pleistocene, artifacts. In particular, the age (1350 ± 150 BP) obtained for the flesher is almost 26,000 years younger than that originally measured (*3*). There are a number of arguments that support this new result.

First, the accuracy of our AMS measurement technique is demonstrated by the results of previous blind tests (*12*), by data obtained in the same test on the known-age secondary standards, and by the concordance with previous dates for the samples in the first two categories. It is unlikely that we inadvertently mixed samples or introduced modern contaminants during the chemical pretreatment, since the samples were processed in random order on a "blind" basis, and yet only those of the third category gave young ages. Also, immediately after reporting the results, the analytical group was asked to burn, graphitize, and measure a second portion of the flesher gelatin. These results confirmed the first measurement.

Second, if the dates for the four artifacts are in error, the most likely explanation would be that the samples were contaminated. If the flesher were 27,000 years old, the new date requires that about 85 percent of our carbon sample was from a modern contaminant. However, the method of protein extraction used is quite specific for proteinaceous material so that it is difficult to imagine such a large fraction of other carbon-containing material could pass through the extraction process. Furthermore, the $\delta^{13}C$ value of −19.7 per mil (Table 1) for the separated protein is evidence against such a massive contamination. This value is typical for the bone collagen of northern herbivores and atypical of any likely contaminants (*14*).

Last, the date obtained here for the flesher is typical of the ages of all other known artifacts of this general type in this region. Thus, it is not an unusual artifact, nor does it represent a general tool type that existed relatively unchanged for nearly 30,000 years.

To conclude, a new radiocarbon analysis of supposed upper Pleistocene artifacts from the Old Crow area in the Northern Yukon shows that these materials can be subdivided into two age groups. The radiocarbon dates for the proposed mammoth bone flakes and cores are all of Pleistocene age, in accord with earlier determinations and with geological expectation. These results provide no new information to help determine whether these specimens are man-made tools or the results of natural phenomena (*15*). However, the four Old Crow artifacts that have counterparts in recent time are all of late Holocene age. In particular, the original age obtained for the Old Crow flesher was seriously in error, probably due to massive contamination of the bone apatite by groundwater carbonates. These four artifacts can no longer be regarded as evidence for a Pleistocene human occupation of northwestern North America.

## REFERENCES AND NOTES


1. A. L. Bryan, Ed., *Early Man in America from a Circum-Pacific Perspective* (Occasional Papers 1, Department of Anthropology, University of Alberta, Edmonton, 1978); R. L. Humphrey and D. Stanford, Eds., *Pre-Llano Cultures of the Americas: Paradoxes and Possibilities* (Anthropological Society of Washington, Washington, DC, 1979); R. Shutler, Jr., Ed., *Early Man in the New World* (Sage, Beverly Hills, CA, 1983).
2. F. H. West, *The Archaeology of Beringia* (Columbia Univ. Press, New York, 1981); D. E. Dumond, *Am. Antiq.* **47**, 885 (1982); D. F. Dincauze, in *Advances in World Archaeology*, F. Wendorf and A. E. Close,

