Reports and Letters

Improved Method for Isolation of Adenosine Di- and Triphosphates

The technique used by Albaum (*1*) for isolation of adenosine polyphosphates from higher plants has been applied in the present studies to conidia of *Neurospora sitophila* (Mont.) Shear and Dodge. In order to extract acid-soluble phosphates quantitatively from conidia, however, it was necessary to repeat the extraction five or six times with three volumes of 5-percent trichloroacetic acid (TCA). This resulted in a large volume of liquid and very dilute solutions of phosphates. Under these conditions, 50 percent or more of the orthophosphate and adenosine diphosphate present in the original TCA extracts was lost in the barium isolation procedure. No efficient means have been found for complete breakage of the conidia; therefore, the volume of TCA could not be diminished by homogenation, which is possible when many other source materials are used.

A search was made, therefore, for a cation that would precipitate phosphates more nearly completely than barium in dilute solutions and that would still lend itself to convenient isolation and purification of adenosine triphosphate (ATP) and adenosine diphosphate (ADP) by adsorption on anionic exchange resin by the method of Cohn and Carter (*2*). Of the several cations tested, Zn^{++} was found to be most suitable. With zinc, the step involving dissolution of the original precipitate in HCl could be eliminated because the zinc phosphates are soluble in 1*N* NH_4OH, and zinc, in this solution, passes readily through the resin column, whereas orthophosphate, ATP and ADP ions are adsorbed.

For the purpose of comparing zinc and barium as precipitating agents, isolation of phosphates from one-half of TCA extracts, usually about 200 ml, was done with barium as described by Albaum (*1*), and from the other half with zinc as follows. Five milliliters of 25-percent zinc acetate solution were added to the extract. The solution was cooled to 0°C and the precipitate was collected by centrifugation. The precipitate was washed once with 15 ml of cold distilled water to remove excess TCA and then resedimented. It was dissolved in 15 ml of 1*N* NH_4OH and passed several times through a 2- to 3-cm column of Dowex No. 1 anionic exchange resin in a 100-ml burette. This was followed by two 15-ml portions of 1*N* NH_4OH to wash out the zinc. NH_4OH was removed by several 15-ml portions of distilled water. Adenosine monophosphate (AMP), ADP, and ATP were eluted differentially according to the method of Cohn and Carter (*2*) and measured spectrophotometrically at 260 mμ using adenylic acid as the standard. Most of the inorganic orthophosphate was eluted along with ADP and was measured by the colorimetric method of Lowry and Lopez (*3*), with which inorganic phosphate can be determined in the presence of labile phosphate esters.

Data are given in Table 1 from two experiments in which the yields of orthophosphate, AMP, ADP, and ATP were compared when the two procedures were used. The zinc precipitation method yielded two to nine times as much of the various phosphate fractions in the final analyses as did the barium method. Precipitation of the phosphates with zinc revealed that ADP and ATP were present in conidia in essentially equal quantities, whereas precipitation with barium would lead one to the conclusion that ADP was present in much smaller quantities than ATP. The ratio of ADP to ATP in conidia was thus considerably higher than that found in other tissues where barium precipitation was used (*1, 4*). It is possible, since prolonged extraction with TCA was necessary, that some ATP was hydrolyzed to ADP. However, the yield of ATP was also always greater with zinc than it was with barium; hydrolysis of zinc-ATP would not account for the observed differences in ADP.

Table 1. Comparative yields of inorganic phosphate, AMP, ADP, and ATP from conidia of *Neurospora sitophila* with barium and zinc as precipitating agents. In experiment No. 1, 4.78 g (dry weight) of spores were extracted; in experiment No. 2, 9.56 g (dry weight) of spores were extracted.

Compound	Expt. 1 Barium (μ*M*)	Expt. 1 Zinc (μ*M*)	Expt. 2 Barium (μ*M*)	Expt. 2 Zinc (μ*M*)
Inorganic phosphate*	20.1	108.6	169.5	407.6
AMP	Trace	Trace	Trace	Trace
ADP	0.5	3.0	1.9	17.5
ATP	2.6	3.9	6.8	15.9

* Calculated as phosphoric acid.

