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

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RECEIVED APRIL 28, 1951

(8) Special Research Fellow.

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

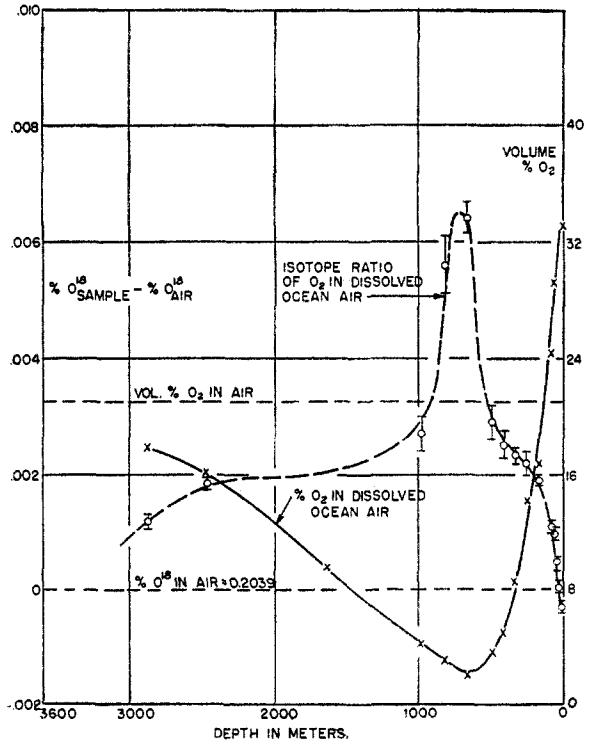

Fig. 1.

(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

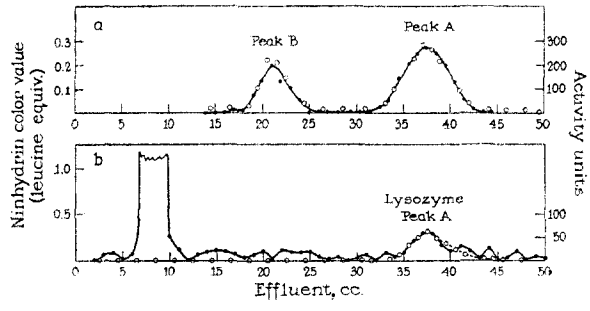

Fig. 1a.—Chromatography of lysozyme: about 5 mg. of lysozyme carbonate was chromatographed. About 60% of the activity initially put on the column appears in peak A, about 40% in peak B.

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).

lysozyme had been made from hens' eggs by the "direct crystallization" procedure[2] about one year prior to these experiments and was very kindly supplied by Dr. C. A. Stetson, Jr. Lytic activity was determined by the procedure of Smolelis and Hartsell[3] employing a dried preparation of *Micrococcus lysodeikticus* generously supplied by Dr. M. McCarty. Upon chromatography of lysozyme chloride freshly prepared by the "adsorption" procedure,[2] the bulk of the protein and of the activity was found in peak A with only a small amount in peak B. Some residual inactive proteins from egg white were present and appeared as fast moving peaks. The preparation could not be purified further by recrystallization, but rechromatography of the material in peak A gave a single peak at the same position. Isoelectric lysozyme, freshly prepared by direct crystallization, appeared to be nearly homogeneous chromatographically after one or two recrystallizations. Over 95% of the enzyme appeared in peak A, with only a small amount in peak B. Inactive proteins were virtually absent. Transformation to lysozyme carbonate did not alter appreciably the chromatographic behavior. Both isoelectric lysozyme and the chloride appeared homogeneous on electrophoresis at *p*H 4.6, 7.8 and 9.2. The advice and coöperation of Dr. G. Perlmann in the performance of the electrophoretic experiments is gratefully acknowledged.

In order to ascertain whether either peak A or B (Fig. 1a) corresponds to the "native" lysozyme found in eggs, whole diluted egg white was chromatographed, with the results visible in Fig. 1b. As may be seen, the bulk of the ninhydrin positive material, mostly protein in nature, emerges as a large unadsorbed peak at about 7 cc., followed by a long irregular zone for about 50 cc. A low peak is visible at 37 cc., corresponding in position to peak A, Fig. 1a, which on bioassay was found to contain all the lytic activity. Upon the addition of a sample of purified isoelectric lysozyme to egg white, and chromatography of the mixture, both the ninhydrin and activity peaks shown at 37 cc. in Fig. 1b rose symmetrically. These experiments demonstrate that by direct crystallization it is possible to prepare a sample of lysozyme which is over 95% homogeneous chromatographically, and the main component of which is chromatographically identical with the enzyme found in egg white.

