# Textbook of BIOCHEMISTRY

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# *Preface*

The expansion of biochemistry continues at so rapid a pace that frequent editions of a textbook in the field become necessary. The fact that previous editions have been received favorably makes this new (fifth) edition possible. For various reasons—not the least one of which is to avoid plaguing the student with more than a minimum number of pages—it has seemed desirable not to expand the book to any appreciable extent; and by omitting portions of the appendix and various items in the body of the book which appeared more important in 1946 than they do today, the size of the new edition has been kept substantially the same, even though a new chapter (Chap. 15, Biological Antagonists) and various other additions have been incorporated.

Apart from these additions, much rewriting has been done. A complete list of the various changes would occupy considerable space; but some of the more important of the new material is mentioned here:

Frequent references to microbiological methods (pp. 63, 142, 156, and elsewhere).

The use of Neurospora in the studies of amino acids and vitamins (pp. 63, 161, 169, 395).

Paper chromatography (pp. 63, 64).

The protein molecule (p. 71).

Nucleoproteins (pp. 76, 77, 80, 83).

Peptidases (pp. 90, 91).

Enzyme inhibitors (pp. 98, 99).

Protein hydrolysates (pp. 112, 114).

Amino acids essential for man (p. 115).

Malnutrition (p. 130).

Vitamin $B_{12}$ (pp. 170–172, 260, 459).

Animal protein factor (pp. 171, 172).

Photosynthesis (pp. 200, 201).

Origin of hydrochloric acid (p. 214).

Absorption of fat (pp. 249, 346, 347).

The anemias (p. 258).

Edema (pp. 261, 262).

Shock (pp. 264, 265).

Immunochemistry (pp. 279–281).

Histamine and antihistaminic reagents (pp. 282, 283).

BAL (British antilewisite) (p. 308).

Hyaluronic acid and hyaluronidase (pp. 284, 471).

Tyrothricin, streptomycin, aureomycin and chloromycetin (pp. 295, 296, 297).

The chapter on chemical respiration (from p. 310 on).

Energy-rich bonds (p. 333).

ATP and phospocreatine (p. 334).
Oxidation of carbohydrate (pp. 338, 339).
List of stable and radioactive isotopes (p. 349).
Theories of fatty acid oxidation (pp. 359, 360).
Formation of tissue protein (p. 372).
Interrelationships of carbohydrate, fat and protein (p. 380).
Formation of homogentisic acid (pp. 385, 387).
Alcaptonuria (p. 386).
Importance of sulfhydryl groups (pp. 388, 391).
Metabolism of lysine (p. 401).
Muscular contraction (pp. 466, 467).
Estradiol (pp. 528, 529).
Therapeutic use of estrogens and androgens (pp. 531, 535).
Hormones and carcinogenesis (pp. 537–539).
Glutamic acid and brain metabolism (p. 552).
Diisopropylfluorophosphate (DFP) (p. 555).

The bibliographies which are to be found at the end of each chapter have also been completely revised.

My thanks are due to the following for help in various ways: Prof. P. M. Apfelbaum (City College of New York), Prof. E. Borek (City College of New York); Prof. H. Burton (University of London); Prof. A. M. Chase (Princeton University); Prof. T. Coolidge (University of Chicago); Prof. H. Dam (Polytechnic Institute, Copenhagen); Prof. L. R. Dragstedt (University of Chicago); Dr. O. H. Gaebler (Henry Ford Hospital, Detroit); Prof. R. A. Gortner, Jr. (Wesleyan University); Prof. D. M. Greenberg (University of California); Prof. P. Handler (Duke University); Prof. J. H. Henderson (Tuskegee Institute); Prof. W. C. Hess (Georgetown University); Prof. J. E. Jorpes (Caroline Institute, Stockholm); Prof. S. E. Kammerling (Bowdoin College); Prof. M. Karshan (Columbia University); Prof. W. D. Langley (University of Buffalo); Prof. G. T. Lewis (Emory University); Prof. H. B. Lewis (University of Michigan); Prof. H. E. Longenecker (University of Pittsburgh); Prof. C. G. Mackenzie (Cornell University); Prof. A. Mazur (City College of New York); Prof. W. H. Moran (University of North Dakota); Prof. M. Muhrer (University of Missouri); Prof. W. A. Perlzweig (Duke University); Prof. G. H. Pritham (Pennsylvania State College); Prof. S. K. Reed (Bucknell University); Prof. R. G. Roberts (Chicago Medical School); Prof. C. S. Robinson (Vanderbilt University); Prof. E. G. Schmidt (University of Maryland); Prof. E. H. Shaw (University of South Dakota); Prof. G. Stone (City College of New York); Prof. E. H. Stotz (University of Rochester); Prof. W. S. Thompson (Kent State University); Dr. W. R. Tweedy (Veterans' Administration); Prof. H. Wagreich (City College of New York); Prof. A. White (University of California); Prof. A. E. Wilhelmi (Yale University); Prof. A. Wormall (University of London).

