Radiocarbon Dating: Fictitious Results with Mollusk Shells

Abstract. *Evidence is presented to show that modern mollusk shells from rivers can have anomalous radiocarbon ages, owing mainly to incorporation of inactive (carbon-14-deficient) carbon from humus, probably through the food web, as well as by the pathway of carbon dioxide from humus decay. The resultant effect, in addition to the variable contributions of atmospheric carbon dioxide, fermentative carbon dioxide from bottom muds, and, locally, of carbonate carbon from dissolving limestones, makes the initial carbon-14 activity of ancient fresh-water shell indeterminate, but within limits. Consequent errors of shell radiocarbon dates may be as large as several thousand years for river shells.*

A number of previous investigators, for example, Blau *et al.* (*1*), Deevey *et al.* (*2*), and Crane (*3*), have noted that mollusk shell samples may yield radiocarbon ages which are too large in comparison with the scale based on wood ages. Comparison of data from various sources (*4–9*) shows that modern fresh-water shells generally have larger anomalous ages than do marine shells.

The observed carbon-14 deficiency of modern fresh-water mollusk shells and of continental waters has been explained mainly in two different ways: (i) as a dilution effect resulting from the incorporation of inactive carbon from limestones and other rocks (*1, 2, 9–12*), and (ii) as a fractionation effect attributed to biological and exchange processes, including the rate of exchange of CO_2 across the air-water interface (*7–9, 13, 14*).

Several other investigations are pertinent although not directly concerned with the isotopic composition of mollusk shells. Baertschi (*13*) and Craig (*14*) referred to the possible isotopic effect on fresh-water carbonates of CO_2 from decaying organic matter. Oana and Deevey (*15*) set up a model allowing for contributions from various sources to the carbon cycle in lakes. They give a mean δ C^{13} of -8.6 per mil for surface waters of four soft-water lakes and show a progressive depletion of C^{13} and C^{14} in deeper waters of the lakes which they studied.

Previous investigations of mollusk shell carbon, briefly summarized above, appear to have neglected two important factors: (i) the contribution of CO_2 from fermentation of bottom muds (Oana and Deevey, *15*), and (ii) a variable humus contribution to dissolved CO_2 and to the food web. Keith, Eichler, and Parker (*16*) and Keith and Anderson (*17*) proposed that the food web, rather than dissolved bicarbonate in the water, may be the main direct source of carbon in shell carbonate. The present investigation is an outgrowth of that hypothesis, and is concerned with the relationship between environment and the C^{13} and C^{14} concentrations in the shell carbonate of a small group of modern mollusks collected alive from widely different environments.

Three marine specimens, one lake specimen, and three river specimens were selected and submitted for radiocarbon analysis to the Michigan Radiocarbon Laboratory. Measurements of the $C^{13} : C^{12}$ ratio were made on split subsamples from the same ground shell samples which were submitted for C^{14} dating.

Shell samples were treated in commercial Clorox solution (5-percent sodium hypochlorite) to remove adhering organic matter, and were then air dried and crushed to -40 mesh size. A subsample was crushed to -80 mesh size and heated for about 20 minutes at 420°C in a stream of purified helium, in order to drive off volatile organic compounds.

The resulting carbonate residue was treated with 100-percent phosphoric acid in an evacuated reaction tube. The evolved carbon dioxide was collected, purified, and analyzed (*18*).

Isotopic compositions, the means of duplicate measurements made at different times, are expressed in the standard δ notation, relative to the Chicago PDB standard carbon dioxide. The carbon isotopic analysis is given as δ C^{13}, in parts per thousand-difference from the standard as:

$$\delta\, C^{13} = 1000 \left(\frac{C^{13}/C^{12}_{\text{Sample}} - C^{13}/C^{12}_{\text{Std.}}}{C^{13}/C^{12}_{\text{Std.}}} \right)$$

Data were corrected for O^{17} content of sample and standard, using Craig's equation (*19*).

The reported radiocarbon ages and the results of the stable isotope measurements are given in Table 1 and are shown in the larger symbols in Fig. 1.