Eds., (Academic Press, New York, 1984), vol. 3, pp. 275–323; R. C. Owen, in *The Origins of Modern Humans*, F. H. Smith and F. Spencer, Eds. (Liss, New York, 1984), pp. 517–563; E. J. Dixon, *North Am. Archeol.* **6**, 83 (1984–85).
3. W. N. Irving and C. R. Harington, *Science* **179**, 335 (1973).
4. R. Bonnichsen, *Pleistocene Bone Technology in the Beringian Refugium* (National Museum of Man, Archaeological Survey of Canada Mercury Series Paper 89, Ottawa, 1979); R. E. Morlan, *Taphonomy and Archaeology in the Upper Pleistocene of the Northern Yukon Territory* (National Museum of Man, Archaeological Survey of Canada Mercury Series 94, Ottawa, 1980); A. L. Jopling, W. N. Irving, F. Beebe, *Arctic* **34**, 3 (1981).
5. O. L. Hughes, *Geol. Surv. Can. Pap. 69–36* (1972); O. L. Hughes *et al.*, *Arctic* **34**, 329 (1981).
6. C. R. Harington, thesis, University of Alberta, Edmonton (1977); C. R. Harington, *Quaternary Vertebrate Faunas of Canada and Alaska and Their Suggested Chronological Sequence* (National Museum of Natural Sciences Syllogeus 15, Ottawa, 1978); C. R. Harington, *Arctic* **33**, 815 (1980).
7. Bone consists of an inorganic matrix bound by long fibrils of connective tissue (*9*). The inorganic material is a phosphatic, calcified (hydroxyapatite) crystal that makes up about 75 to 80 percent of the bone mass. The rest is organic proteinaceous material, of which 90 to 95 percent is the protein collagen. Carbon from either the inorganic or the organic fractions of the bone may be extracted for radiocarbon dating, and the terms "apatite" and "collagen" dates have come to be used to describe dates taken on these respective fractions. Since the extractive methods vary considerably, this terminology is not very accurate, as the carbon obtained may not be entirely from the apatite or from the collagen. The carbon indigenous to the apatite can be seriously contaminated with extraneous carbon, and the extracted "collagen" may also include some of the other proteins found in minor quantities in bone, and possibly some degradation products of the organic molecules.
8. C. V. Haynes, *Arct. Anthropol.* **8**, 3 (1971); R. D. Guthrie, *Q. Rev. Archeol.* **1**, 2 (1980).
9. A. A. Hassan and D. J. Ortner, *Archaeometry* **19**, 131 (1977); A. A. Hassan, J. D. Termine, C. V. Haynes, *Radiocarbon* **19**, 364 (1977); R. E. Taylor, *ibid.* **22**, 969 (1980).
10. For illustrations of these tools see W. N. Irving and C. R. Harington (*3*).
11. R. Longin, *Nature (London)* **230**, 241 (1971); B. S. Chisholm, D. E. Nelson, K. A. Hobson, H. P. Schwarcz, M. Knyf, *J. Archeol. Sci.* **10**, 355 (1983).
12. D. E. Nelson, J. R. Southon, J. S. Vogel, R. G. Korteling, T. L. Ku, *Nucl. Instrum. Methods* **233**, 139 (1984); D. E. Nelson, J. S. Vogel, J. R. Southon, T. A. Brown, *Radiocarbon*, in press.
13. J. S. Vogel, J. R. Southon, D. E. Nelson, T. A. Brown, *Nucl. Instrum. Methods* **233**, 289 (1984); J. S. Vogel *et al.*, in preparation.
14. Most present-day Northern plants are of the $C_3$ variety with $\delta^{13}C$ values of about −25 per mil; the collagen and flesh of animals eating these plants are typically about −20 and −25 per mil, respectively [B. S. Chisholm, D. E. Nelson, H. P. Schwarcz, *Science* **216**, 1131 (1982)]. The diet of late Pleistocene Beringian herbivores was also predominantly $C_3$ plants [M. Bombin and K. Muelenbachs, *Quaternary Res.* **23**, 123 (1985)]. The value found for the flesher is thus exactly that expected for the bone collagen of a caribou, and several per mil different than the values expected for most other organic materials.
15. R. E. Morlan, in *A Question of Bone Technology*, G. M. LeMoine and A. S. MacEachern, Eds. (Univ. of Calgary, Calgary, 1983), pp. 241–269; R. E. Morlan, *Quaternary Res.* **22**, 160 (1984); R. E. Morlan, in *New Evidence for the Pleistocene Peopling of the Americas*, A. L. Bryan, Ed. (Univ. of Maine, Orono, 1985), pp. 27–48.
16. We thank T. Brown for aid with the AMS measurements, M. Stuiver and his staff for the stable isotope measurements, and the staff of the McMaster Tandem Accelerator Laboratory for the use of their equipment. Supported in part by the Natural Sciences and Engineering Research Council of Canada.