The identities of ADP and ATP were confirmed by paper chromatograms developed according to the method of Cohn and Carter (*2*). Both compounds exhibited the same chromatographic properties as yeast ATP and ADP.

It would appear that use of zinc in place of barium might give a better quantitative value for ATP and ADP in tissues and cells other than fungus spores, especially when the quantity of cells is small and when large volumes of TCA must be used for quantitative extraction of the phosphates. A complete report on the application of the modified method as described here to studies on phosphorus metabolism in germinating fungus spores will be submitted for publication elsewhere.

Robert G. Owens
Boyce Thompson Institute for Plant Research, Yonkers, New York

References

1. H. G. Albaum, in *The Biology of Phosphorus* (Michigan State College Press, East Lansing, 1952), p. 37.
2. W. E. Cohn and C. E. Carter, *J. Am. Chem. Soc.* **72**, 4273 (1950).
3. O. H. Lowry and J. A. Lopez, *J. Biol. Chem.* **162**, 421 (1946).
4. W. W. Umbreit, R. H. Burris, J. F. Stauffer, *Manometric Techniques and Related Methods for the Study of Tissue Metabolism* (Burgess, Minneapolis, Minn., 1945).

9 May 1955

Radiocarbon Concentration in Modern Wood

Redeterminations of the absolute C^{14} concentration in wood carbon and of its variations since the industrial revolution became widespread in the late 19th century were carried out (*1*) by means of proportional counting of acetylene as described previously (*2*). No direct determination of the counting efficiency was made, but the counting rates were compared with those from material containing a mass-spectrometrically determined amount of artificial C^{14}, obtained from the National Bureau of Standards through the courtesy of H. H. Seliger.

Indications of a decrease in the specific C^{14} activity of wood at time of growth during the past 50 yr had been found previously (*3*). The decrease amounted to about 3.4 percent in two trees from the east coast of the United States. A third tree, from Alaska, investigated at that time, showed a smaller effect. The decrease can be attributed to the introduction of a certain amount of C^{14}-free CO_2 into the atmosphere by artificial coal and oil combustion and to the rate of isotopic exchange between atmospheric

Table 1. Relative carbon-13 and carbon-14 concentration (*9*).

Sample	Lab. No.	Years of growth, annual rings	δC^{13} ± 0.01 (%)	δC^{14} corrected for age (%)	$\left[\frac{1+\delta C^{14}}{1+2\delta C^{13}}-1\right]\times 10^2$, corrected for age
Spruce, Alaska, from Moses Point area	W-213	1875–1880	− 1.14	− 2.15	+ 0.12 ± 0.2
	W-212	1945–1950	− 1.89	− 5.48	− 1.77 ± 0.3
White pine, Harvard Forest near Petersham, Mass.	W-206	1840–1850	− 0.41	− 0.93	− 0.12 ± 0.2
	W-208	1936–1946	− 0.08	− 3.55	− 3.40 ± 0.3
	W-207	1946–1953	+ 0.07	− 2.76	− 2.89 ± 0.3
Incense cedar, Yosemite Park, Calif., from 4000 ft elevation	W-214	1895–1900	− 0.21	− 0.32	+ 0.10 ± 0.3
	W-216	1940–1944	− 0.50	− 2.93	− 1.85 ± 0.3
	W-215	1950–1953	− 0.51	− 2.07	− 1.05 ± 0.3
Cedrela, Peruvian Amazon region, altitude 1000 ft	W-218	1890–1896	− 0.30	− 0.69	− 0.10 ± 0.3
	W-219	1943–1946	− 0.51	− 1.07	− 0.05 ± 0.4
	W-217	1948–1953	− 0.32	− 1.70	− 1.05 ± 0.4
Venus mercenaria, Nantucket Sound, collected alive in 45 ft of water	W-209 (flesh)		+ 0.11	− 6.57	− 6.79 ± 0.5
	W-210 (shells)		− 1.33	− 2.95	− 5.45 ± 0.5
Sargassum from sea surface 36° 24′N, 69° 37′W	W-211 (weed)		+ 0.85	− 2.32	− 3.95 ± 0.5

CO_2 and the bicarbonate dissolved in the oceans.