It has been found that, after standing in the dry state at room temperature for about five months, a sample of lysozyme carbonate which had been virtually homogeneous chromatographically when freshly prepared, gave results similar to those shown in Fig. 1a. Isoelectric lysozyme, and the chloride, however, remained unaltered. The nature of the transformations responsible for the production of a chromatographically distinct lysozyme is being investigated.

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RECEIVED APRIL 18, 1951

(2) H. L. Fevold and G. Alderton in "Biochemical Preparations," John Wiley and Sons, Inc., New York, N. Y., 1949, Vol. I, p. 67.

(3) A. N. Smolelis and S. E. Hartsell, *J. Bact.*, **58**, 731 (1949).

THE PHOTOCHEMICAL REACTIONS OF DIAZOMETHANE WITH CARBON TETRACHLORIDE AND BROMOTRICHLOROMETHANE

Sir:

The reaction of diazomethane with carbon tetrachloride in the presence of light gives 1,3-dichloro-2,2-bis-(chloromethyl)-propane (pentaerythrityl chloride). With bromotrichloromethane 1,3-dichloro-2-chloromethyl-2-bromomethylpropane is obtained. This interesting reaction resembles the known photochemical reactions of diazomethane with ethers and alcohols.[1] It is remarkable in that four molecules of diazomethane react with one molecule of carbon tetrachloride in the presence of a large excess of the latter.

Diazomethane (10.5–12.6 g., about 0.25 mole), generated by the addition of a slurry of N-nitroso-N-methylurea (35. g., 0.34 mole) in methanol to a magnetically stirred aqueous potassium hydroxide solution (40%, 70 ml.), was swept by a stream of nitrogen into carbon tetrachloride (185 g., 1.2 mole) in an apparatus illuminated internally by a mercury discharge lamp. Addition of the diazomethane required a two-hour period, and its color disappeared from the solution after an additional hour. Products obtained were methyl ether (3.0 g.; 0.065 mole; mol. wt., 46.6; vapor pressure at −80°, 33; m.p. of N-methylpyridinium iodide prepared from methyl iodide obtained by the reaction of this product with concentrated hydriodic acid, 116–117°), polymethylene (0.05 g.) and 1,3-dichloro-2,2-bis-(chloromethyl)-propane (m.p. 96.3–97°; 3.89 g.; m.p. of a mixture with an authentic sample prepared by the method of Mooradian and Cloke, 96–97°).[2]

Anal. Calcd. for $C_5H_8Cl_4$: C, 28.59; H, 3.85; Cl, 67.39; mol. wt., 209. Found: C, 28.59; H, 4.03; Cl, 67.31; mol. wt., 208.

The reaction by the same procedure of diazomethane (10.5–12.6 g., about 0.25 mole) with bromotrichloromethane (457 g., 2.25 moles) gave a substance presumed to be 1,3-dichloro-2-chloromethyl-2-bromomethylpropane (m.p. 103.7–104°, 3.4 g.).

Anal. Calcd. for $C_5H_8Cl_3Br$: C, 23.64; H, 3.17; Ag equivalent, 63.6; mol. wt., 254. Found: C, 23.67; H, 3.47; Ag equivalent, 65.8; mol. wt., 257.

Studies of the reactions of diazomethane and other aliphatic diazo compounds with other organic halides are being continued.

(1) Meerwein, Rathjen and Werner, *Ber.*, **75**, 1610 (1945).

(2) Mooradian and Cloke, THIS JOURNAL, **67**, 942 (1945).

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RECEIVED MAY 19, 1951

LIBERATION OF AMINO ACIDS BY MILD ACID HYDROLYSIS OF HOG BLOOD GROUP A and O SUBSTANCES[1]

Sir:

Heating at 100° for two hours at *p*H 1.5 to 1.8 has been shown to destroy blood group activity while increasing the capacity of blood group A, B and O substances to precipitate with Type XIV

(1) Aided by grants from the United States Public Health Service and the William J. Matheson Commission.