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.

THE ROCKEFELLER INSTITUTE
FOR MEDICAL RESEARCH HARRIS H. TALLAN
NEW YORK 21, N. Y. WILLIAM H. STEIN

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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).

GEORGE HERBERT JONES LABORATORY
UNIVERSITY OF CHICAGO W. H. URRY
CHICAGO 37, ILLINOIS J. R. EISZNER

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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.

antipneumococcal horse serum.[2] Dialysates of such heated preparations contained most of the methylpentose and a portion of the non-hexosamine nitrogen of the blood group substances.[2] Much of this dialyzable non-hexosamine nitrogen is now found to consist of six free amino acids, aspartic acid, glutamic acid, lysine, serine, threonine and glycine. These were identified and the amounts liberated determined quantitatively by treatment of the dialysate with dinitrofluorobenzene, extraction of dinitrophenyl (DNP) derivatives with ether and chromatographic separation and identification of the DNP derivatives on silica gel using combinations of the fractionation methods developed by Sanger[3,4] and Blackburn.[5] Further identification of the various amino acids was provided by filter paper chromatography of their DNP derivatives. Although slight differences in *p*H greatly affect the total amounts of the amino acids liberated from one preparation to another, aspartic acid is always found in largest amount, followed generally in the order given by lysine, glycine, serine, threonine and glutamic acid. On a mole basis, the aspartic acid liberated approximates the sum of the other free amino acids. An interpretation for the present findings is provided by the report by Partridge[6] and by Blackburn[7] that peptide bonds joining aspartic acid and asparagine to other amino acids in insulin and in wool are labile and are preferentially split by mild acid hydrolysis with the liberation from these proteins of free aspartic acid. That five other amino acids are liberated from the blood group substances as well and that aspartic acid is present in largest amount suggests that a portion of the blood group molecule consists of one or more peptide chains with aspartic acid in alternate positions, the remaining positions being occupied by the other five amino acids. Such a unique structural pattern composed largely of polar amino acids with alternate aspartyl or asparaginyl residues could account for the resistance of the polypeptid chain of the blood group substances to the proteolytic enzymes and may be important in determining the specificity and blood group activity of these substances.

(2) E. A. Kabat, H. Baer A. E. Bezer and V. Knaub, *J. Exp. Med.*, **88**, 43 (1948).
(3) F. Sanger, *Biochem. J.*, **39**, 507 (1945).
(4) R. R. Porter and F. Sanger, *ibid.*, **42**, 287 (1948).
(5) S. Blackburn, *ibid.*, **45**, 579 (1949).
(6) S. M. Partridge and H. F. Davis, *Nature*, **165**, 62 (1950).
(7) S. Blackburn, *Biochem. J.*, **47**, xxviii (1950).

DEPARTMENTS OF BACTERIOLOGY AND NEUROLOGY
COLLEGE OF PHYSICIANS AND SURGEONS
COLUMBIA UNIVERSITY AND THE HELEN VAN VUNAKIS
NEUROLOGICAL INSTITUTE ELVIN A. KABAT
PRESBYTERIAN HOSPITAL
NEW YORK, N. Y.

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BOOK REVIEWS

Structural Chemistry of Inorganic Compounds. Volume I. By WALTER HÜCKEL, Dr. Phil., Professor of Pharmaceutical Chemistry, Tubingen University, formerly Professor of Organic Chemistry in the University and Technische Hochschule of Breslau. Translated by L. H. LONG, B.Sc., Ph.D. (London), Ph.D. (Cantab.), A.R.C.S., D.I.C., Lecturer in the Department of Chemistry, University College, Exeter. Elsevier Publishing Company, Inc., 250 Fifth Avenue, New York 1, N. Y., 1950. xii + 437 pp. 18 × 26 cm. Price, $9.00.

The present volume is a translation of the first half of a book published in Germany in 1948. The second and concluding volume of the translation is promised by the publisher for the summer of 1951. Most of the book was written during World War II and so the literature from 1940 on was not all available to the author. (This is evident for example in the critical discussion of "mesomerism" (resonance) as applied to the additivity rule for interatomic distances, pp. 431–434, which should be modified in the light of the important contribution of Schomaker and Stevenson (1941). The discussion of $MoCl_2$ does not include Brosset's solution of this structural problem (1945, 1947).)

The author's object is "to furnish inorganic chemistry with . . . a structural and constitutional theory in one embracing representation." He classifies substances not by the position of their elementary constituents in the periodic table but by the kinds of chemical bonds that occur in the substances. Volume I contains a long (and, in the reviewer's opinion, dull and unnecessary) introductory discussion of some epistemological problems in chemistry. This is followed by a treatment of coördination compounds, including considerable advanced, interesting descriptive material. The discussion of polynuclear complexes includes the iso-, poly- and heteropoly-acids, metaphosphates and silicic acid. Some of these difficult and complicated fields have been studied intensively by German chemists, and non-specialist, English speaking readers will appreciate the present introduction to these topics.

The periodic system, atomic structure and spectra, etc., are then presented. Why this section includes such subjects as isotope separation, nuclear physics, and ortho- and para-hydrogen is not evident. Methods for investigating the nature of chemical bonds are then treated. The volume concludes with a discussion of chemical binding, using the united atom, molecular orbital point of view. Volume II will be mainly concerned with the structure and constitution of inorganic compounds, and will contain more descriptive material.

The following paragraph summarizes the reviewer's opinion of this book. It is not recommended to any class of readers. The selection and ordering of topics is not appropriate for the stated aim of the author. The effort to give an encyclopedic account of fundamental material has failed; too many topics are considered and, in many cases, the presentations are too long and not very good. The prose style is complicated and it is almost invariably difficult to discern the author's meaning. The chapter on coördination compounds is exempted from these criticisms.

The volume concludes with a note by the translator—an intemperate attack on the theory of resonance. This attack may have the good effect of stimulating (or irritating) theoretical chemists interested in resonance to explain more carefully the conditions required for resonance between several electronic structures.

GATES AND CRELLIN LABORATORIES OF CHEMISTRY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA 4, CALIFORNIA NORMAN DAVIDSON