Reports

Carbon-14 Dating with the Liquid Scintillation Counter: Total Synthesis of the Benzene Solvent

Abstract. Samples are analyzed for natural radiocarbon content by a total synthesis of benzene from their organic constituents. The benzene is employed as the solvent in a liquid scintillation counter. The instrument used permits 15 grams of carbon to be counted with an efficiency of 40 percent and a background of 13 counts per minute.

The discovery of the method of radiocarbon dating by W. F. Libby and his associates in 1947 has been recognized as a very important event in many branches of science, especially archeology and geology. Analysis of organic materials for their C^{14} activity allowed determination of ages of many thousands of years. However, the radioactivities measured are fairly low, and elaborate procedures of sample preparation and counting must be followed in order to obtain significant results (*1*). Libby's original method of producing samples to be counted as solid elemental carbon was improved upon by the introduction of the organic material in the form of a gas directly into a proportional region counting tube. This type of procedure has allowed the sensitivity of the method to be pushed to 50,000 years (*2*).

More recently, the introduction of the liquid scintillation counter has led to the development of several dating methods in which preparation of liquid materials from the organic samples to be dated are utilized (*3*). Liquid scintillation counting has the advantage of using liquid samples which are much easier to handle and purify than the gaseous or solid materials that are necessary in the other counting arrangements. Also, it is possible to submit more carbon to analysis as compact liquids than as the much less dense gases. The disadvantages of present radiocarbon dating by liquid scintillation counting methods are that the preparation of the samples is more complicated and less efficient than the gas methods and that it has not been possible to synthesize the solvent completely, in reasonable yields, from the carbons in the material to be analyzed. The procedures now described in the literature require either dilution of the ordinary scintillator solvent system with another miscible liquid that has been prepared from the specimen to be dated or modification of existing organic compounds with carbon dioxide whose natural radiocarbon content is to be analyzed. Both of these conditions lessen the effectiveness of the basic method.

A liquid scintillation procedure has been worked out which improves the usefulness of radiocarbon dating. The method provides a solvent which contains 92 percent carbon, all of which is obtained from the sample to be dated. The solvent is benzene and the steps of the total synthesis employed in the method are listed below:

$$\text{Organic material} + O_2 \rightarrow CO_2 \quad (1)$$

$$CO_2 + 2NH_4OH \rightarrow (NH_4)_2CO_3 + H_2O \quad (2)$$

$$(NH_4)_2CO_3 + BaCl_2 \rightarrow BaCO_3 + 2NH_4Cl \quad (3)$$

$$2BaCO_3 + 5Mg \rightarrow BaC_2 + BaO + 5MgO \quad (4)$$

$$BaC_2 + 2H_2O \rightarrow C_2H_2 + Ba(OH)_2 \quad (5)$$

$$3C_2H_2 \rightarrow C_6H_6 \text{ (at } 650°C) \quad (6)$$

Steps 1 through 5 are a standard procedure for the production of acetylene (*4*). Step 4 was changed in that the reaction is carried out in a helium atmosphere rather than in a vacuum. The violence of the exothermic reaction varied so widely with the batch of powdered magnesium used that it was impossible to assure that the closures on the stainless steel tube would not blow out during the reaction. The procedure actually followed was to first evacuate the tube with the mixed powder, then introduce helium gas and continue it flowing through, and finally to ignite the open end with a magnesium ribbon fuse. In step 6 the acetylene was circulated repeatedly through a Vycor tube heated to 650°C, and the pyrolysis products were trapped out by a trap cooled by dry ice and located in the circuit. Most of the liquid collected in the trap is benzene. After all the acetylene has been subjected to the heated tube the organic liquids in the trap are separated by a fractional distillation. The benzene is further purified by two more distillations and the final yield, at this time, is approximately 10 percent. It is anticipated that the yield can be substantially improved with further work. This material is then used as the solvent, and small amounts of PPO (2,5-diphenyloxazole) and POPOP [1,4-di(2-(5-phenyloxazolyl))benzene] are added as scintillator and wave shifter, respectively. In those cases where only a limited quantity of the material to be dated is available it is reasonable to use what benzene can be obtained and then to bring the solvent system up to volume with ordinary laboratory benzene, which is ancient in comparison to the 5568-year half-life of C^{14} and will not, therefore, contribute to the activity observed. Radon and fission product contamination are effectively removed by the distillations of the benzene and do not constitute a problem.