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BENJAMIN HARROW

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# *Abbreviations*

(For general reviews consult the *Ann. Rev. Biochem.*, *Ann. Rev. Physiol.*, *Physiol. Rev.*, and *Harvey Lectures*. References to original papers are given. For further references see *Chemical Abstracts*.)

*Adv. Enzym.* = Advances in Enzymology
*Adv. Protein Chem.* = Advances in Protein Chemistry
*Am.* = American
*Am. J. Physiol.* = American Journal of Physiology
*Analyt. Chem.* = Analytical Chemistry
*Annals N. Y. Acad. Sciences* = Annals of the N. Y. Academy of Sciences
*Ann. Rev. Biochem.* = Annual Review of Biochemistry
*Ann. Rev. Physiol.* = Annual Review of Physiology
*Arch. Biochem.* = Archives of Biochemistry
*Arch. Internal Med.* = Archives of Internal Medicine
*Biochem. J.* = Biochemical Journal
*Biochem. Z.* = Biochemische Zeitschrift
*Bull. N. Y. Acad. Med.* = Bulletin of the N. Y. Academy of Medicine
*Chem. Eng. News* = Chemical and Engineering News
*Chem. Rev.* = Chemical Reviews
*Federation Proceedings* = Federation of American Society for Experimental Biology
*Ind. Eng. Chem.* = Industrial and Engineering Chemistry
*J. Am. Chem. Soc.* = Journal of the American Chemical Society
*J. Am. Med. Assoc.* = Journal of the American Medical Association
*J. Biol. Chem.* = Journal of Biological Chemistry
*J. Chem. Educ.* = Journal of Chemical Education
*J. Gen. Physiol.* = Journal of General Physiology
*J. Nutrition* = Journal of Nutrition
*J. Physiol.* = Journal of Physiology
*J. Soc. Chem. Ind.* = Journal of the Society of Chemical Industry
*Nutr. Rev.* = Nutritional Reviews
*Physiol. Rev.* = Physiological Reviews
*Proc.* = Proceedings
*Proc. Nat. Acad. Sci. U. S.* = Proceedings of the National Academy of Sciences of the United States of America
*Proc. R. S.* (London), Series B = Proceedings of the Royal Society (London)
*Proc. Soc. Exp. Biol. Med.* = Proceedings of the Society for Experimental Biology and Medicine
*Symp. Quant. Biol.* = Symposia on Quantitative Biology
*Trans.* = Transactions
*Z. physiol. Chem.* = Zeitschrift für physiologische Chemie

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Chapter 26

# *Chapter 1. Introduction*

Without attempting a definition of biochemistry, we may say that it deals, among other things, with the chemical processes which go on in living matter. Chemical processes common to plants come under the heading of "plant biochemistry"; and this phase of the subject is barely touched upon in the present volume. A short chapter on photosynthesis (Chap. 9) merely scratches the surface. For further information, see, for example, Tottingham: *Plant Biochemistry*.

In this volume we devote ourselves to animal biochemistry, and when the word "biochemistry" (or "physiological chemistry") alone is used, we usually mean "animal biochemistry."

While it is difficult to trace origins, one is tempted to speak of Lavoisier (1743–1794) not only as the father of modern chemistry, but also as the father of biochemistry, for he was certainly one of the first, if not the first, to appreciate the true nature of respiration. His classical researches into oxidation, and the central role played by oxygen in the process, led him to investigate "burning" in the body, and he came to the conclusion that oxygen is consumed in the reaction, that carbon dioxide is eliminated, and that heat is evolved. He also realized that the temperature of the body is the result of the oxidation of foodstuffs. Later, in the hands of Voit, Pettenkofer, and Rubner in Germany, and Atwater and Benedict in this country, animal calorimetry (Chap. 20) became a science in the modern sense.

Liebig (1803–1873) and Wöhler (1800–1882), two organic chemists, had much to do with the further development of the subject; for their researches led them, from time to time, to analyze material of vegetable and animal origin. Liebig arrived at the conclusion that "the nutritive materials of all green plants are inorganic substances"; and Wöhler's dramatic synthesis of urea, the principal end-product of nitrogenous metabolism in the body, did much to destroy the notion that animal products were endowed with a "vitalism" which made them fundamentally different from "lifeless" substances. The work of Chevreul (1786–1889) on the chemical constitution of fats, and later the researches of Kossel and Emil Fischer on proteins, and of Emil Fischer on carbohydrates, gradually led to an understanding of the chemical composition of foods and the chemical composition of the cell.

