sponse rate. Similarly, the duration of the pause in both procedures is a direct function of the ratio requirement. The procedure provides a means of investigating other schedules of intermittent reinforcement by the removal of conditioned aversive stimulation (*7*).

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Absence of Carbon-14 Activity in Dolomite from Florida Bay

Abstract. A sample of dolomite crystals concentrated from Recent carbonate sediments in Florida Bay gave a carbon-14 age greater than 35,000 years. Since Recent sedimentation in Florida Bay began less than 4000 years ago, the dolomite must be derived from older rocks, and Taft's hypothesis that dolomite is forming today is incorrect.

The occurrence of dolomite crystals in the Recent carbonate sediments in western Florida Bay was recently reported by W. H. Taft, who expressed the opinion, based on textural evidence, that the dolomite had been formed by Recent diagenesis (*1*). Because Taft also observed that the sediments contained quartz grains of approximately the same size as the dolomite crystals, there is the possibility that the dolomite, like the quartz, is clastic material derived from older rocks. Since the episode of Recent sedimentation in Florida Bay began about 3000 to 4000 years ago (*2*), it is possible to test whether the dolomite is of Recent origin by determining the carbon-14 content of the carbonate from the dolomite. The fact that dolomite separated from one sample of Florida Bay sediment showed no measurable carbon-14 activity indicates that the dolomite in this sample was of detrital origin.

A surface sample of about 3000 grams of carbonate sediment from Ox Foot Bank at the western end of Florida Bay (near latitude 25°00′ N, longitude 81°00′ W) was collected by Eugene Shinn. Dolomite crystals similar to those described by Taft were present in the sample. The sediment was separated into size fractions by wet sieving for the particles coarser than 44 μ and by repeated decantation for the finer sizes. Preliminary x-ray diffraction examination showed that the sample consisted mostly of aragonite and calcite with minor amounts of dolomite and quartz, and that the largest concentration of dolomite was in the 20- to 75-μ sizes. The 20- to 75-μ material was treated by adding dilute hydrochloric acid gradually, and x-ray diffraction runs were used to determine when the aragonite and calcite had been reduced to less than 1 percent of the amount of dolomite in the concentrate. During the acid treatment, the approximately constant ratios of x-ray peak heights between dolomite and quartz indicated that at least 90 percent of the dolomite present at the beginning was recovered. For the carbon-14 determination, the dolomite concentrate was treated with an excess of strong acid to evolve carbon dioxide, and the residual liquid was analyzed for calcium and magnesium by Versenate titration. The molar ratio of calicum to magnesium was 1 to 1.03, which indicates that, within the accuracy of analysis, the material dissolved for the carbon-14 measurement was composed entirely of dolomite. The amount of calcium and magnesium in the residual liquid showed that 6.7 g of dolomite were converted to carbon dioxide.

Carbon-14 counting, carried out 30 days after the sample preparation to allow a minor amount of radon activity to decay, showed that the dolomite contained no measurable carbon-14 activity. The count rate observed for the dolomite sample and background determinations made before and after agreed within the 2-percent error expected from the counting statistics. The age of the dolomite must be greater than 35,000 years, because a sample younger than this would have given a count rate different from the background rate by two standard deviations. Stated in another way, the carbon-14 determination was sufficiently sensitive to have detected a significant difference in count rate if as much as 2.4 percent of the sample had been as young as 4000 years.

Carbon-14 ages on the bulk carbonate sediment in the original sample gave 1750 ± 150 years, and the coarse shell fragments separated from the sample gave 1660 ± 130 years. If the dolomite crystals had grown in this environment during the last few thousand years, they would have had access only to carbonate that contained the usual amount of carbon-14 activity. Therefore it must be concluded that the dolomite crystals are derived from older rocks and mixed with the Recent sediment. Because the presence of carbon-14 activity would be a positive demonstration of the Recent origin of a carbonate material, it is suggested that the radiocarbon measurement used for the dolomite from the Bonneville salt flats by Graf *et al.* (*3*) is a much more reliable means of identifying a recently formed carbonate mineral than is the textural evidence used by Taft (*1*) and more recently used by Miller (*4*).

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Carbon Dioxide Fixation in Lobster Nerve

Abstract. Aspartic, glutamic, malic, and citric (isocitric) acids were isolated by chromatographic methods from lobster nerves incubated with Ringer's solution containing C^{14}-bicarbonate. All the compounds were labeled; the bulk of the radioactivity appeared in the aspartic acid. The findings suggest the operation in lobster nerves of the citric acid cycle including CO_2 fixation.

In nerve, carbon dioxide has long been regarded as a regulator of its internal *p*H; in addition, it produces an increased membrane potential (*1*). Recently, a significant carbon dioxide fixation, presumably via the citric acid cycle, has been demonstrated in vivo in the mammalian brain. Intracarotid infusion into cats of C^{14}-bicarbonate resulted in labeling of cerebral glutamic and aspartic acids and of glutamine (*2*).