In order to obtain more quantitative data concerning the effect, mass-spectrometric C^{13} determinations were made and used to correct for isotope fractionation in nature and in the laboratory. For this purpose a few cubic centimeters of C_2H_2 of each sample were converted to CO_2 by recycling over hot CuO and sent to Urey's laboratory at the University of Chicago, where Harmon Craig kindly undertook the mass-spectrometer measurements.

Eleven samples of wood from four different trees, each sample consisting of a small range of annual rings, were investigated; for comparison, three samples of marine carbon were also measured. Two independent sets of counting equipment were used. By repeated counting of the samples for 48 hr in alternating sequence, it was possible to correct for gradual changes in the counting sensitivity of the counters. The total counting time for these determinations amounted to 4 mo, and the number of counts observed ranged from 10^5 to 10^6 for each sample.

Table 1 lists the observed C^{13} and C^{14} concentrations for the individual samples relative to respective standard concentrations. The C^{13} standard is arbitrarily chosen as 2.5 percent below the Chicago C^{13} standard (*4*), approximately the average C^{13} value of wood. In other words, in defining

$$\delta C^{13} = \left[\frac{C^{13}/C^{12}_{\text{sample}}}{C^{13}/C^{12}_{\text{standard}}} - 1\right] \times 100,$$

the ratio of the standard is taken as equal to 0.975 times the ratio of Craig's standard and approximately equal to 0.01096. Column four of Table 1 lists, in percentages, the observed deviations in the C^{13} concentration of the combusted C_2H_2 samples from this standard.

Correspondingly, δC^{14} is the measured C^{14}/C^{12} enrichment with respect to a C^{14} standard. The C^{14}/C^{12} ratio of the standard chosen here is the average of four values of

$$R/1 + 2\delta C^{13},$$

where R is the measured C^{14}/C^{12} ratio in pre-1900 wood, corrected for age. The four values are those of samples W-206, W-213, W-214 and W-218. The absolute value of this ratio can be calculated from the C^{14}/total C ratio given in Table 2. This gives

$$C^{14}/C^{13}_{\text{standard}} = 1.252 \times 10^{-12}$$

Column five lists, in percentages, the observed deviation in the C^{14} concentration, corrected for age, from the C^{14} standard as defined in the previous paragraph. Deviations of the C^{14} concentrations normalized to standard C^{13} content are listed in column six, again using the fact that isotopic fractionation, either in nature or in the laboratory, affects C^{14} twice as much as it does C^{13}.

The four samples of 19th century wood gave results (after correction for age and C^{13} variation) that deviated by not more than 0.12 percent from their mean. In particular, the lower C^{14} concentration observed in the Alaskan spruce sample is paralleled by a correspondingly low C^{13} content, so that after taking into account the depletion of the heavier carbon isotopes, the same activity is derived as for the other three determinations.

Results from wood grown more recently, however, show marked variations, always in the direction of a lower C^{14} content. The tree from the east coast of the United States shows the largest effect, and the results are in good agreement with those previously reported for wood from that area (*2*). The smaller effects noted in the other three trees indicate relatively large local variations of CO_2 in the atmosphere derived from industrial coal combustion, and that the worldwide contamination of the earth's atmosphere with artificial CO_2 probably amounts to less than 1 percent. Hence the rate by which this CO_2 exchanges and is absorbed by the oceans must be greater than previously assumed.

At present it is not possible to make conclusive interpretations concerning the reasons for the differences (in excess of experimental uncertainties) between individual samples grown at the same time.

Carbon of marine origin is known to show a lower C^{14} content than expected when one assumes complete equilibration with the atmosphere (*3, 4*). No satisfactory explanation can yet be given for this fact.

In order to obtain the absolute value of the C^{14} concentrations, two samples of known C^{14} content were counted for comparison. A solution of Na_2CO_3 containing $(7.29 \pm 0.15) \times 10^{-13}$ g of C^{14} per

Table 2. Absolute carbon-14 concentrations. The results indicate counting efficiencies of approximately 70 percent for counter I and 90 percent for counter II, assuming a C^{14} half life of 5568 yr.

