These compounds exhibit an order of inhibition of carbonic anhydrase previously observed only among the heterocyclic sulfonamides and in animals promote the renal excretion of sodium. In addition, however, they produce also a marked increase in chloride excretion and cause a diuresis not unlike that observed with organic mercurial compounds. One compound of this type, 6-chloro-7-sulfamyl-1,2,4-benzothiadiazine-1,1-dioxide (II, R = H) has been selected for clinical trial and assigned the generic name of chlorothiazide. Preliminary results[3] in man substantiate the important pharmacological findings observed in our laboratories.

Chlorosulfonation of *m*-chloroaniline at 150° in the presence of sodium chloride[4] yielded 6-amino-4-chlorobenzene-1,3-disulfonyl chloride (III) which with ammonium hydroxide gave 6-amino-4-chlorobenzene-1,3-disulfonamide (IV) (m.p. 251–252°; *Anal.* Calcd. for $C_6H_8ClN_3O_4S_2$: C, 25.22; H, 2.82; N, 14.71. Found: C, 25.48; H, 2.81; N, 14.68). Reaction of III with acetic anhydride afforded 6-acetylamino-4-chlorobenzene-1,3-disulfonyl chloride (m.p. 137–139°; *Anal.* Calcd. for $C_8H_6Cl_3NO_5S_2$: C, 26.21; H, 1.65; N, 3.82. Found: C, 26.39; H, 1.77; N, 3.79) which with alcoholic ammonia gave a mixture of 6-acetylamino-4-chlorobenzene-1,3-disulfonamide (I, R = CH_3) (m.p. 261–262° dec.; *Anal.* Calcd. for $C_8H_{10}ClN_3O_5S_2$: C, 29.31; H, 3.08; N, 12.82. Found: C, 29.49; H, 3.25; N, 12.85) and 6-chloro-3-methyl-7-sulfamyl-1,2,4-benzothiadiazine-1,1-dioxide (II, R = CH_3) (m.p. 332° dec.; *Anal.* Calcd. for $C_8H_8ClN_3O_4S_2$: C, 31.02; H, 2.60; N, 13.57. Found: C, 31.27; H, 2.53; N, 13.50) that was separated by recrystallization from water. When heated with formic acid under reflux, IV was converted to 6-chloro-7-sulfamyl-1,2,4-benzothiadiazine-1,1-dioxide (m.p. 342.5–343° dec.; *Anal.* Calcd. for $C_7H_6ClN_3O_4S_2$: C, 28.43; H, 2.05; N, 14.21. Found: C, 28.65; H, 2.23; N, 14.11).

(3) R. V. Ford and C. L. Spurr, "Electrolyte Excretion Patterns Due to Chlorothiazide, A New Orally Effective Diuretic Agent," Presented at the Southern Society for Clinical Research, New Orleans, January 26, 1957.

(4) O. Lustig and E. Katscher, *Monatsh.*, **48**, 87 (1927).

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CAROTENE, 3-C^{14}- AND 4-C^{14}-LEUCINE

Sir:

3-C^{14}- and 4-C^{14}-*d,l*-leucines were synthesized by conventional methods from carboxyl labeled isobutyric acid and carbonyl labeled acetone, respectively, and incorporated into culture media in which *Phycomyces blakesleeanus* was grown. The culture medium consisted of glucose (2.5% w./v.), asparagine (0.125%), and 3-C^{14}-leucine or 4-C^{14}-leucine (0.125%), together with inorganic salts and thiamine.[1] Thirty petri dishes were inoculated with an active spore culture of *P. blaskesleeanus*. Cultures were grown at room temperature for 7 days, harvested and extracted.[1] The extract was saponified, chromatographed and the carotene crystallized without carrier, with yields 7 and 9 mg., respectively. Both were recrystallized with resulting yields of 5.8 and 6.0 mg.

