Both hemoglobins may be dissociated into two equal or nearly equal molecules by change in *p*H.[4,5,6,7] When a mixture of HbA and S is first taken to a dissociating *p*H and then returned to a non-dissociating *p*H, the lack of new species, either electrophoretically[7] or chromatographically,[8] suggests two possibilities: that heterologous recombination does not occur or that dissociation is asymmetric[7] $\alpha_2\beta_2 \rightleftarrows \alpha_2 + \beta_2$. According to Itano dissociation in acid solution is asymmetric and the expected hybrids do form.[9] When labelled and unlabelled hemoglobins are hybridized,[8] the hybrids contain both labelled and unlabelled chains and any peptide may be assigned to the proper chain. In the experiments to be described, the N-terminal peptides have been used to define the aberrant chain in HbS.

HbA* (7,000 c.p.m./mg.) and HbS* (2,600 c.p.m./mg.) were prepared by incubating the appropriate reticulocyte-rich bloods with uniformly C^{14}-labelled L-leucine.[8,10]

As required, both radioactive or non-radioactive hemoglobins were purified chromatographically[11] to prevent interference from minor hemoglobin components.

In Experiment I, oxyhemoglobin A* diluted 1:1 with HbA was mixed with an equal amount of HbS. The solution was dialyzed at 3° against 0.1 *M* sodium acetate buffer at *p*H 5.0 for 24 hr. and further dialyzed for 24 hr. prior to chromatography with Developer No. 12 at *p*H 7.22. Chromatographic separation of 50 mg. of the mixture then was carried out with Developer No. 1. The main portion of the zone of HbS (now radioactive, 850 c.p.m./mg.) was taken, combined with 150 mg. of HbS as a carrier, and dinitrophenylated. In Experiment II, HbS* was hybridized with HbA at *p*H 11.0 in 0.05 *M* sodium phosphate, 0.15 *M* NaCl at 3° for 24 hours. The N-terminal peptides were isolated chromatographically, estimated spectrophotometrically,[1,2] and assayed for radioactivity with these results.

	Hybrid HbS* from HbS and HbA*		Hybrid HbA* from HbS* and HbA	
	c.p.m./ μmole	μmoles	c.p.m./ μmole	μmoles
DNP-val-leu	47.5[a]	2.21	32.2	2.33
di-DNP-val-his-leu	6.2	0.52	3.4	1.08
di-DNP-val-his	7.2	1.09	1.8	0.56
Dinitroaniline	0.6	2.79	0.2	5.36

[a] Contents of planchette rechromatographed and reassayed: DNP val-leu, 47 c.p.m./μmole.

Because the N-terminal dipeptide DNP-val-leu is radioactive and the N-tripeptide di-DNP-val-his-leu is substantially inactive, the α chains must have exchanged and are the chains common to both hemoglobins. The β chains differ and are aberrant in HbS.

Thus, sickle cell anemia is associated with a mutation of the gene which controls the synthesis of the β chains of hemoglobin.

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(10) We wish to thank Dr. P. A. Sturgeon, Children's Hospital, Los Angeles, for supplying us with the blood samples and the hematological data. The blood for experiment I was obtained from an individual with an acquired hemolytic anemia. We wish to thank Professor H. M. Dintzis for aid in the preparation of the labelled hemoglobins.

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BOOK REVIEWS

Advances in Enzymology and Related Subjects of Biochemistry. Volume XX. Edited by F. F. NORD, Fordham University, New York, N. Y. Interscience Publishers, Inc., 250 Fifth Ave., New York, N. Y. 1958. vii + 488 pp. 15.5 × 23.5 cm. Price, $12.50.

This publication is a continuation of an annual series of volumes started in 1941. A wide variety of topics is included, ranging from a possible relation between optical activity and aging to antibiotics and plant diseases. Much of the material is concerned with topics where modern chemical theory and methods can be applied to problems of enzyme chemistry.

