Natural Radiocarbon Measurements by Acetylene Counting

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During the past year a new standardized method for the determination of radiocarbon for age determinations has been developed and applied in the U.S. Geological Survey. The results of 65 determinations already carried out will soon be submitted for publication in this journal. The present report (*1*) describes the technique involving acetylene counting and the experiences with it.

The chemical steps in the preparation of the acetylene gas are the following:

$$1)\ \left.\begin{array}{ll}\text{Organic matter (wood, peat, etc.)} & +\,O_2\\ \text{Carbonate (shells)} & +\,HCl\end{array}\right\}\rightarrow CO_2.$$

2) $CO_2 + 2NH_4OH \rightarrow CO_3(NH_4)_2 + H_2O.$

3) $CO_3(NH_4)_2 + SrCl_2 \rightarrow SrCO_3 + 2NH_4Cl.$

4) $2SrCO_3 + 5Mg \rightarrow SrC_2 + 5MgO + SrO.$

5) $SrC_2 + 2H_2O \rightarrow C_2H_2 + Sr(OH)_2.$

Steps 1 and 2 are carried out in essentially the manner described by Libby (*2*) but using one-third to one-half of the quantities. Instead of calcium chloride, strontium chloride is used in step 3 to precipitate the carbonate. Barium chloride can be used equally well, but preference was given to strontium chloride because of the possibility of a high radium content in commercial barium chloride (*3*).

Strontium carbonate, after washing and drying, can be directly reduced with an excess of magnesium powder (80 mesh) to strontium carbide. If enough material is available, a mixture of 30 g of $SrCO_3$ to 35 g of magnesium is used, yielding about 2.2 lit STP of C_2H_2 corresponding to a yield of 90 to 100 percent. The reaction is carried out inside an evacuated stainless-steel tube (2.8 by 38 cm) connected directly to a vacuum line through an O-ring seal. The mixture is ignited from the outside with a torch, and the reaction is completed in less than 5 min. The reaction product is then dumped into about 1 lit of water inside an evacuated system. The gases formed are dried by passing them through a trap cooled by an acetone mixture and a Drierite column. The acetylene is condensed in a liquid nitrogen trap, and some hydrogen that also forms is pumped off intermittently. After purification by passing over cooled charcoal, the acetylene is stored for 2 to 3 wk to permit the decay of the few hundred radon atoms that are sometimes present in the gas sample.

The gas counter is connected to a vacuum line. Its case and the "two mil" center wire is of stainless steel. For the past 9 mo no air has been admitted inside the counter. The inside diameter is 7 cm, and the effective volume is almost exactly 1 lit. It is surrounded by a mercury mantle of thickness 1.5 cm, by 11 cosmic-ray counters (2 by 20 in.), and by an iron shield of thickness 21 cm such as is described by Libby (*2*).

The principle of the low-level counting technique applied is that developed by Libby (*2*), which is the elimination of most of the cosmic-ray background by a ring of cosmic-ray counters in anticoincidence with the sample counter. The linear amplifier of a NICC methane counter model 117 is used for the amplification of the pulses. These are subsequently fed into an RCL anticoincidence circuit Mark 15 model 40 and registered. For control purposes, the total counts in the sample counter and in the 11 anticoincidence counters are also counted continuously. A separate negative high-voltage supply keeps the counter case at a potential of − 2000 v against ground. The case is insulated by a Teflon sheet. The center wire obtains a positive voltage of 3000 v from the NICC counter so that the resulting total counting voltage is 5000 v. A 1000-w Sola constant-voltage transformer is used to maintain constant line voltage.

Table 1 gives the counts obtained with acetylene made from contemporaneous material and from coal. According to Libby (*2*), the specific activity of contemporaneous wood carbon is 15.3 ± 0.1 disintegrations/min. The effective counter volume contains 0.918 g of carbon in the form of acetylene and, therefore, 75.5 percent of the C^{14} disintegrations are recorded. Measurements for calibration purposes were combined in an effort to detect effects from industrial coal combustion on the specific activity of contemporaneous carbon (*4*). This work has not yet been concluded, and the results will be published at a later date.