Comparable literature data on C^{13} and C^{14} content of modern shells are shown by the smaller symbols in Fig. 1. Included are data for a clam shell from Long Island Sound and nine marine gastropod shells (samples L-317 and L-576 A, B, E, F, G, H, I, J, K) reported by Broeker and Olson (*7, 8*). The same authors report exact C^{13} and C^{14} data for three specimens of modern fresh-water shells, and the data are given in Table 2 and Fig. 1, corrected as necessary, according to the note of Broecker and Olson (*8*).

The most noteworthy feature of the results is that the analyzed modern mollusk shells from river environments are not only deficient in C^{13}, relative to marine shells, as noted by Keith *et al.* (*16*), but are also extremely deficient

Table 1. Carbon-13 and carbon-14 content of modern mollusk shells.

Sample No.	Species	Michigan analysis No.	Radiocarbon age (t)[*] (years)	δC^{13}[†] (per mil)	δC^{14}[‡] (per mil)	Δ[§] (per mil)
			Marine samples			
61–155	*Strombus*	M–1220	300 ± 150	−0.45	−36	−83
62–132	*Tivela*	M–1221	40 ± 150	+1.12	− 5	−57
62–196	*Haliotis*	M–1222	125 ± 150	+0.63	−16	−66
		Means	155	+0.43	−19	−69
			Lacustrine sample			
61–16	*Lampsilis*	M–1223	440 ± 150	−5.23	−54	−91
			Fluvial samples			
61– 11	*Elliptio*	M–1224	1890 ± 200	−11.45	−209	−230
61–127	*Actinonaias*	M–1225	1010 ± 150	−13.83	−118	−138
62– 48	*Ligumia*	M–1226	2300 ± 200	−13.91	−248	−265
		Means	1733	−13.06	−192	−211

[*] t = uncorrected radiocarbon age, as reported by the Michigan Radiocarbon Laboratory.

[†] δC^{13} = per-mil difference from Chicago PDB standard carbon dioxide.

[‡] $\delta C^{14} = [(A - A_0)/A_0]1000$, where A and A_0 represent activity of sample and of contemporary wood. The above values of δC^{14} were calculated from the reported radiocarbon ages (t) by using a C^{14} half life of 5568 years (Libby, 22)[||] and the relationship $\lambda t = -\mathrm{In}\ (\delta C^{14} + 1000)/1000$, where $\lambda = \ln (1/2)/5568 = 1.24 \times 10^{-4}$.

[§] Δ = normalized C^{14} content, in per-mil difference from contemporary wood, after correction for fractionation effects as suggested by Broecker and Olson (*8*).

[||] A C^{14} half life of 5730 years was recommended by the 5th Radiocarbon Dating Conference (Godwin, *23*). The question has been re-examined by Libby (*24*) and there appears to be no great advantage in changing the half life until some of the discrepancies are cleared up.

in C^{14}, relative to modern wood, and give uncorrected radiocarbon ages in the range 1010 to 2300 years. The fluvial shells exhibit wide variations in carbon isotope composition but nevertheless are distinctly different, as a group, from the marine shells. Our single lacustrine shell, and the lake outlet sample of Broecker and Olson, have carbon isotopic compositions intermediate between those of the marine shells and the fluvial shells.

Table 2. Corrected data of Broecker and Olson (*7*, *8*) on C^{13} and C^{14} content of modern fresh-water shells. δC^{13}, δC^{14}, and Δ are defined as in Table 1, footnote. Corrections applied, Broecker and Olson (*8*, p. 177).

Sample No.	Species and location	δC^{13} (per mil)	δC^{14} (per mil)	Δ (per mil)
	Lake outlet sample			
L-487F	*Margaritifera**, Truckee River, outlet of Lake Tahoe, Calif.	−4.8	−8	−48
	Fluvial samples			
L-415F	Unidentified mollusk, Bear River, Utah	−8.4	−122	−151
L-415BB	*Anodonta*, Humboldt River, Nevada	−11.9	−189	−210

* Note that C^{13} is relatively concentrated in the shell (δC^{13} = −4.8 per mil) and depleted in the *Margaritifera* meat (δC^{13} = −22), relative to dissolved CO_2 in the water (δC^{13} = −10.2), Broecker and Olson (*8*).