The counter used was a Tri-Carb liquid scintillation spectrometer (*5*). This instrument utilizes two matched photomultipliers connected to a coincidence analyzer that rejects most of the pulses that have their origin in electronic noise, largely within the photomultipliers. The noise level is further reduced by cooling these tubes to 6°C, which is just above the freezing point of benzene. The spectrometer is also equipped with two channels and scaling units, which were set to view the same portion of the spectrum and can be used in this way as continuous checks on the proper operation of the instrument. Windows permitting approximately 40 percent of the C^{14} beta particles to be counted were used and the background was about 13 count/min. This will eventually be lowered by use of quartz sample vials and more shielding. At this time the sample chamber is arranged to fit a counting vial that holds 20 ml, but this volume may be increased to 100 ml with minor modifications.

The method of benzene synthesis presented here is at least as simple as the other liquid scintillation counter methods described in the literature. It is inherently more sensitive than any of

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

the gas-counting methods and those liquid counting methods which include carbon that does not come from the sample being dated. Furthermore, the benzene method does not require a high vacuum rack or liquid nitrogen, which is not available in most places in the world.

The existing assembly is now capable of providing radiocarbon dates. Work is in progress to set up the operation on a routine basis and to increase the efficiency of the steps in the procedure to increase the yield. It is anticipated that the background can also be reduced considerably (*6*).

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References and Notes

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6. Assistance from the Robert A. Welch Foundation of Houston, Tex., is acknowledged.

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Effects of Temperature and Anions on Titration Curves of Frog Muscle

Abstract. Titration curves of frog muscle at 2°C and 25°C were determined in vivo. Lowered temperature decreases respiration without change of colloidal charge or of ion distribution as estimated from charge. Anions which are inhibitors or metabolites combine with muscle colloids, changing the distribution of other anions and cations.

Biological structures are amphoteric colloids which combine with organic and inorganic anions and cations, as well as with hydrogen ions. These ion-binding properties apply both to soluble and insoluble proteins (*1*). They have been studied in titration curves of colloidal surfaces (*2*). Many anions are strongly reactive and have marked effects on the titration curves of proteins and enzymes and on the binding of other anions and cations. The reactive anions include metabolic substrates as well as inhibitors. The effects are especially strong in reactions with insoluble protein aggregates.

Enzymes may occur in solution or as components of insoluble colloidal aggregates (*3*). According to the Michaelis-Menten formulation, combination with ions or molecules conforms to the mass action law, and is a condition for reactions with substrates or inhibitors (*4*). From these considerations, combinations with such substances would produce changes in electrolyte distribution simultaneously with changes in enzyme reactions. Rates of respiration and states of ion distribution both become dependent on the physicochemical state of the colloid and the chemical composition of the environment. It follows that the relation between cellular respiration and ion distribution is not direct, but that it involves colloidal structures of varying physicochemical states.

Experimentally, respiration may be changed by inhibitors or by temperature. In the first case, states of intracellular and extracellular colloids are simultaneously changed. Temperature changes, however, need not change the colloid, especially in *p*H ranges where protein titration curves are nearly independent of temperature. Experiments on the influences of temperature and of 2-4-dinitrophenol on the surface titration curves of frog muscle in vivo are reported here.