Sample W-9 was remeasured during the 4 mo from Oct. 1953 to Mar. 1954 at intervals of 3 to 4 wk, and the four coal samples were measured during the same period of time. Table 2 shows the internal consistency of the individual readings. From this it appears that the observed fluctuations do not appreciably exceed the fluctuations to be expected from statistics. The number of counts in the sample counter in coincidence with the surrounding cosmic-ray counters varies between the extremes of 107 and 113 counts/min. The variations clearly show a period of 27 days, corresponding to the period of the solar component of cosmic radiation. The expectation that this variation of the cosmic-ray flux would reflect in the counter

Table 1. Total counts per minute and statistical standard error σ, determined in a counter of 1 lit effective volume filled with acetylene at a pressure of 72.3 cm of Hg made from contemporaneous and from "dead" carbon. Counting voltage 5000 v. The C^{13} concentration in the counter gas in terms of the deviation from limestone standard δ was determined by Harmon Craig.

Sample	Description	Age or years of growth from tree rings	Total counts/min	σ counts/min	δC^{13} percent deviation from limestone standard (*5*)
W- 9	Hemlock, New Hampshire, cut 1950, obtained from E. G. Hutchinson	1840 to 1845	12.938	0.015	−2.68
W-13	Same tree as W-9	1945 to 1950	12.73	.05	−2.85
W-24	Spruce, Moses Point, Alaska, T. L. Péwé, coll., 1950	1890 to 1900	12.87	.05	−3.01
W-23	Same tree as W-24	1939 to 1950	12.75	.05	−3.81
W-14	*Venus mercenaria* shells, Nantucket Sound, coll. alive in 45 ft of water, H. J. Turner, coll.	1953	12.98	.05	−0.85
W-39	*Mya* shells, Sagadahoc Bay, Maine, W. H. Bradley, coll.	1950	12.82	.05	
W-25	Red Abalone shell, Santo Tomas, Baja California	1950	12.83	.05	
W-41	Land snail shells, *Triodepsis albilabris*, Ansonia, Conn., Barry McGuire, coll.	1952	12.62	.05	−2.61
W-34	Bituminous coal, Kentucky	Carboniferous	2.328	.04	
W-63	Same as W-34	Carboniferous	2.370	.03	
W-70	Lignite, Washington, D.C.	Cretaceous	2.325	.05	
W-80	Same as W-70	Cretaceous	2.387	.03	
	Average for coal		2.342	.012	

background seemed to be verified by initial runs, but it was not confirmed by later measurements. There is no noticeable correlation between coincidence counts and counter background.

In addition to the statistical counting error, the possibility of errors due to isotope fractionation and of contamination with carbon from atmospheric CO_2 in the chemical steps of sample preparation has to be considered. Craig (*5*), who has carried out mass-spectrometric C^{13} determinations of six acetylene samples, has found that a depletion of the heavier isotopes occurs, corresponding to a loss of C^{14} ranging from 1.5 to 3.5 percent of C^{14}. This loss will introduce the possibility of an error in the age of a sample of about ± 80 yr. Such an error is insignificant for old samples but may be important for precision measurements on young materials. A mass-spectrometric C^{13} determination is then required. The possibility of exchange with or adsorption of atmospheric CO_2 was demonstrated by allowing $SrCO_3$ prepared from coal to stand for 3 days in the open air. The acetylene prepared from it gave a counting rate 0.2 count/min above background. Although effects of that magnitude can easily be avoided, particular care has to be taken to avoid contamination with contemporaneous carbon in the case of very old samples.

Table 2. Deviation from the mean counting rate of all the individual readings taken, after counting times ranging from about 100 to 1000 min expressed in terms of the standard counting error of the individual readings. The figures in parentheses give the theoretical statistical expectation. None of the readings deviated by more than 3 σ.

Sample	Total no. readings	No. readings deviating from mean by		
		Less than 1 σ	More than 1 σ	More than 2 σ
Coal	32	21 (21.6)	11 (10.4)	0 (1.5)
W-9	19	14 (12.8)	5 (6.1)	3 (0.9)

The contamination of the counter gas with a few hundred radon atoms can occasionally be observed, which may introduce up to 0.1 count/min. Contamination with alpha activities, however, can easily be controlled by observing the alpha disintegrations at a reduced voltage of about 4000 v. The counting rate is surprisingly insensitive to traces of impurities such as air or CO_2 in the counter gas.

Comparison with other methods for C^{14} age determinations show (Table 3) that scintillation counting under optimal conditions will give results of greater precision with equal counting times. Compared with scintillation counting, however, the method described here has the following advantages: (i) a small amount of sample material is required; (ii) the acetylene

Table 3. Comparison of the different methods for the determination of natural radiocarbon (*6*).

