carbons of glycerol can be converted to formaldehyde to some extent cannot be definitely answered at present. The demonstration that formaldehyde is produced and utilized by *P. arabinosum* during the fermentation of glycerol indicates that formaldehyde or some compound in equilibrium with it is an active intermediate. The mechanism of propionate fermentation is under further investigation.

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RECEIVED APRIL 23, 1951

ENZYMATIC FORMATION OF BARBITURIC ACID FROM URACIL AND OF 5-METHYLBARBITURIC ACID FROM THYMINE

Sir:

The oxidation of uracil to barbituric acid and the formation of 5-methylbarbituric acid from thymine have been observed with a partially purified enzyme preparation obtained from strains of *Corynebacterium* isolated by enrichment culture from soil samples. A strain of *Mycobacterium*[1] was also found to metabolize uracil and thymine in the same way and because of its rapid growth was used in enzyme studies. The organism was grown for about 40 hours at 30° with shaking on a medium containing 0.1% uracil or thymine, 0.15% K_2HPO_4, 0.05% KH_2PO_4, and 0.02% $MgSO_4 \cdot 7H_2O$ in distilled water.

The enzyme was prepared by grinding the cell paste with alumina[2] and extracting with tris-hydroxymethylaminomethane buffer (0.02 *M*, *p*H 9.0). The clear supernatant obtained by high-speed centrifugation of the extract was fractionated with ammonium sulfate and the precipitate obtained between 35 and 50% saturation was collected.

With enzyme prepared from thymine-grown cells,[3] uracil was oxidized with the consumption of one atom of oxygen per one mole of substrate. The optimum *p*H of the reaction was about 9.0 and the presence of methylene blue was necessary. The ultraviolet absorption spectrum of the oxidation product was indistinguishable from that of barbituric acid (Fig. 1). The isolated crystalline material melted with decomposition at 243° as did an authentic sample of barbituric acid and a mixture of the two. The behavior of the uracil oxidation product on ion exchange (Fig. 1) and paper chromatography (propanol–water, 10:3) (R_f = 0.22) further indicated identity with barbituric acid.

With thymine as substrate, a product was obtained which on the basis of its ultraviolet absorption spectrum[4] appears to be 5-methylbarbituric acid. This product was differentiated from the uracil oxidation product by ion-exchange (Fig. 1) and paper chromatography (R_f = 0.36). Analogous to the relative behavior of uracil and thymine, the oxidation product of thymine follows the oxidation product of uracil (*i.e.*, barbituric acid) in the ion-exchange chromatogram and migrates farther on paper. Final identification awaits comparison with an authentic sample of 5-methylbarbituric acid.