Fourteen frogs (*Rana pipiens*) were studied, seven at 25°C and seven in a cold room at 2°C. The frogs were pithed and the anterior thigh muscles were exposed. The overlying perimysium was scraped off to expose the sarcoplasm of the muscle fibres as confirmed by histologic examination. An electrometric method was used to determine the density of charge of the colloidal surface at a series of *p*H's between 7.4 and 2.2 (*5*). This is equivalent to a titration curve of the surface (*2*). The curve was then redetermined with the same series of isotonic saline solutions to which had been added 2-4-dinitrophenol at a concentration of 0.001*M*. The effects of *dl*-lactate, bicarbonate ion, sodium iodoacetate, and sodium cyanide were determined at *p*H 7.4 (Table 1). The compounds were chemically pure or reagent grade preparations.

In the method, described elsewhere in detail (*2*), two potential differences are determined between a reference site and the muscle surface. The reference junction is established by placing one leg in a 0.15*M* NaCl solution, while at the experimental site a solution of buffered 0.15*M* NaCl is applied by means of a moistened cotton pellet. Both reference and experimental junctions are connected through KCl-agar junctions to saturated KCl-calomel half cells, and a potential, E_1, is measured. A 0.015*M* NaCl solution is then applied at the experimental site, and the potential E_2 is measured. The difference $(E_2 - E_1)$ is termed the dilution potential, E_d. The displacement of potential observed with the dilute solution is expressed in millivolts. Depending on the sign of the colloidal charge, it is related to the charge density as follows:

$$E_d = E_d^0 + kx \qquad (1)$$

where x is negative colloidal charge, expressed as equivalents per kilogram of water (above the isoelectric point), or

$$E_d = E_d^0 - ky \qquad (2)$$

where y is the positive charge (below the isoelectric point) expressed in the same way. E_d° is the theoretical dilution potential at zero colloidal charge, and k is a constant which depends on temperature (*6*). Equations 1 and 2 have been derived for amphoteric colloidal surfaces consisting of one or more colloidal components. Potential differences refer to macroscopic sections of the muscle surface of about 1 to 4 mm² (*2*). These contain heterogeneous components of the sarcoplasm.

Results of the potentiometric determinations are presented (Fig. 1, Table 1). Values of x and y at two temperatures are plotted against *p*H to yield titration curves of the surface. The points have been fitted with the same curve, which shows an isoelectric point of about 3.25 (zero x and y). Curves observed with 0.001*M* dinitrophenol have likewise been plotted at two temperatures. These show displacements toward low levels of x; the displacements at 2°C are greater than those at 25°C. They resemble the displacements caused by picric acid at connective tis-

Table 1. Effects of anions on colloidal charge of muscle surface at 25° and 2°C. Change of colloid charge density values are means and change of potential values are means and standard deviations, of four to seven measurements.

Concn.* (*M*)	Temp. (°C)	Change of potential ($E_d - E_d'$)† (mv)	Change of colloid charge density ($x - x'$)‡ (equiv/kg water)
		2-4-Dinitrophenol	
0.001	25	4.4 ± 1.1	0.022
0.001	2	6.2 ± 0.6	.034
		Iodoacetate	
0.001	25	4.0 ± 0.5	0.020
0.01	25	4.6 ± 0.4	.023
0.01	2	6.0 ± 1.3	.030
		dl-*Lactate*	
0.01	25	3.4 ± 0.9	0.017
0.01	2	3.5 ± 0.3	.019
		NaCN	
0.001	25	3.9 ± 0.5	0.020
0.01	25	4.6 ± 0.2	.023
0.01	2	4.7 ± 1.1	.026
		$NaHCO_3$	
0.025	25	3.9 ± 0.3	0.020

* In isotonic NaCl-phosphate buffer at *p*H 7.4. † E_d is control value of dilution potential (−1.2 mv at 25°, and −1.6 mv at 2°). E_d' is the value of dilution potential in presence of given solution. ‡ Value of $(x - x')$, the change of colloid charge density, was calculated from Eq. 1, with the following values of k: 197 at 25°C, and 182 at 2°C.