Measurement	Counts/min		C^{14} total C (atoms)	
	Counter I	Counter II	From NBS determination	From counting rate
C^{14} standard: corrected average of 4 wood samples	11.969 ± 0.03	15.759 ± 0.10		$(1.238 \pm 0.03) \times 10^{-12}$
Background from "dead" carbon	2.061 ± 0.02	2.267 ± 0.05		
Spiked carbonate				
Sample 1	22.230 ± 0.10	29.379 ± 0.15	$(2.472 \pm 0.05) \times 10^{-12}$	
Sample 2	30.857 ± 0.15	41.253 ± 0.25	$(3.577 \pm 0.07) \times 10^{-12}$	
Background from "blank" carbonate	2.407 ± 0.03	2.754 ± 0.05		

milliliter was obtained from the National Bureau of Standards. The solution was prepared from a carbonate with a C^{14} ratio determined mass-spectrometrically at four different laboratories with results agreeing within 3 percent. Ten milliliters and 15 ml of this solution were added to 22.335 g and 23.148 g of Na_2CO_3, respectively, for conversion to C_2H_2. A third sample, serving as a blank, was prepared from the same carbonate without the addition of a spike. The results are listed in Table 2.

Assuming 5568 yr for the C^{14} half life (*5*), one obtains from the C^{14} ratio of $(1.238 \pm 0.03) \times 10^{-12}$ for standard wood carbon, a specific activity of 14.7 ± 0.4 disintegrations/min, which is in good agreement with the value of 15.3 ± 0.5 as determined by Anderson and Libby (*6*). The lower values reported by Hayes *et al.* (*7*) and by Fergusson (*8*) of 12.9 ± 0.2 and 12.5 ± 0.2 disintegrations/min, respectively, can be brought into agreement with the reported measurement only by assuming a correspondingly longer half life.

Hans E. Suess

U.S. Geological Survey,
Washington, D.C.

References and Notes

1. Publication authorized by the director, U.S. Geological Survey. The assistance of Meyer Rubin in carrying out the measurements is greatly appreciated. Thanks are also due to Harmon Craig, not only for providing the necessary C^{13} analyses, but also for valuable discussions concerning the geochemistry of carbon isotopes. Corrinne Alexander carried out the technical procedure of sample preparation.
2. H. E. Suess, *Science* 120, 5 (1954).
3. ———, *Proc. Conf. on Nuclear Processes in Geologic Set*, Williams Bay, 1953.
4. H. Craig, *Geochim. et Cosmochim. Acta* 3, 53 (1953); *J. Geol.* 62, 115 (1954).
5. W. F. Libby, *Radiocarbon Dating* (Univ. of Chicago Press, Chicago, 1952).
6. E. C. Anderson and W. F. Libby, *Phys. Rev.* 81, 64 (1951).
7. F. N. Hayes, D. L. Williams, A. B. Rogers, *ibid.* 92, 512 (1953).
8. G. F. Fergusson, *Nucleonics* 13, 18 (1955).
9. The samples were obtained through the following: Alaska spruce, T. L. Péwé, U.S. Geological Survey; white pine, A. C. Redfield, W.H.O.I.; incense cedar, J. C. Preston, National Park Service; cedrela, J. Arnold, Univ. of Chicago, and B. F. Kukachka, U.S. Department of Agriculture; sargassum, D. R. Norton, U.S. Geological Survey. The sample of *Venus mercenaria* was collected by H. J. Turner.

7 June 1955

Sharks in Fresh Water

In a recent number of *Science* [**121**, 759 (27 May 1955)] is a note on the occurrence of sharks and sawfish in Lake Sentani, Dutch New Guinea. In it I read "How these salt-water fish became acclimated to fresh water in a lake 500 ft above set level is unknown."