The activities of medium and carotene were determined in triplicate. Samples were burned in a Pregl furnace and the CO_2 was precipitated from sodium hydroxide as $BaCO_3$. The carbonate was plated on 1-inch copper planchets and counted in a gas-flow counter. Counts were recorded on a Tracer-Lab Autoscaler. Activities were corrected for self-absorption.

The presence of leucine in the culture medium visibly stimulates production of β-carotene by the mold, but the incorporation of leucine carbon into carotene is insignificant for 1-C and limited for 2-C.[1] The loss of the carboxyl carbon was to be expected, but the limited incorporation of 2-C made it impossible for the leucine to provide the whole of the requisite C_5 repeating unit in the carotene molecule.

From media in which 6.4% of the total carbon is represented by leucine carbon, we find an enrichment of 3.6-fold for the 3-C, and of 8.7 for 4-C, in the carotene. The specific activities of the leucines and carotenes are shown below, in cts. per min. per mg. $BaCO_3$, together with the activities of the media determined by independent combustion and by calculation based on combustion of the leucine alone. The activities are the averages for

Specific activity	3-C^{14}-Leucine	4-C^{14}-Leucine
Leucine	1442	2350
Medium calcd.	94.0	149
Medium exptl.	96.8	159
Carotene	343	1392

the six carbons of leucine and for the forty of carotene. The true activities of carbons 3 and 4 are six times greater and the extent to which they are incorporated is given by the expressions 343/1442 × 40/6 or 1.59, and 1392/2350 × 40/6 or 3.96, respectively.

Up to this point, we have introduced no assumptions. In all our work, the primary carbon source has been glucose which comprizes *ca.* 90% of the carbon of the medium. With labeled glucose, the specific activity of the carotene is less than that of the glucose, and the extent to which the glucose carbon is discriminated against varies with the non-glucose carbon source, *e.g.*, yeast autolysate,[2] glycine.[3]

If the assumption is made that formation of carotene derivable from non-leucine sources is unaffected by the leucine, the labeled carotene derived from leucine has been diluted by an equal quantity of inert endogenous carotene.

The figures 1.59 and 3.96 may therefore be multiplied by a factor of *ca.* two. This makes it possible for the 4-C of leucine to appear eight times in

(1) C. O. Chichester, T. Nakayama, G. Mackinney and T. W. Goodwin, *J. Biol. Chem.*, **214**, 515 (1955).

(2) C. O. Chichester, P. S. Wong and G. Mackinney, *Plant Physiol.*, **29**, 238 (1954).

(3) C. O. Chichester, T. Nakayama and G. Mackinney, *J. Biol. Chem.*, **221**, 819 (1956).

the carotene molecule, and the position of this key carbon in the C_5 repeating unit of carotene can readily be envisaged. Whether leucine provides an iso C_3 fragment cannot be determined as yet, but it is clear that the stimulatory effect of leucine on carotene formation is due to the incorporation of leucine fragments into the molecule.

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BOOK REVIEWS

Synthetic Polypeptides. Preparation, Structure, and Properties. Volume V. By C. H. BAMFORD, A. ELLIOTT and W. E. HANBY, Courtaulds Ltd., Research Laboratory, Maidenhead, Berkshire, England. Physical Chemistry. A Series of Monographs. Edited by Eric Hutchinson. Academic Press, Inc., Publishers, 111 Fifth Avenue, New York 3, N.Y. 1956. xiii + 445 pp. 16 × 23.5 cm. Price, $10.00.

The special interest in the synthetic polypeptides lies not only in their relation to proteins but in the fact that they constitute a class of high polymers exhibiting with unusual clarity the relation between properties and molecular structure.

In general this book can be read by all who have a basic interest and training in the natural sciences and by all who are concerned with high polymers.

The polymers which are the subject of this book are the synthetic linear polypeptides derived from amino acids. In the earlier chapters difficulties in the preparation and study of the polypeptides are discussed together with the preparation from various beginning materials and the behavior of the final products toward different solvents. Chapter IV has to do with chain configurations in the polypeptides, bond lengths and angles, folding of chains, hydrogen bonding, etc.

