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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(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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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.