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BOMB RADIOCARBON IN METABOLICALLY INERT TISSUES FROM TERRESTRIAL AND MARINE MAMMALS

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Abstract. We report here radiocarbon measurements of monkey eye lens nucleus proteins and a narwhal tusk, biological tissues which have sampled the bomb radiocarbon signal in different ways. The results confirm the metabolic inertness of eye lens nucleus proteins and demonstrate the feasibility of measuring radiocarbon in small samples of biological tissue using accelerator mass spectrometry (AMS). The narwhal tusk provides a unique record of the radiocarbon activity in Arctic Ocean waters over most of the 20th century.

Introduction

The detonation of thermonuclear weapons in the atmosphere during the 1950s and early 1960s nearly doubled the radiocarbon (^{14}C) activity of tropospheric carbon dioxide by 1964 [Nydal and Lövseth, 1983]. Although this bomb radiocarbon signal has steadily decreased since the ratification of the limited atmospheric test ban treaty in October 1963, the current radiocarbon activity of atmospheric carbon still exceeds pre-bomb levels by about 15-20% [Levin et al., 1985]. The radiocarbon levels of dissolved inorganic carbon (DIC) in ocean surface waters also increased, but more slowly and to a much smaller extent [Broecker et al., 1985]. Mixing with subsurface waters acted to dampen the bomb radiocarbon signal in surface waters, and the long residence time of radiocarbon in the atmosphere delayed the peak maximum in the ocean nearly 10 years [Druffel and Suess, 1983]. Numerous studies have utilized the radiocarbon "spike" derived from nuclear weapons testing to investigate various oceanographic [Broecker et al., 1985; Druffel and Suess, 1983], geochemical [Hedges et al., 1986], and biological [Mok et al., 1986] processes. We have investigated the bomb radiocarbon signal in metabolically stable tissues of a terrestrial and a marine mammal in order to assess the utilization of the bomb "spike" as a tool for studying protein turnover rates and for providing radiocarbon records in regions where few or no previous radiocarbon measurements are available.

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Experimental

We utilized amino acids isolated from the ocular lens nucleus from Rhesus monkeys to investigate bomb radiocarbon activities in a metabolically inert tissue from a terrestrial mammal. Proteins in the ocular lens nucleus are considered to be some of the most inert in mammalian tissues [Harding, 1985]. They are synthesized *in utero* and thereafter are apparently never involved in active metabolic processes, although some controversy exists as to whether low rates of protein synthesis continue throughout life [Ozaki, 1985]. A number of post-translational modifications are detectable with increasing mammalian age in the lens nucleus proteins and these have been attributed to their metabolic isolation [Harding, 1985]. In contrast, active protein synthesis occurs in the lens cortex throughout life. The lens nucleus from a series of known age Rhesus monkeys sacrificed in 1981 was carefully removed by dissection [Bada and Brown, 1985] and then hydrolyzed in 6M HCl for 6 hours at 100°C. The amino acids in the hydrolysates were isolated by cation exchange chromatography, and further purified by treatment with activated charcoal to remove a black-tarry material which remained after desalting. As the quantities of amino acids which were obtained (1.0 to 1.6 mg) were too small for β-counting techniques, the radiocarbon activities were determined using accelerator mass spectrometry (AMS) [Batten et al., 1986].

A narwhal tusk was used to examine the incorporation of bomb radiocarbon into an inert tissue of a marine mammal. Only male narwhals have a tusk, which is actually a maxillary tooth that erupts during the first few years of life and grows continuously throughout the animal's lifetime. Tusks can reach a length of over 2 m and are characterized by regularly spaced dentinal growth layer groups (GLG) which are deposited at a rate of about 1 GLC per year [Bada, et al., 1983]. Once deposited, the growth layers are isolated from metabolic processes. The narwhal tusk used for this study was collected in 1978 during an Inuit ice edge hunt, Northern Baffin Island, Northwestern Territories, Canada. We sampled the tusk at 8 equally spaced intervals from the butt end to the tip. The year of formation of each sample was ascertained from the total number of growth layer group counts, and the animal's age calculated from the extent of aspartic acid racemization measured in an unerupted maxillary tooth [Bada et al., 1983]. The tusk samples were first treated with

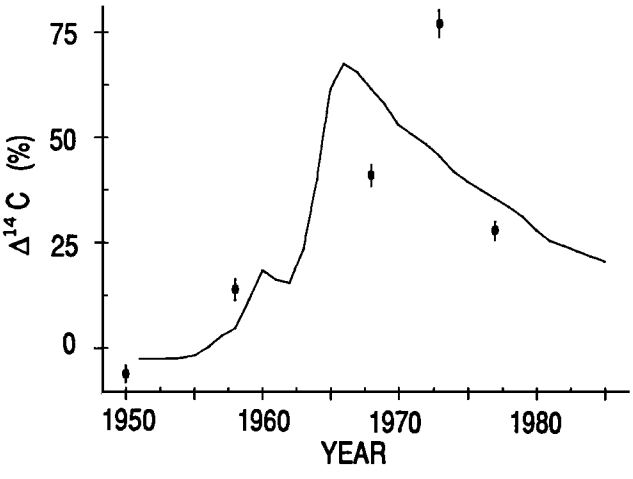

Fig. 1. Radiocarbon in rapidly turned over tissues and hair in humans [Mok et al., 1986, ——] and in the monkey eye lens nucleus amino acids (•) described in this study. $\Delta^{14}C$ is calculated relative to the 1950 NBS-1 oxalic acid standard.

dilute HCl to remove carbonates and then combusted at 600°C in a stream of oxygen to pyrolyze the organic material to carbon dioxide. The radiocarbon activity of the liberated carbon dioxide was determined using the conventional β-counting techniques described elsewhere [Druffel and Suess, 1983; Mok et al., 1986].