Chapters V and VI are concerned with Infrared Spectroscopy and the effect of various agents (Hydrogen bonds, Deuterium) on this method of examination.

Chapters VII, VIII and IX deal with X-ray studies on various types of polypeptides.

In Chapter X are discussed the properties of synthetic polypeptides—molecular weights, carboxylic acid effects, solubilities of various types, dyeing properties, optical rotation and molecular orientation.

Chapter XI is a brief review of the biological properties of the synthetic polypeptides—enzymic hydrolysis, interaction with viruses and bacteria and blood clotting.

The final chapter takes up the fibrous proteins in their relation to the synthetic polypeptides, the silks, the keratins of wool and hair, their amino acid residues with their sequence and the physical structure as revealed by X-ray and infrared observations.

There is a 16 page appendix which includes the discussion of Dichroism in partially oriented polymers, use of atomic models, etc. Finally there is an excellent subject index of nineteen pages.

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W. R. BLOOR

Annual Review of Physical Chemistry. Volume 7. H. EYRING, Editor, University of Utah, C. J. CHRISTENSEN, Associate Editor, University of Utah, and H. S. JOHNSTON, Associate Editor, Stanford University. Annual Reviews, Inc., Palo Alto, Stanford, California. 1956. vii + 503 pp. 16 × 23 cm. Price, $7.00.

In these times of tremendous increase in volume of scientific literature, Volume 7 of the Annual Review of Physical Chemistry is very welcome even to a specialist in a field. The topics covered in this edition are: Cryogenics; Heterogeneous Equilibria and Phase Diagrams; Solutions of Nonelectrolytes; Statistical Mechanics; Radiation Chemistry; Quantum Theory; Ion Exchange; Polymers; Kinetics of Reactions in Solution; Kinetics of Reactions in Gases; Combustion and Flames; High Temperature Chemistry; Thermochemistry and Thermodynamics of Substances; The Solid State; Isotopes; Magnetic Resonance; Surface Chemistry and Catalysis; Molecular Electronic Spectroscopy; Vibration-Rotation Spectroscopy; Experimental Molecular Structure.

The literature coverage is for the year 1955. Because of space limitations and other reasons, none of the authors claim complete coverage of the literature. Even with such restrictions almost 3300 references are cited. In some of the topics the coverage is more complete than in others. In the article on quantum theory the reviewers limited themselves to a timely and pertinent survey of crystal field theory. Their discussions mainly concerned the transition metal complexes. Such limitations are not serious and probably are desirable when one considers the matter from the point of view of the whole series of reviews, since in previous years other topics in quantum theory have been adequately covered. The style and method of presentation of the reviewers of course vary from individual to individual but on the whole the material is well presented. The editors and authors are to be complimented on a job well done. This review should be on the shelves of all scientists interested in physical chemistry.

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WALTER S. KOSKI

Medicinal Chemistry. Volume III. A Series of Reviews Prepared under the Auspices of the American Chemical Society. Editors: F. F. BLICKE and R. H. COX. Authors: T. P. Carney, P. L. deBenneville, V. Papesch, E. F. Schroeder, A. Stempel, and J. Z. Aeschlimann. John Wiley and Sons, Inc., 440 Fourth Avenue, New York 16, N.Y. 1956. vi + 346 pp. 15.5 × 23.5 cm. Price, $10.50.

The four review monographs dealing with the subjects Methadone and Related Analgesics, Quaternary Ammonium Germicides, Non-mercurial Diuretics, and Synthetic Analogues of Physostigmine are written by experts in their field for experts. This volume, like its precursors, should be of interest not only to the synthetic organic chemist, but also to the pharmacologist. The mass of detail is, for the most part, skillfully handled, with concise discussions of the text upon which the special reader may amplify through reference to one of the numerous citations of the original literature. The task of thoroughly covering many homologous series of compounds is skillfully handled by the use of detailed tables containing original literature references.

The format, type style and freedom from errors are notable. In the last connection, only a few typographical errors were encountered.

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