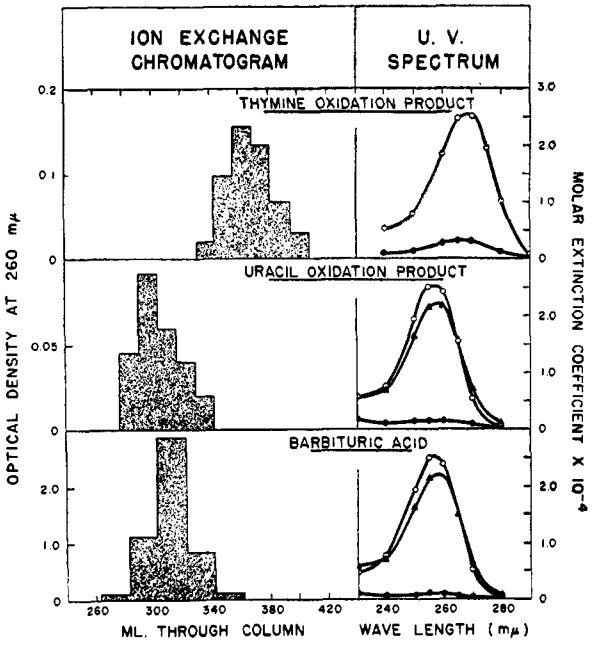

Fig. 1.—Ion-Exchange Chromatogram: 20 μmoles of thymine or uracil, 2.0 ml. of enzyme (2.4 mg. of protein), 2.0 ml. of 0.1% methylene blue, 15.0 ml. of tris-(hydroxymethyl)-aminomethane buffer (0.2 *M*, *p*H 8.7) and 2.0 ml. of H_2O. The incubation was at 30° for about 30 minutes. When the increase in absorption at 260 mμ reached a plateau, the incubation mixture was chilled and a 10 ml. aliquot was adsorbed on Dowex-1 (Cl^- form) column, 9.5 cm. × 1 sq. cm., and eluted with $NH_4OH.NH_4Cl$ buffer (0.2 *M*, *p*H 9.9). The thymine oxidation product was eluted between 255 and 370 ml. and the uracil oxidation product between 220 and 330 ml. Recovery based on u.v. adsorption was 107 and 88%, respectively. The presence of impurities in the reaction mixture interfered with the chromatographic pattern. Therefore, an aliquot (310–317.5 ml.) of the eluate of the thymine oxidation product containing 0.3 μmole and an aliquot (249–253.2 ml.) of the eluate of the uracil oxidation product containing 0.3 μ mole were each diluted with equal volumes of "tris" buffer and rechromatographed under the same conditions. The results are illustrated here: recovery, 100 and 50%, respectively. 5 μmoles of barbituric acid (Eastman Kodak recrystallized) were dissolved in 10.0 ml. of "tris" buffer (0.1 *M*, *p*H 8.7). Recovery: 90%. Ultraviolet spectrum: ▲—▲ *p*H 12.0, O—O *p*H 7.4, ●—● *p*H 2.3. The absorption coefficient of the oxidation product of thymine was calculated on the assumption that 100% of the substrate was converted to this compound when the increase in absorption reached a plateau.

This enzyme does not attack isobarbituric acid, postulated by Cerecedo[5] to be the initial product of uracil metabolism in mammals. It is also inert toward 4-methyluracil, dihydrothymine, dihydrouracil, 2-thiouracil, 2-thio-5-methyluracil and cytosine.

Bacterial oxidation of pyrimidines has been observed concurrently by others.[6,7] Wang and Lampen obtained an unidentified product from uracil and thymine oxidation by cell-free extracts,

(1) Isolated from rabbit feces by Drs. Schatz, Savard and Pintner and identified and kindly furnished by Dr. T. Stadtman.

(2) H. McIlwain, *J. Gen. Microbiology*, **2**, 288 (1948).

(3) Results with uracil-grown cells were essentially the same except that the growth of the organism was less favorable.

(4) R. E. Stuckey, *Quarterly J. Pharm. Pharmacol.*, **15**, 370 (1942).

(5) L. R. Cerecedo, *J. Biol. Chem.*, **88**, 695 (1930).

(6) T. P. Wang and J. O. Lampen, *Federation Proc.*, **10**, 267 (1951).

(7) F. J. S. Lara and C. B. van Niel, personal communication.

the spectral characteristics of which suggest identity with the products reported here.

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RECEIVED APRIL 28, 1951

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ISOTOPIC COMPOSITION OF OXYGEN IN AIR DISSOLVED IN PACIFIC OCEAN WATER AS A FUNCTION OF DEPTH

Sir:

Seventeen samples of water collected in the Pacific Ocean at 32° 10′ N. and 120° 19′ W. on 18 Feb. 1951 from 0000 to 0600 PST at depths ranging down to 2870 meters were evacuated and the evolved air collected for analysis by a Toepler pump system. After analysis of the air for its oxygen content, the samples were sent to Evanston, Illinois, where the O^{18}/O^{16} ratio was measured on all of the samples.[1] A Consolidated-Nier isotope ratio mass spectrometer was used to which had been added a double molecular leak input system, using features recommended on the one hand by Halsted and Nier[2] and on the other by McKinney, McCrea, Epstein, Allen and Urey.[3] In addition, the number 2 amplifier was replaced by a vibrating reed electrometer and the galvanometer of the mass spectrometer by a photoelectric recording potentiometer. Relative accuracy in the case of air was 0.03% in the isotope ratio, an accuracy which decreased with decrease of oxygen percentage.