9 October 1985; accepted 3 February 1986

# Amplification and Expression of Genes Associated with Multidrug Resistance in Mammalian Cells

KATHLEEN W. SCOTTO, JUNE L. BIEDLER, PETER W. MELERA

**In multidrug resistance, which is observed clinically and in tissue culture, cells that are challenged with certain cytotoxic drugs develop resistance not only to the selective agent but also to other, seemingly unrelated, agents. The multidrug-resistant phenotype is associated with DNA sequence amplification and with the overproduction of a number of cytosolic and membrane glycoproteins. The differential amplification and altered expression of at least two related genes, termed multidrug-resistant associated genes has been shown in multidrug-resistant Chinese hamster cells. In multidrug-resistant mouse and human cells, genes homologous to those in Chinese hamster cells are also amplified. The level of expression of these genes varied and did not correlate with their copy number. Furthermore, in Chinese hamster cells, the development of resistance to a single drug and multidrug resistance were closely related, but uncoupled, events. The overexpression of the multidrug-resistant genes was better correlated with the degree of resistance to the selective agent than it was with the extent of multidrug resistance.**

THE ESTABLISHMENT AND MAINTENANCE of drug resistance in mammalian cells is mediated by the overexpression of specific genes (*1*). Generally, the drugs irreversibly bind to specific housekeeping enzymes, and resistance is achieved by the overproduction of these target enzymes due to amplification of the genes that encode them (increased gene dosage). The RNA expression is generally less than or equal to, but not greater than, the degree of amplification, and the resulting phenotypes are monospecific; the cells are resistant only to the selective drug or its analogue.

In contrast, cytotoxic drugs that interact with structural proteins or DNA, rather than with housekeeping enzymes, elicit a more complicated response (*2–4*). Cells become refractory to both the selective agent and other, seemingly unrelated, compounds. This more general cross-resistance is apparently mediated by alterations in membrane transport that decrease the intracellular accumulation of these agents (*5*). The phenotypes of these multidrug-resistant cells are complex. (i) Independently selected cell lines display different levels of cross-resistance to the same drugs. (ii) Multidrug-resistant cells contain increased amounts of membrane glycoproteins such as gp150-180 (*6*), p-glycoprotein (*7*), gp180 (*8*), or P180 (*9*); the overproduction of p-glycoprotein in Chinese hamster cells is mediated by gene amplification (*10*). (iii) Some multidrug-resistant cells overproduce cytosolic proteins such as V19 (*11*) in addition to membrane glycoproteins. (iv) Tumorigenic cells selected for multidrug resistance display a reverse transformed phenotype and cannot cause tumors in animals (*12, 13*). The variability and complexity of the multidrug-resistance phenotype suggest that this clinically relevant form of drug resistance may involve a change in gene expression at more than one genetic locus.

We used vincristine-resistant sublines of the Chinese hamster lung (CHL) cell line DC-3F for our studies (*2*) because these cells contain cytological manifestations of gene amplification, such as homogeneously staining regions (HSR) and abnormally banding regions (ABR) (*13, 14*), and elevated gp150-180 (*13*) and V19 (*11*). We could then analyze these cells with methods optimized for the detection of amplified DNA sequences (*15, 16*). We identified at least two related genes, the amplification and overexpression of one or all of which was associated with the multidrug-resistant phenotype. Genes homologous to these were also amplified and overexpressed in multidrug-resistant mouse and human cell lines. In contrast to other drug-resistant cell systems, the level of expression of these genes could exceed that of their amplification and, although this increased expression may correlate with the increase in resistance to the

K. W. Scotto, Laboratory of Biochemistry and Molecular Biology, Rockefeller University, New York, NY 10021.
J. L. Biedler, Graduate Program in Cell Biology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021.
P. W. Melera, Graduate Program in Molecular Biology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021.