	Solid carbon screen-wall counter (*2*)	Liquid paraffin 100 ml scintillation counter (*6*)	Gas counting 1 lit acetylene (*7*)
Sample size (wt. of carbon)	8 g	47 g	1 g
Counting efficiency (percent)	5.4	25	75
Net background (counts/min)	4	26	2.34
Net counts/min from modern carbon	6.7	182	10.72
Statistical error for modern carbon in years after 2 days of counting	± 120	± 17	± 50
Maximum age limit in years (4 σ) 2 days of counting	25,000	44,000	38,000

preparation requires less than 12 man-hours of labor; and (iii) the remarkable stability of the counting system and the reproducibility of the absolute counting rates eliminate the need for more than one check run every 2 to 3 wk.

Thanks are due to W. F. Libby for much helpful advice in general matters of C^{14} counting, and to N. Sugarman for valuable discussions on proportional counting.

References and Notes

1. Publication authorized by the director, U.S. Geological Survey.
2. W. F. Libby, *Radiocarbon Dating* (University of Chicago Press, Chicago, 1952).
3. W. R. Champion, U.S. Geological Survey, has kindly carried out radium determinations of commercial $SrCl_2$ and $BaCl_2$ and found the $BaCl_2$ usually about 100 times higher in Ra than the $SrCl_2$, which contains about 10^{-14} g Ra per gram $SrCl_2$.
4. H. E. Suess, paper presented at the NSF conference, Oct. 1953, Williams Bay, Wis.
5. Harmon Craig, *Geochim. et Cosmochim. Acta* **3**, 53 (1953).
6. See also J. R. Arnold, *Science* **113**, 155 (1954).
7. While this work was in progress, A. R. Crathorn [*Nature* **172**, 632 (1953)] attempted the use of acetylene as a counter gas for C^{14} determinations. For unknown reasons, however, a background of 30 counts/min was obtained with a 3-lit counter. This makes the use of his particular system impractical.

Metallo-Flavoproteins and Electron Transport

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ALTHOUGH the importance of metals in biological oxidations has been generally recognized (*1, 2*), there have been, until very recently, relatively few documented instances of the presence of metals in those oxidizing enzymes that have been isolated and characterized. Copper has been found to be the functional group of monophenol oxidase (tyrosinase) (*3*), polyphenol oxidase (laccase) (*4*), and ascorbic oxidase (*5*), while iron in the form of its porphyrin complex has been established as the functional group of catalase (*6*), peroxidase (*7*), yeast lactic dehydrogenase (*8*), and the cytochromes (*1, 9, 10*). This article summarizes some recent developments that provide additional confirmation of the pivotal role of metals in electron transport.

Metallo-flavoproteins are a group of enzymes containing both metal and flavin in firm linkage with the protein and in definite proportions. Thus far, seven enzymes have been characterized as metallo-flavoproteins, and three metals—copper, iron, and molybdenum—have been implicated as the functional metal. For simplicity of presentation, it may be desirable first to describe the general catalytic properties of each of these flavoprotein enzymes before considering the special function of the metal in the electron-transport sequence.

Acyl CoA dehydrogenases. Animal tissues contain two enzymes that catalyze the reversible oxidation of fatty acyl CoA's to their corresponding trans α,β-unsaturated derivatives (*11, 12*). Both have now been isolated from beef liver mitochondria in essentially homogenous state. One, which we shall refer to in this article as the *green enzyme,* is specific for the short-chain acyl CoA's from C_3 to C_8, while the other, which we shall refer to as the *yellow enzyme,* is active on all acyl CoA's from C_4 to C_{18}. Both enzymes contain flavinadenine dinucleotide as prosthetic flavin but differ in the nature of the prosthetic metal. The green enzyme contains copper (2 atoms per molecule of flavin), while the yellow enzyme may contain iron.

The absorption spectrum of the green enzyme shows the characteristic flavin bands at 355 and 432 mμ and, in addition, a new band at 680 mμ which is referable to copper or its appropriate complex. The deep green color of the enzyme stems from this band in the red end of the spectrum. When the enzyme is reduced by its substrate, both the flavin and copper bands disappear, and they reappear when the reduced enzyme is oxidized by molecular oxygen or other oxidizing agents. The absorption spectrum of the enzyme after removal of copper by mild procedures closely resembles that of a typical flavoprotein—that is, a flavo-