The results of the oxygen analysis and isotope ratio study are shown in Fig. 1 where the length of the vertical lines on the isotope ratio points represents the relative uncertainty in the data. The correlation between the rise in the O^{18}/O^{16} ratio and the decline in oxygen percentage is not only extremely striking, but strongly suggestive of a postulate that the same fundamental mechanism is responsible for both effects. The conclusion is almost inescapable that marine vegetation, plankton, bacteria and other sea life which consume oxygen must preferentially metabolize O^{16} at a higher rate than O^{18} to produce this marked isotope fractionation.

The bearing of these results on the enhanced O^{18} content of the atmosphere, photosynthesis in the oceans, and other geochemical and oceanographic problems will be discussed in later publications.

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RECEIVED APRIL 30, 1951

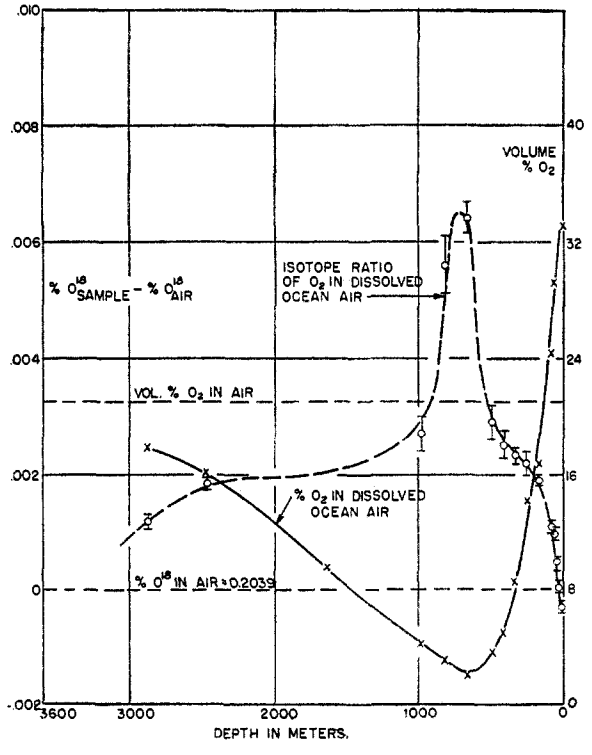

Fig. 1.

(1) This work was partially supported by the Air Force Cambridge Research Laboratories under contract AF19(112)-157 with Northwestern University.

(2) R. E. Halsted and A. O. Nier, *Rev. Sci. Instruments*, **21**, 1019 (1950).

(3) C. R. McKinney, J. M. McCrea, S. Epstein, H. A. Allen and H. C. Urey, *ibid.*, **21**, 724 (1950).

STUDIES ON LYSOZYME

Sir:

When a several times recrystallized preparation of lysozyme carbonate is chromatographed on a column of IRC-50, two ninhydrin positive peaks (A and B, Fig. 1a) are obtained, both of which contain lytic activity. The experimental conditions are the same as those employed earlier in the chromatography of ribonuclease,[1] except that elution is performed with a 0.2 *M* sodium phosphate buffer of *p*H 7.18. The preparation of

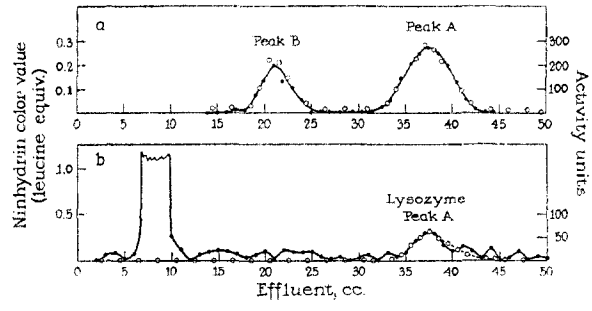

Fig. 1a.—Chromatography of lysozyme: about 5 mg. of lysozyme carbonate was chromatographed. About 60% of the activity initially put on the column appears in peak A, about 40% in peak B.

Fig. 1b.—Chromatography of egg white: about 0.2 cc. of egg white was diluted to 1 cc. with buffer and the entire sample chromatographed: ●———● ninhydrin color value; O———O lysozyme activity.

(1) C. H. W. Hirs, W. H. Stein and S. Moore, THIS JOURNAL, **73**, 1893 (1951).