The total variation of C^{14} content of all the samples is much greater than would be expected from fractionation effects. By fractionation alone, the observed 17-per-mil range of δC^{13} should be accompanied by a change of about 34 per mil in δC^{14} (Craig, *14*); the observed range of δC^{14} is about 250 per mil. Furthermore, comparison of the means and standard deviations of δC^{14} and of Δ (corrected C^{14} abundance) shows that correction for fractionation effects does not appreciably reduce the spread of the fluvial shell data nor bring them into line with the lacustrine or marine shell data. The means (and standard deviations) of the pertinent data for five fluvial shells and fourteen marine shells (Tables 1 and 2) are: fluvial shell: δC^{14}, −177 (S.D. 56); Δ, −199 (S.D. 53); marine shell: Δ, −59 (S.D. 11).

It seems probable, therefore, that the variability of carbon isotopic composition of fresh-water shells, and the extreme C^{14} deficiency of river shells, are due mainly to dilution effects rather than to fractionation effects. It is proposed that a most important dilution effect is that due to the incorporation of C^{14}-deficient carbon from ancient land-plant remains in humus.

The prior explanation, first suggested by Godwin (*10*), in which the principal dilution of modern carbon was attributed to incorporation of inactive carbon from limestones, is inadequate in two respects: (i) it is applicable mainly to regions in which limestones are abundant, and (ii) it does not explain the total carbon isotopic compositions of mollusk shells, including both C^{13} and C^{14}.

The first point, (i) above, must be qualified to the extent that ancient carbonates may be dissolved from rocks other than limestones (*9*). The effect will of course be much greater in limestone areas. Two of the samples analyzed for the present study are from areas in which limestones are abundant. One is the Lake Erie site at Put-in Bay, Ohio; shell from that site has an intermediate isotopic composition. The other is the Meramec River site in Missouri, the shell from which exhibits extreme deficiency of both C^{13} and C^{14}.

It seems clear that the grouping of the fresh-water shells by isotopic composition is mainly a function of "river environment" versus "lake environment," rather than a function of "limestone areas" versus "limestone-free" areas.

The problem posed by C^{13} content of the shells is illustrated in Fig. 1, in which the approximate range of carbon isotopic composition of atmospheric carbon dioxide is shown schematically by a rectangle at the left of the diagram, and the isotopic composition of limestones is represented by two arrows at the right of the diagram. The line of the upper arrow represents the mean C^{13} content of fresh-water limestones and the line of the lower arrow lies along the mean C^{13} content of marine limestones (unpublished data of Keith and Weber). In both cases the C^{14} content of ancient limestones is effectively zero, that is, far to the right of the C^{14} range shown on the diagram.

It will be noted that the analyzed river shells contain less C^{13} than either the atmospheric carbon reservoir or the limestone reservoir, in spite of the fact that C^{13} is *concentrated* in mollusk shells relative to dissolved bicarbonate