This finding raised the question as to whether carbon dioxide fixation is a general metabolic property of nervous

tissue. Therefore, our investigations were extended to a study of carbon dioxide fixation in invertebrate nerves. The lobster nerve was chosen because of its large concentration of aspartic acid (*3*) which might act as a sink for any incorporated carbon dioxide. Furthermore, metabolic changes in these nonmyelinated nerve fibers, with the participation of large surface areas during activity, should be greater than in myelinated nerves with saltatory conduction.

The nerves from the first two pairs of the walking legs of several lobsters were removed and incubated at 15°C in lobster Ringer's solution containing C^{14}-bicarbonate (*4*). After 30 minutes of incubation, the nerves were removed and homogenized in 0.3*M* trichloroacetic acid (0.3 ml for every four nerves) in order to terminate any enzymic reaction. The homogenate was neutralized and transferred to a graduated centrifuge tube together with the wash water, and the volume was brought to 3 ml with water (for every four nerves). A portion (0.1 ml) of the fine protein suspension was removed for protein determination (*5*), and the remainder of the mixture was centrifuged. A sample of the supernatant fluid was taken for the determination of the radioactivity, and the rest was transferred to a Dowex-1-acetate column (3 mm by 10 mm). Water (6 ml), used in washing the protein precipitate, followed by 9 ml of 0.05*N* acetic acid, was added to the column after the original supernatant fluid had passed through the column. The eluents were combined and dried, and the residue was taken up in a total volume of 5 ml of water (fraction O). Subsequent elutions were performed with 9 ml of 0.1*N* acetic acid (glutamic acid), 9 ml of 0.25*N* acetic acid (aspartic acid) (*6*), and 9 ml of 0.5*N* HCl. These eluents were reduced to 2 ml. Portions of the various fractions were removed for determination of the radioactivity (Table 1). Of the total radioactivity of the protein-free supernatant fluid, about 70 to 80 percent was recovered in the various eluents. More than 50 percent of the recovered radioactivity was found in the aspartic acid, whose purity was ascertained by paper chromatography and paper electrophoresis. Some radioactivity was also found in glutamic acid.

Close to 40 percent of the radioactivity was recovered in the 0.5*N* HCl eluent. The solution showed an absorption at 260 mμ. A sample was chromatographed on paper with a solvent system containing sodium phosphate, ammonium sulfate, and *n*-propanol (*7*). The strips of paper were cut into three sections corresponding to: (i) R_F 0 to 0.4, (ii) 0.4 to 0.6, and (iii) 0.6 to 0.85. The three sections were extracted with water, and the absorption spectra and radioactivities of the extracts were determined. Fraction i contained nearly all the substances absorbing at 260 mμ. The radioactivities were present in the ratio 1:1:6 in fractions i, ii, and iii, respectively. The results indicate that the material absorbing at 260 mμ does not contain the bulk of radioactivity.

Table 1. Carbon dioxide fixation in lobster nerve. In each experiment, the extract of a varying number of nerves (up to 40) was distributed over several columns (see text). The eluents were pooled and concentrated, and the radioactivities were determined with a thin-window Geiger counter (Nuclear Chicago model D47).

Fraction	Activity (count/min)					
	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5	Sum
Fraction O	510	420	130	240	310	1,610
0.10*N* HAc (glutamate)	270	150	120	180	630	1,350
0.25*N* HAc (aspartate)	4,810	8,540	3,570	13,680	23,460	54,060
0.5*N* HCl	2,820	6,780	2,560	5,870	14,990	33,020
Total	8,410	15,890	6,380	19,970	39,390	90,040
Activity (%)						
in aspartate	57.2	53.7	56.0	68.5	59.6	60.0
in HCl fraction	33.5	42.7	40.2	29.4	38.1	36.7

With 80 percent ethanol containing 3 percent of ammonia, a solvent system utilized for developing paper chromatograms of organic acids (*8*), most of the radioactivity in the HCl fraction was located in a spot corresponding to malic and succinic acids. Further column chromatography of this material suggested that malic acid contained most of the radioactivity. In addition, a small portion of the radioactivity was found in citric or isocitric acid, or both, as identified by paper and column chromatography.

The occurrence of the intermediates of the citric acid cycle and of the metabolically related amino acids in the lobster nerve and the labeling of these compounds by added $C^{14}O_2$ suggest that this basic metabolic cycle operates in this tissue. The demonstration of carbon dioxide fixation in lobster nerve offers direct experimental support for the suggestion of an "anabolic" role of carbon dioxide in nerve metabolism (*9*). The question arises about the significance of carbon dioxide in the metabolism of the nerve in addition to its effect on the physicochemical environment of such tissue (*10*).

The carbon dioxide fixation in lobster nerve may provide one of the means by which the intermediates of the citric acid cycle might be generated and make this aspect of nerve metabolism autonomous. Whether carbon dioxide fixation provides a regulatory mechanism for the rate of the citric acid cycle, and thereby directly or indirectly is related to the function of the nervous system, must be determined by further studies.

At present, our results do not clarify the enzymic mechanism of the fixation of carbon dioxide. The presence of the malic enzyme, and also of the phosphoenolpyruvate carboxylase, can be demonstrated in homogenates of the lobster nerve (*11*).

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