There is no mystery about this at all. That certain species of sharks and rays, including sawfishes, enter fresh water freely and travel long distances into lakes and rivers has been known for a long time. It is now 75 years since I first read of the Ganges shark (*Carcharias gangeticus*) and its attacks on people bathing in the Hugli and Ganges rivers. Long ago A. B. Meyer, a celebrated German naturalist, recorded [*Nature* **13**, 167 (1875)] that sawfish (*Pristis microdon*) up to 20 ft in length were abundant in Laguna de Bay, Luzon. In 1870 Günther recorded this same sawfish in the Amazon and its tributaries, in the Zambezi below the great falls, and in the great rivers of Borneo and Sumatra.

For the past 35 years I have been familiar with the presence of sharks and sawfish in the rivers and lakes of the tropics. In the Philippines, the Ganges shark and the sawfish mentioned previously occur in all rivers of any size and in all fresh-water lakes where there is a good-sized outlet, unimpeded by a dam or waterfall, leading to the sea. They pass through rapids to reach the upper Agusan at least 150 mi from the sea by the winding river. I found the Papuans familiar with sawfish along the Sepik River, New Guinea, more than 300 mi from the sea; these fish undoubtedly go very far beyond that point. Far from being astonished at the presence of sharks and sawfish in Lake Sentani, I would be surprised if they did not occur there. There is no obstacle to the free passage of sharks and sawfish to and from Lake Sentani and the sea, especially in the rainy season. At that time several drainage systems become one vast sheet of water.

One must remember certain facts concerning fishes found in fresh water in the rainy tropics. First, an astonishing variety of marine fishes migrate up rivers and into lakes, many of them going to the remote interior as long as they are not stopped by waterfalls. Eels, mullet, and gobies may ascend in this way to elevations of 5000 ft. Second, in time of high water many rapids unsurmountable to migrating fishes during ordinary water stages become readily passable and are no longer a serious obstacle to them, no matter what Europeans or North Americans may think about it. Some kinds of these fishes remain but a short time, but most kinds remain until they are adult or nearly so and must return to the sea to breed.

The sharks and sawfishes mentioned do not breed in fresh water but, like many other marine fishes, find lacustrine life very attractive. The ecological conditions are very favorable to their existence and there is a great abundance of easily taken food, an altogether attractive situation. Unless cut off by huge permanent fish corrals, as at the outlet of Laguna Bombon, Luzon, sharks return to the sea to breed.

I have found that forest people, such as the Mandayas and Monobos in Mindanao, believe that the sharks they see in their rivers are females and that the sawfish are the males of the same fish. However this singular belief does not seem to be held by any people who are real fisherfolk, depending on fish for their main food supply.

Albert W. C. T. Herre

School of Fisheries,
University of Washington, Seattle

13 June 1955

Physiology of a Primary Chemoreceptor Unit

Progress in the physiology of chemoreception has been hampered by the fact that in most reported experiments the criteria of sensory excitation have been limited to behavioral responses of the animals studied. In some work on vertebrates (*1*), the responses of chemoreceptors have been approached somewhat more directly by recording afferent discharges in sensory nerve fibers that supply the receptor cells. However, these studies still leave in some doubt the nature of the response of the primary receptor surface to specific chemical stimuli.

Chemoreceptor cells of insects have special advantages for experimental work in this area of sensory physiology. These advantages include accessibility of the receptors, absence of a mucus coating over the receptor surface, the fact that the axon of the primary receptor serves as afferent fiber, and the unusually prominent development and sensitivity of the chemical senses of insects. Recent histological studies on single chemosensory hairs of insects have given new encouragement to the hitherto unsuccessful attempts to record electric activity associated with functions of the primary chemoreceptor cells of insects. Gabrowski and Dethier (*2*) found that the distal processes of two neurons extend to the tip of each tarsal chemosensory hair of the blowfly (*Phormia*), and similar observations have been made on the labellar chemosensory hairs of flies (*3*). Attempts to record electric changes in the region of the neurons at the base of the labellar sensory hairs met with some success (*4*), but the results were erratic owing to local shunting of potentials from the small fibers and unpredictability of electrode placement. The present method of recording potential changes between the small localized sensory surface on the tip of the chemosensory hair and the body of the animal has proved to be convenient and reproducible, and may have applications to other organisms and other sense organs.

A 15-mm length of 1-mm (outside