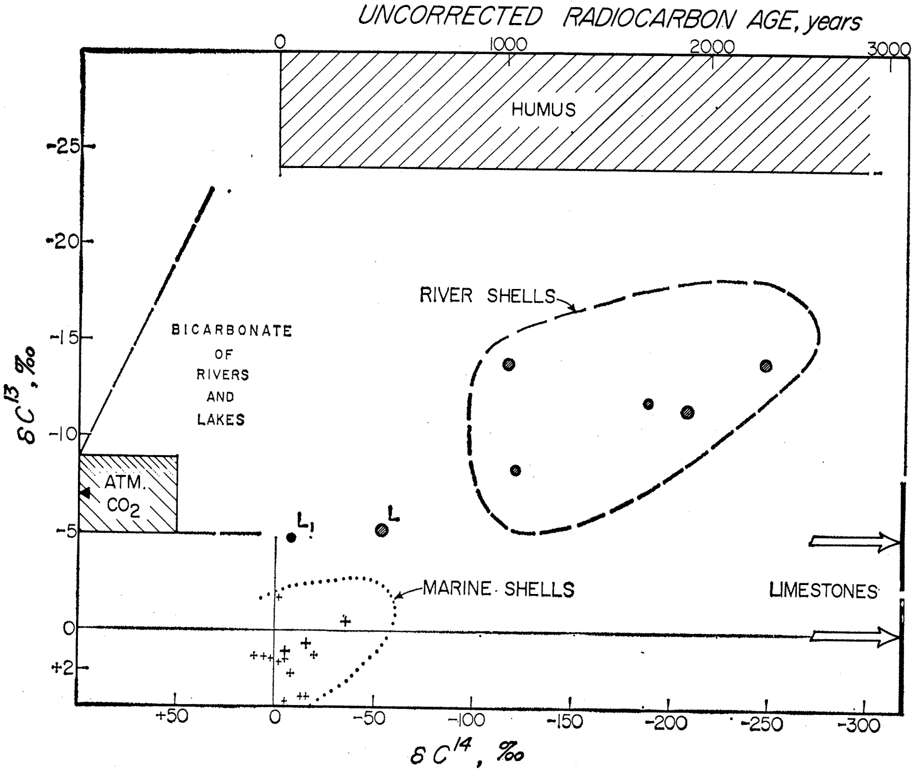

Fig. 1. Carbon isotopic composition and radiocarbon age of mollusk shells. Carbon-13 content is in per-mil difference from the PDB standard; carbon-14, in per-mil difference from contemporary wood. Specimens L and L_1 are from lake water environments (see text).

Table 3. Details of sample sites.

Sample No.	Mollusk and site
	Table 1 samples
61–155	*Strombus gigas*, Key Largo, Dade County, Florida at about 1.5 m depth (gastropod)
62–132	*Tivela stultorum*, Balboa Beach, Newport, Orange County, Calif., at about 4 m depth.
62–196	*Haliotis Fulgens*, La Jolla, San Diego County, Calif., at about 6 m depth (gastropod)
61–16	*Lampsilis siliquoidea*, Lake Erie at Put-in Bay, Ohio: (Silurian limestone and dolomite, mainly of the Lockport formation)*
61–11	*Elliptio dilatatus*, Grand River at Saranac, 20 miles east of Grand Rapids, Mich.: (L. Pennsylvanian sandstones, shale and coal of the Pottsville group)
61–127	*Actinonaias carinata*, French Creek near Meadville, northwest Pennsylvania: (Upper Devonian shale and sandstone, some glacial deposits)
62–48	*Ligumia recta latissima*, Meramec River at Meramec State Park, near Sullivan, eastern Missouri: (Cambrian and Ordovician limestone and shale)
	Table 2 samples (Broecker and Olson)
L–487F	*Margaritifera*, Truckee River, outlet of Lake Tahoe, at site near Truckee, Calif., and 10 to 15 miles downstream from the lake: (Tertiary and younger volcanic rocks)
L–415F	Unidentified mollusk, Bear River near Woodruff, northeastern Utah: (Eocene continental beds of the Wasatch formation, including sands and clays and a thick sequence of rhyolitic ash beds containing lenses of fresh-water limestone in places)
L–415BB	*Anodonta*, Humboldt River at Route 40 bridge near Dunphy, 40 miles southwest of Elko, northern Nevada: (Tertiary volcanic rocks and Quaternary alluvium)

* All Table 1 samples were collected alive in 1959 and 1960. Specimens 61–155 and 62–196 are gastropods; the others are pelecypods. The predominant rocks in the immediate vicinity of the lake and river sample sites are indicated in parentheses.

in the water; (see, for example, the footnote to Table 2). Therefore, it appears that a full explanation of carbon isotopic variation in fresh-water mollusk shells requires allowance for contributions from a third major carbon reservoir, deficient in both C^{13} and C^{14}. It is proposed that the third major source is humus, with δ C^{13} around −25 per mil or less, comparable to that of land plants, and with variable C^{14} deficiency.

Representation of the variable C^{14} content of humus (Fig. 1) is based upon the observation of several investigators (for example, *8*, *20*) that humus samples give appreciable radiocarbon ages, increasing to 3000 years and more in some deeper soil horizons.

