a framework which does not exaggerate their importance. This is an item too often neglected and it argues much for the sincerity of the author that he takes upon himself such obligation of balancing the new and spectacular against the old and dulled. It is suitable for a three-semester course in organic chemistry and yet so arranged that it can be left safely in the hands of a student for general reading preparatory to advanced work. No literature references or questions are included in the text and the saving of space so achieved is turned to good purpose in the inclusion of material and argument. This reviewer hopes that the merit of the work will be rewarded with widespread use. It is deserving of the widest support and is the best general text in organic chemistry that he has seen for many years.

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BLOOD GROUPING

Blood Grouping Technic. By F. SCHIFF and W. C. BOYD. Interscience Press, 1942.

THIS book by two outstanding investigators in the field of blood grouping is an authoritative compilation of methods of grouping, based in large degree on the experience of the authors themselves. While the book is supposed to be a translation and amplification of Schiff's small manual which enjoyed a well-deserved popularity for a long time in Germany, it is really a new book. The arrangement of the material is quite different and the book is nearly three times as large as the German manual on account of the inclusion of much new material. It is unfortunate that Dr. Schiff did not live to see this fine book in print, but the work was capably brought to its completion by Dr. Boyd.

The monograph opens with a brief introductory chapter outlining what is known of the individual differences in human blood and the secreting factor. In the succeeding extensive chapter, the general technic of blood grouping is described in great detail, including methods for determining the four blood groups, the subgroups of groups A and AB, the M-N types and the Rh-type. Detailed instructions are given for carrying out tests not only on blood by direct agglutination and hemolysis, but also on organs and secretions by the technics of absorption, inhibition and complement fixation. In addition, full directions are given for the preparation of suitable grouping sera, including immune sera in rabbits against agglutinogens A, B, M and N. In the remaining chapters of the book, further refinements in the technic are presented from the point of view of the specialized requirements in relation to the practical applications of the tests in blood transfusion, in forensic medicine in cases of disputed parentage and for the examination of blood stains and in anthropology.

Of considerable interest is the section discussing the anthropological significance of the blood groups, especially since Dr. Boyd has made significant contributions to this subject. Among the theories mentioned to explain the differences in the present distributions of the blood groups genes throughout the world the most plausible is that favored by Bernstein and Candela that there were originally two or more races, each belonging predominantly to one or two of the four blood groups, and that the present distribution in white races arose by crossing between the original races. Boyd himself, however, proposes a monophyletic theory and postulates that man originally had a blood group distribution represented approximately by the frequencies p (A) = 0.35, q (B) = 0.15 and r (O) = 0.65, and that as man spread to the four corners of the world, isolated groups by chance lost largely one or two of the three genes. The weak point in this theory is that no attempt is made to explain how the original blood group distribution arose, nor to apply to man the observations on apes and lower monkeys. To the reviewer the polemic between the sponsors of the monophyletic and polyphyletic theories appears largely academic, since undoubtedly numerous times in man's history there must have been periods of migration, isolation and inbreeding succeeded by periods of invasion and mixing of races. Accordingly, as the authors of the manual will probably agree, the choice between the two theories would depend to a great extent on the time selected as the onset of man's history as a distinct species.

All in all this book on blood grouping technic constitutes a valuable contribution and one destined to serve as a standard and authoritative reference work on the subject for a long time to come.

A. S. WIENER

SPECIAL ARTICLES

ENZYME SYSTEMS CONTAINING ACTIVE SULFHYDRYL GROUPS. THE ROLE OF GLUTATHIONE[1]

UP to now there has been published scattered information on this subject, and from time to time there have appeared observations on the presence of -SH groups essential for enzyme activity among some hydrolytic enzymes, certain lipid-splitting enzymes, several pneumococcal and streptococcal hemolysins, and a few oxidation enzymes. A comprehensive study of the presence of -SH groups essential for

[1] From the Chemical Division, Department of Medicine, the University of Chicago.

enzyme activity was considered therefore necessary. The activity of the enzyme systems was measured in the presence of iodoacetamide, chloromercuribenzoic acid and organic arsenicals.[2] Reactivation was studied by addition of glutathione after the enzyme had remained in contact with the inhibitor for about 20 minutes.