The first chapter by W. Kuhn is concerned with the proposition that changes in optical activity of metabolites may be related to aging. The analysis seems to be sound but the basic assumptions appear most improbable to the reviewer. For instance it is assumed that although an enzyme brings about predominantly the synthesis of a given enantiomorph, there is always *some* synthesis of the mirror image. Another improbable assumption is that the enantiomorph not produced normally in appreciable quantity must somehow damage the organism. Few facts are given that might support the theory of change in optical activity and many of the references are out of date. In the opinion of the reviewer the thesis advanced in this chapter is most unconvincing.

The second chapter by H. Theorell is concerned with the mechanism of action of alcohol dehydrogenase, a DPN-requiring enzyme. The chapter summarizes a modern physicochemical approach to the problem of mechanism of action of this enzyme. One mechanism proposed previously by the author is critically examined and found to be inadequate and, although advances are reported, the problem is considered as still remaining not completely solved. A valuable discussion of the use of fluorescence in determining the dissociation constant of the complex between DPN and apo-enzyme is included.

The third chapter by E. A. Barnard and W. D. Stein deals with the role of imidazole in biological systems. This chapter is a very valuable summary of presently available information, much of which is critically evaluated. The specific catalytic role of imidazole groups in reactions involving acylation, phosphorylation and hydrolytic cleavage is stressed. The present difficulties in making positive identification of imidazole as an active center in a given enzyme are examined, and a suggestion is made for obtaining a new type of more specific imidazole-blocking group. The chapter is on the whole well written, but there are occasional points of slight obscurity.

The fourth chapter on uridine diphosphogalactose by H. Kalckar in substance constitutes a very good review of the role of this material in enzymatic reactions. The review is marred, however, by a rather poor style and instances of obscurity and unacceptable grammar.

The fifth chapter on the substrate and mode of action of neuraminidase by A. Gottschalk is a short but informative section on the occurrence and action of this enzyme and the nature of the substrates upon which it acts. The use of the word *terminal* in referring to the position of attachment of sialic acid to polysaccharide may be misleading, since the sialic acid residues seems to occur in multiple sites as terminal branches, and not solely as terminating groups at the end of polysaccharide chains.

The sixth chapter on the constitution of the respiratory chain in animal tissue by E. C. Slater treats the subject of biological oxidation in animal tissues. The major purpose of the presentation is stated to be a consideration of evidence for new components in the respiratory chain located in the mitochondria. The exposition stresses the past and present views of the author. The citations from the literature are incomplete and the article is probably more suitable for the specialist than for the non-specialist.

The seventh chapter on the enzymology of the plastids by N. M. Sissakian should have received much more careful editing. The English is poor. Some of the discussion is very vague; for instance there is no clear statement concerning the location of the enzymes of the Krebs tricarboxylic acid cycle in plant cells. An illustration of what seems to constitute inadequate standards on the part of the author is the inclusion of eight electrophoretic diagrams which in the opinion of the reviewer contribute absolutely no information. On the whole, this chapter does contain some worthwhile material but is not of a high standard of excellence.

The eighth chapter on enzymatic transformation of steroids by microörganisms, by E. Vischer and A. Wettstein, is a well written and excellent review, informative and comprehensive. The various steroid transformations catalyzed by microbial enzymes are listed. The enzymes themselves are discussed, and a little is said about reaction mechanisms. The authors state their hope that the chapter will stimulate interest in the purification of some of the enzymes that are mentioned.

The ninth chapter on the mechanism of hydrolysis by choline esterases and related enzymes by D. R. Davies and A. L. Green is a well-written summary of present day knowledge of the field. Some new mechanisms are proposed by the authors, who may be somewhat arbitrary however in setting up a list of the facts that they believe must be explained by any proposed mechanism for hydrolysis by esterases. The particular mechanism suggested by them for esterase hydrolysis may be somewhat too complex.