Inactive carbon from humus can be added to fresh-water systems in two principal ways: by the pathway of carbon dioxide from humus decay, and by the incorporation of humus particles and derived materials in the food web of aquatic animals. In order to understand the resultant differences of carbon isotopic composition, it must be appreciated that large clear lakes and muddy streams represent two widely different extremes of environment. It is to be expected that there will be all intermediate gradations of chemical and biological characteristics of the water, but, in general, rivers and their biologic communities will be affected by a greater humus contribution than will lakes and lake communities. [In this context, the reason will be apparent for classifying the Truckee River sample of Broecker and Olson as a "lake outlet" sample (Table 2, and L_1 of Fig. 1). The outlet of a large, clear lake, such as Lake Tahoe, without major inflowing streams, will be dominated for many miles downstream by the chemical and biological characteristics of the lake water.]

Table 4. Principal external sources of carbon added to continental aqueous environments.

Source	δC^{13} (per mil difference from marine carbonates)	δC^{14} activity (relative to modern wood)
Atmospheric CO_2 dissolved in surface waters	−7 to −9	High
Fermentative CO_2 from bottom muds	−5	Probably varies from above to below the activity of modern wood*
Humus and derived CO_2	−25 to −30	Low and variable
Marine limestones	Zero	Extremely low, to zero
Compare seston and CO_2 from its oxidation	−30	Normal (?)

* The C^{14} activity of fermentation-produced CO_2 and of the organic material at various levels in mud profiles should be checked. A 25-per-mil enrichment of C^{13}, from $\delta = -30$ per mil in the mud to $\delta = -5$ per mil in evolved CO_2 (Oana and Deevey, *15*, p. 258), should be accompanied by an enrichment of about 50 per mil in C^{14} content of the CO_2. This may be more than offset, however, by the probable occurrence of progressively less active carbon at increasing depths in a bottom mud profile, and by the additional factor that part of the organic material of the mud may be derived from ancient humus.

Conversely, lake waters, particularly the deeper waters of stratified lakes, probably will have a greater contribution of CO_2 produced by fermentation in bottom muds. Approximate isotopic characteristics of the carbon from various external sources are shown in Table 4, in order of C^{14} activity.

Seston and the CO^2 produced by its oxidation are not included in the main part of the table because the isotopic composition of suspended and settled organic remains is a dependent variable, partly a function of external carbon contributions and partly a function of fractionation accompanying photosynthesis of plankton. Fermentative CO_2, on the other hand, although derived from bottom muds, is included in the main part of Table 4 because the anaerobic part of the mud environment does not have free interchange with the overlying water and therefore can be considered as an external source of carbon dioxide.

If the summary in Table 4 is approximately correct, it is obvious that any consideration of radiocarbon content of samples from fresh-water systems must allow for exchange rates and for contributions from all four of the principal external sources of carbon. Extreme differences in isotopic composition can result from variations in proportional contributions.

It follows that the initial C^{14} content of shell specimens and other organic materials originating in rivers or lakes cannot be exactly known or assumed. Normalization of C^{14} content by use of measured C^{13} content may be desirable in order to remove fractionation effects, but the large and variable effects due to dilution of modern carbon with inactive carbon from the humus and limestone reservoirs will remain indeterminate. Resultant errors in radiocarbon age may be as much as 3000 years for shell samples from rivers, and several hundred years for lacustrine and marine shell samples. Maximum error is to be expected in shell specimens from animals which lived in humus-laden streams which were actively cutting into old flood plains or old soil profiles (*21*).