Results and Discussion

The results of the monkey lens nucleus measurements are shown in Figure 1 along with the historical radiocarbon activities for terrestrial carbon in the northern hemisphere as recorded in human blood, hair and organs. In general, the radiocarbon activities ($\Delta^{14}C$) of the amino acids isolated from the monkey lens nucleus approximately equal the radiocarbon level in rapidly cycled carbon in humans at the time of birth, with the exception of the 1973 sample. Based on the extrapolation of the radiocarbon levels for tissues with rapid turnover rates, a value of about 20% would have been expected if there was active protein synthesis in the lens nucleus at the time when the animals were sacrificed in 1981. The results clearly show that pre-bomb radiocarbon levels are present in the monkeys born before extensive atmospheric nuclear testing whereas post-bomb values exist in those born after the test ban treaty in 1963. The pre-bomb $\Delta^{14}C$ value of -6±2% measured in the monkey born in 1950 provides independent verification that the lens nucleus proteins are metabolically inert. We estimate that less than 2-8% of the radiocarbon derived from weapons testing has been incorporated into the lens nucleus proteins of this animal. Although we have no clear explanation for the anomalously high value for the 1973 sample, we do not believe it invalidates our conclusions. One possibility is that the proteins in the diet of the mother of this animal during pregnancy were derived from foods stockpiled in the mid 1960s.

The narwhal tusk radiocarbon measurements are given in Figure 2 as are the oceanic radiocarbon activities recorded in coral and in Arctic Ocean samples collected in 1985. In contrast to both the monkey lens analysis (Figure 1) and the record in corals, the bomb signal in the narwhal tusk is very weak. In the layers deposited before the major nuclear explosions in the late 1950s and early 1960s, the radiocarbon activities ranged from -10 to -18%. Similarly depleted radiocarbon activities have been found in the tissues of other prebomb polar marine mammals and birds [Tauber, 1979; Mabin, 1986]. During and after the interval of atmospheric weapons testing, $\Delta^{14}C$ values in the narwhal tusk rose to a maximum of -2.0% by the late 1960s, and then decreased to values nearly as low as those recorded in prebomb layers. In comparison, the bomb signal in corals was more pronounced and has declined only slightly since reaching a maximum value around 1970. Thus, radiocarbon in Arctic Ocean waters as recorded in the narwhal tusk was less affected by bomb radiocarbon than other oceanic waters even though the largest atmospheric detonations were carried out in the Arctic regions of the USSR [Carter and Moghissi, 1977]. Present day subsurface (>200 m) waters in the Canadian Arctic Ocean have radiocarbon levels which range from -5.0 to -15.0%, while surface waters range from -2.5 to +3.0% [Ostlund et al.,, 1987]. Deep convective mixing in the Arctic Ocean serves to dilute the radiocarbon in surface waters to a much larger extent than at lower latitudes (e.g., as recorded in corals). Since there are no data on radiocarbon in Arctic Ocean waters prior to 1979 [Ostlund et al., 1987], the narwhal tusk provides a unique time history of Arctic Ocean radiocarbon values over the last five decades. Unfortunately, due to the migratory behavior and feeding habits of the narwhal, this record cannot be interpreted solely as a reflection of Arctic Ocean mixing processing in one localized area, i.e., the Baffin Island region where the narwhal was killed. Similar studies using metabolically stable tissues from other cetaceans should provide important long term records of radiocarbon activities in various oceanic areas.

The monkey lens results suggest that the artificial bomb radiocarbon signal may have a variety of applications in mammalian biology. Using the radiocarbon activities in the ocular lens nucleus and other metabolically stable proteins such as dentin, it should be possible to determine whether an

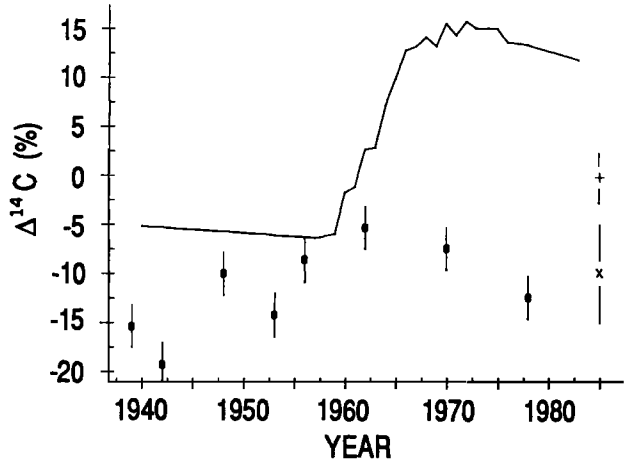

Fig. 2. Radiocarbon in Florida corals [Druffel and Suess, 1983, ——], in Canadian Arctic DIC from the upper 200 meters (+) and between 200 to 2500 meters (X) depth [Ostlund et al., 1987], and in the narwhal tusk layers (•) analyzed in this study. Note expanded $\Delta^{14}C$ scale in comparison to Figure 1.

animal was born prior to the period of bomb radiocarbon production. This technique could be valuable in establishing the minimum age of animals which are difficult to age by other biochronological methods such as dentinal growth counts or aspartic acid racemization. It should be possible to extend this bomb radiocarbon based aging method to other species such as non-aquatic birds and reptiles. In addition the radiocarbon activity in proteins with poorly known turnover rates could be used to evaluate whether they are inert or metabolically active. For example, further studies of the radiocarbon activities in a lens cross-section would help define the region where there is active protein synthesis. This type of analysis could easily be extended to the proteins and other organic components in bone, skin, and the brain.

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