Enzymes for Carbohydrate Metabolism: Some evidence for the presence of -SH groups in pyruvate oxidase could be found in the inhibition produced by 2,6-dichlorophenol indophenol, and quinone reported by Barron[3] and in the increase of the rate of pyruvate oxidation and of acetoacetate synthesis by ground avian liver on addition of glutathione (Table I). The

TABLE I

THE EFFECT OF GLUTATHIONE (GSH) (0.01 *M*) ON THE OXIDATION OF PYRUVATE AND ON THE FORMATION OF ACETO-ACETATE
FIGURES GIVE CMM. PER HR. GROUND PIGEON LIVER; BUFFER, NaCl-PHOSPHATE, PH 7.4; CONCENTRATION OF PYRUVATE, 0.01 M

	Control	GSH
O_2 uptake	241	371
Pyruvate utilization	174	378
Aceto-acetate formation	40.2	114.6

presence of -SH groups in the protein seems necessary not only for pyruvate oxidation, but also for pyruvate dismutation, and pyruvate condensation (CO_2 fixation ?), (Table II). In these experiments, pyruvate oxidation was determined by measurement of O_2 uptake; dismutation by measurement of CO_2 production in bicarbonate-Ringer and N_2: CO_2 as gas phase; and condensation by measurement of ketoglutarate, aceto-acetate, acetyl methylcarbinol and carbohydrate synthesis. Iodoacetamide, chloromercuribenzoic acid and organic arsenicals inhibited these reactions; glutathione reactivated them. Besides pyruvate, the oxidation of malate and of ketoglutarate were inhibited by these reagents and reactivated by glutathione.

Native myosine (adenosine triphosphatase) was inhibited by chloromercuribenzoic acid and reactivated by glutathione.

Enzymes for Nitrogen Metabolism: d-Amino acid oxidase, l-glutamic acid oxidase, monoamine oxidase and transaminase were inhibited by iodoacetamide, chloromercuribenzoic acid and trivalent arsenicals, and reactivated by glutathione (Table II). Diamine oxidase was not inhibited by these -SH reagents.

Enzymes for Alcohol Oxidation: Dixon[4] found that

[2] The organic arsenicals used were: p-carboxyphenyl arsine oxide, 3-amino, 4-hydroxy phenyl dichloro-arsine HCl, p-amino phenyl dichloro-arsine HCl, p-carbamylphenyl arsine oxide, and p-arsine oxide, kindly furnished by Dr. Harry Eagle. p-Chloromercuribenzoic acid was kindly provided by Dr. L. Hellerman.

[3] E. S. G. Barron, *Jour. Biol. Chem.*, 113: 695, 1936.

[4] M. Dixon, *Nature*, 140: 806, 1940.

TABLE II

ENZYME SYSTEMS CONTAINING ACTIVE -SH GROUPS THEIR INHIBITION WITH IODOACETAMIDE (0.001 *M*), CHLOROMERCURIBENZOIC ACID (0.001 *M*), AND ORGANIC TRIVALENT ARSENICALS (0.0001 *M*), AND THEIR REACTIVATION WITH GLUTATHIONE (0.01 *M*)

Enzyme system	Inhibition (per cent.)			Reactivation (per cent.)
	Iodoacetamide	ClHg benzoic acid	Organic arsenical	Glutathione
Carbohydrate metabolism				
Pyruvate oxidation	80	92	87	41
Pyruvate condensation (α ketoglutarate synthesis)			87	
Pyruvate condensation (acetoacetate synthesis)			63.5	complete
Pyruvate condensation (carbohydrate synthesis)			95	93
Pyruvate dismutation		96	35.6	93
Pyruvate condensation (acetyl methyl carbinol formation	64.5	85	56	complete
α Ketoglutarate oxidation	90	70	98.5	98
Malate oxidation		complete	69.5	80
Native Myosine (adenosine triphosphatase)		90		complete
Nitrogen metabolism				
d-amino acid oxidase		complete	90	78
l-glutamate oxidase	none	87		90
Transaminase		49	81	95
Monoamine oxidase	33.3	82	71	complete
Fat metabolism				
Stearate oxidase (liver)	30	complete	complete	
Stearate oxidase (bacteria)	80	complete	72	
Oleate oxidase (bacteria)	52	complete	complete	
β Hydroxybutyrate oxidase (heart)		complete	complete	complete
Lipase	none	38	62	72

the oxidation of ethyl alcohol by yeast alcohol oxidase was inhibited by iodoacetate while its oxidation by liver alcohol oxidase was not affected. The same results were found by using the purified activating proteins and measuring the rate of diphosphopyridine nucleotide reduction.

The oxidation of choline by liver choline oxidase was found by Block and Barron[5] to contain -SH groups essential for activity. Similar findings were observed on the oxidation of glycerol by bacteria.[6]

Enzymes for Fat Metabolism: The presence of -SH groups was found necessary for the activity of the following enzymes concerned with the metabolism of fats: the oxidation of stearate by rat liver extract, and by bacteria (*B. coli*), the oxidation of β hydroxybutrate by animal tissues. Pancreatic lipase was partially inhibited by chloromercuribenzoic acid and by organic arsenic while it was not affected by iodoacetamide (Table II). In these experiments, the oxidation of stearate was measured by the O_2 uptake; the oxidation of β hydroxybutyrate, by the rate of reduction of diphosphopyridine neucleotide; pancreatic lipase activity by titration with NaOH.

[5] B. Block and E. S. G. Barron. To be published.