The tenth chapter on the biosynthesis of dicarboxylic amino acids and enzyme transformations of amides in plants by W. L. Kretovitch is for the most part readable and informative, although the English style is not excellent. Some of the tables are very poorly set up, and in one or two cases the data are so meagre as to make questionable their inclusion in a table at all. The interesting hypothesis of Steward and Thompson concerning a possible ring form for asparagine is mentioned and it is suggested that the occurrence of this molecular species may account for the fact that the metabolic activity of asparagine is generally lower than that of glutamine. Although the references to non-Russian papers may be incomplete, the inclusion of a very large number of Russian references is to be commended, since these often are overlooked in Western countries.

The eleventh chapter on pectic substances and pectic enzymes by H. Deuel and E. Stutz is relatively well written, informative and comprehensive. An addendum has been added to bring the work up-to-date insofar as possible.

The twelfth chapter on antibiotics and plant diseases contains much interesting material on the use and attempted use of antibiotics to control diseases of plants and to fight spoilage of harvested crops, such as potatoes. The chapter will undoubtedly be of considerable value to those interested in this new application of biochemistry. The review is well written, informative and apparently quite comprehensive.

When viewed in its entirety this volume certainly suffers from a lack of editing. There are many instances of poor construction, poor style and bad grammar. The ludicrous error occurs of using *urinediphospho galactose* instead of *uridine diphosphogalactose* as a running title. In the case of a work composed by a cosmopolitan group of authors, for many of whom English presumably is not the mother tongue, more care should be taken by the editor in smoothing out the rough spots and removing at least the most glaring mistakes. It can be stated however that this volume will be of general interest to all biochemists and a worthwhile addition to the libraries of those particularly interested in enzymology.

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Number-Average Molecular Weights. Fundamentals and Determination. By ROBERT U. BONNAR, MARTIN DIMBAT and FRED H. STROSS, Shell Development Company, Emeryville, California. Interscience Publishers, Inc., 205 Fifth Avenue, New York 1, N.Y. 1958. x + 310 pp. 16 × 23.5 cm. Price, $7.50.

As is indicated by the title, this book is concerned with a discussion of the theory and experimental methods that are employed to determine the number average molecular weights of a variety of chemical substances. An introductory chapter surveys the various methods available and discusses very briefly the advantages and disadvantages of each. The methods discussed are cryoscopic, ebullioscopic, osmotic pressure, lowering of the vapor pressure, gas density and functional group analysis. An individual chapter is devoted to a more detailed discussion of each method and one chapter is concerned solely with a theoretical discussion of the calculation of molecular weight when using the cryoscopic or ebullioscopic methods. About eighty-five per cent. of the book is devoted to a discussion of the ebullioscopic, cryoscopic and osmotic pressure methods with the other techniques receiving proportionately less attention.

The limitations of each of the primary methods are presented, particularly the molecular weight ranges that can be covered. The various types of equipment that can be utilized and that are available are considered. Thus, for example, five different types of osmometers are considered in detail and the advantages and disadvantages of each are discussed. In this and the other techniques emphasis appears to be given to designs which allow for rapidity of measurements. The major merit of this book and a very significant contribution indeed, lies in the detailed discussion and analysis of experimental procedures and possible sources of error. The latter consideration involves discussion of the more obvious sources of error and the equally important more subtle ones. Procedures are given as to how these errors can be systematically detected and in many instances advice is given as to how they can be avoided. These descriptions will be of invaluable aid to the experimentalist working in this field.

Characteristic of the methods used to determine molecular weights is the fact that though all measurements are made at finite concentrations, it is inevitable that extrapolation be made to infinite dilution to determine the required quantity, unless one is dealing with an ideal solution. Although some consideration is given to the theory of non-ideal solutions and references are given to the more standard texts on the subject, major emphasis is focused on the statistical methods of data analysis. Though the contribution of statistics in analyzing the reliability of data is well recognized, the appropriate more detailed thermodynamic analyses may be of more importance to the problem. The suggestion by the authors that the Flory–Huggins formulation be used to express the activity of the solvent in dilute solutions should be taken cautiously as it is known to be inapplicable in most dilute polymer solutions.