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Radiocarbon Activity of Shells from Living Clams and Snails

As a logical extension of an artificial tracer study on snail shells (*1*), measurements were made of the natural carbon-14 activity of shells from living clams and snails collected from a variety of environments. Three samples were measured, representing growth in lime-poor, fairly soft, and lime-rich waters (Table 1). Samples were collected by D. W. Taylor in September and October 1959, and measured at the U.S. Geological Survey's radiocarbon laboratory. Sample W-1003 (*2*), from lime-poor water, had approximately 10 percent less C^{14} than the conventional modern standard (95 percent N.B.S. oxalic acid standard); W-1006 (*3*), from fairly soft water, was 22 percent deficient; and W-1004 (*4*), from lime-rich water, was 32-percent deficient. These deficiencies correspond to errors of approximately 800, 2000, and 3000 years if no correction is made for initial activity in computing sample ages. These measurements agree essentially with those of Broecker and Walton (*5*) and Keith and Anderson (*6*).

Table 1. Radiocarbon activity of shells.

Sample No.	Environment	δC^{14} (per mil)
W-1003	Spring pool in basalt, lime-poor water	−96.6
W-1006	Snake River, fairly soft water	−224.9
W-1004	Portneuf River, in limestone area, lime-rich water	−323.3

The tracer study (*1*) indicated that a snail can incorporate 10- to 12-percent inorganic carbon in making its shell and "the remainder of the carbon in the shell carbonate must come from food or the atmosphere, and the resulting C^{14}/C^{12} ratio will be essentially the same as in those sources." The control laboratory snails ate "modern" food (lettuce), whereas the natural snails ate food with a C^{14}/C^{12} ratio similar to their environment and so showed the greater deficiency in C^{14}. The limiting value of a system in contact with the atmosphere is 50-percent "dead" carbon, theoretically possible, but not yet measured anywhere. Even such a great deficiency in C^{14} as this will produce a constant error in the dating of unknown nonmarine carbonate samples of one C^{14} half life (5568 or 5730 years). Of greater significance is the postdepositional alteration and replacement of the original shell composition, which can add or subtract material. Neither visual nor mineralogic criteria can be used to predict the degree of this alteration (*7*). This can be determined only by mass spectrometric isotopic studies (*8*).

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Mutations: Incidence in Drosophila melanogaster Reared on Irradiated Medium

Abstract. *An increase in the rate of mutation has been found in* Drosophila melanogaster *reared on a basic medium that was irradiated with a sterilizing dose (150,000 rads) of cobalt-60 gamma rays. In Muller-5 tests, sex-linked recessive lethals occurred only in the* F_2 *progenies of the male test flies obtained from breeding the parent flies on irradiated medium, while visible changes occurred in experimental cultures from both the control and irradiated media. The frequency of sex-linked recessive lethals was 0.35, 0.55, and 0.8 percent in three independent experiments. Visible changes were two to six times more frequent in the irradiated series than in the controls.*

Earlier studies on the effects of irradiated culture media (White's basic medium, potato mash, and fruit juices) on mitosis in root meristems of *Hordeum*, sp. (barley), *Vicia faba* (broadbean), and *Allium cepa* (onion) have revealed that the products of such irradiation may have radiomimetic effects (*1*). In view of the obvious bearing of these data on assessing the wholesomeness of food sterilized by irradiation, we studied *Drosophila melanogaster* to ascertain whether there is an increase in mutations in flies that are fed irradiated food.

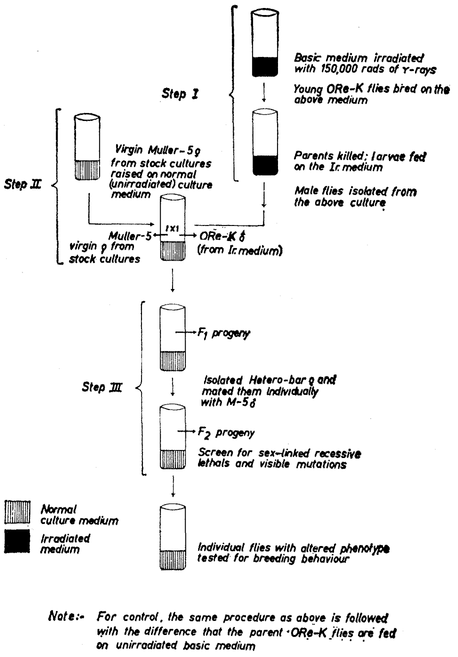

Fig. 1. Diagrammatic representation of the experimental procedure.