[6] E. S. G. Barron, *Bol. Soc. Quim. Peru*, 6: 7, 1940.

Esterases: Nachmanson[7] has shown that acetyl choline esterase is inhibited by mild oxidizing agents and iodoacetic acid. In agreement with these findings, 3-amino-4-hydroxyphenyl arsine oxide (6.6×10^{-5} *M*) produced 57 per cent. inhibition. In contrast with this esterase, the hydrolysis of mono-n-butyrin by human serum esterase was inhibited by ClHg benzoic acid by only 18 per cent., addition of glutathione bringing partial reactivation; hog liver esterase was inhibited by 31 per cent. with p-carbamyl phenyl arsine oxide, and by 9 per cent. with ClHg benzoic acid; pancreatic esterase was not affected by the -SH reagents.

Proteins containing no -SH groups essential for enzymatic activity: The following enzymes were not affected by the above-mentioned -SH reagents: polyphenol oxidase, arginase, citric oxidase, uricase, catalase, lactic oxidase, liver alcohol oxidase, histaminase, potato phosphorylase, carbonic anhydrase, acid phosphatase, peanut fat oxidase, pepsin, cytochrome oxidase and flavoproteins.

Since such a large number of enzyme systems contain in their protein moiety -SH groups essential for enzyme activity, the role of glutathione becomes of great importance. Glutathione, by maintaining these groups in their reduced form would maintain the enzyme activity of those systems possessing essential -SH groups.

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T. P. SINGER

AUXIN ACTION

MUCH is already known about the results of the action of auxins and similarly behaving compounds in inducing roots to grow on cuttings, in producing parthenocarpy and in affecting the growth of the whole plant, especially at the tips of herbaceous stems where growth activity is most intense. However, the fundamental mechanism is still to be revealed. The purpose of this article is to make known that discovery and to support it with sound evidence.

The mechanism is fundamentally the release of diastase, and possibly sucrase and other enzymes, from the protein colloidal substances to which they are normally attached. Enzymes are partly and considerably inactivated by adsorption onto a colloid as shown by Eyster.[1] A 100 ml solution containing 50 ml 1 per cent. soluble starch and 5 ml 1 per cent. diastase (Merck) and enough water to make a total volume of 100 ml required only 15 minutes for digestion past the last iodine staining stage as shown by the I_2KI test. A similar solution with 1 gram of dry activated charcoal, added immediately after the diastase was added, required 234 minutes (almost four hours). While ethyl alcohol, ether and chloroform decreased slightly the enzymatic activity of diastase in the absence of a colloidal carrier, they markedly accelerated the activity in the presence of charcoal. This indicates that the narcotics had a stronger influence in releasing the diastase from the charcoal (*i.e.*, enzyme from colloid) than it had in reducing the actual digestive action of the unbound enzyme. It was concluded that associated colloids dominate the effects of chemical agents and of environmental factors on enzymes.

Indole-3-acetic acid, β-(indole-3)-propionic acid, γ-(indole-3)-n-butyric acid and α-naphthaleneacetic acid increase the action of diastase when associated with activated charcoal in proportion to their concentrations. Table 1 presents the data for indole-propionic acid, which was being used at the time of the discovery of the mechanism of growth substances. In each case 50 ml of 1 per cent. soluble starch solution, 5 ml of 1 per cent. diastase solution, and enough indole-propionic acid to give the stated concentration were diluted to 100 ml with distilled water. The concentration of indole-propionic acid is based on the final solution volume of 100 ml. The diastase solution was added last and then there followed immediately the addition of exactly 1 gram of dry charcoal. Each mixture was placed in 125 ml bottles. The temperature of the room and of the component solutions before mixing was close to 25° C. The experiment was done in the evening in the presence of four functioning 100-watt electric light bulbs at an average distance of 8 feet. The influence of light will be clarified later in this article.

TABLE 1

INFLUENCE OF VARIOUS CONCENTRATIONS OF INDOLE-PROPIONIC ACID ON THE ENZYMATIC ACTION OF DIASTASE ASSOCIATED WITH CHARCOAL

Concentration of indole-propionic acid	Time required for digestion of soluble starch past the last iodine staining stage
0 parts per million	265 minutes
5 " " "	252 "
10 " " "	245 "
25 " " "	230 "
50 " " "	188 "
75 " " "	160 "
100 " " "	126 "
150 " " "	60 "

Table 2 presents data to show the effect of indole-propionic acid on isolated diastase; *i.e.*, diastase in the absence of charcoal. In this case 50 ml of 1 per cent. soluble starch solution, 1 ml of 1 per cent. diastase solution and enough indole-propionic acid to give the stated concentration were diluted to 100 ml. No charcoal was added. The conditions of temperature and light were the same as in the preceding ex-

[7] D. Nachmanson and E. Lederer, *Bull. Soc. Chem. Biol.*, 21: 797, 1939.

[1] *Plant Physiology*, 18: in press.