Nor must the influence of the illustrious Pasteur (1822–1895) be overlooked. His extensive researches into the nature of fermentation led Buchner (1860–1917) to our modern conception of enzymes (Chap. 6), the cellular catalysts which are responsible for much of the activities within the body. Further, Pasteur's work on fermentation was a prelude to much activity in the field of muscle metabolism (Chap. 17), and, even more recently, to work dealing with the metabolism in the brain (Chap. 26).

Researches by such pioneers as Arrhenius, van't Hoff, and Ostwald on electrolytic dissociation and osmotic pressure led physical chemists, as well as organic chemists, to turn their attention to biological phenomena. The results were very fruitful; among others, Sörensen developed our concept of *p*H (Chap. 4), Loeb examined the colloidal behavior of proteins (Chap. 4), and L. J. Henderson and Van Slyke developed their ideas regarding "body neutrality" (Chap. 16).

Side by side, physiologists and clinicians were contributing much of great value to the biochemist in such fields as digestion (Chap. 10), absorption (Chap. 12), blood (Chap. 13), and metabolism (Chaps. 17, 18, 19, 21).

Nor can we overlook the impetus to further work given by the founding, in 1879, of the first journal devoted to biochemistry, the *Zeitschrift für physiologische Chemie*. In 1906 three other journals were started: the *Journal of Biological Chemistry* in this country, the *Biochemical Journal* in England, and the *Biochemische Zeitschrift* in Germany. To this day, the bulk of the important literature in biochemistry still appears in these four journals.

Present-day activity in biochemistry is very varied; but some of the most sensational successes in recent times have been in the fields of vitamins, hormones, enzymes, and nucleoproteins. A number of vitamins and hormones have been not only isolated but even synthesized, and some of them have turned out to be relatively simple substances (Chaps. 8, 25). Enzymes are probably all proteins, and therefore very complex (Chap. 6). Not the least interesting discovery is that several vitamins are probably "mother substances" of enzymes active in metabolism (Chap. 20). And chromosomes and some viruses, at least, are being more and more associated with nucleoproteins (Chap. 5).

These activities must be coupled with the activities in the field of chemotherapy, with its sulfonamides and its amazing penicillin (Chap. 14).

At the beginning of the present century there was already an active laboratory of biochemistry in this country. Chittenden was its founder at Yale. One of his pupils, Mendel, succeeded him, and another, Gies, became professor of the subject at Columbia. Folin was appointed to a chair of biochemistry at Harvard in 1907. The guiding spirits in the medical schools quickly recognized the importance of the subject, and chairs of biochemistry sprang up all over the country.

To such an extent has biochemistry developed, here and abroad, that a substantial portion of *Chemical Abstracts* is devoted to abstracts of biochemical articles. In the attempt to summarize a field which has

become so extensive and diversified, the *Annual Review of Biochemistry* was founded in 1932.

In this book, the chemical composition of the cell is discussed in the opening chapters. The substances which have been identified include carbohydrates, lipids, proteins, and enzymes. Water and inorganic salts, also present, are more appropriately considered in the section devoted to metabolism. Hormones too are discussed towards the end of the book in connection with coordinating mechanisms of the body.

The chapters dealing with synthesis in the plant kingdom, foods, and vitamins are preliminary to a discussion of digestion and absorption. The digested materials are carried by the blood to the various cells of the body. A discussion of this "carrier," the blood, together with methods by which foods are oxidized, leads to chapters on blood, respiration, various phases of metabolism, and the mechanism of biological oxidation. The elimination of substances from the body leads to a discussion of urine. The dramatic clinical results obtained with sulfonamides and penicillin have suggested immunochemistry and chemotherapy (Chap. 14). The nervous system and the glands of internal secretion (which manufacture hormones) represent the coordinating links of the body; and the concluding chapters are devoted to them.

## EFERENCES

*Chittenden:* The Development of Physiological Chemistry in the United States, 1930.

*Von Meyer:* History of Chemistry, 1891.

*Lieben:* Geschichte der physiologischen Chemie, 1935.

*Mettler:* History of Medicine (1947).

# *Chapter 2. Carbohydrates*

The carbohydrates include substances which are constituents of the cell, compounds which are important foods, and products which find industrial application. They include polyhydroxyaldehydes and polyhydroxyketones, and compounds which can be converted into such aldehydes or ketones by hydrolysis.

The simplest of these compounds is glycolaldehyde, $CHO.CH_2OH$, which exhibits many of the properties of carbohydrates. However, carbohydrates are all optically active, containing asymmetric carbon atoms, which makes glyceraldehyde, $CHO.CHOH.CH_2OH$, the more logical "mother" substance.

**Classification.** The more important of these carbohydrates may be classified as follows:

| | |
|---|---|
| *Monosaccharides* | Pentoses, $C_5H_{10}O_5$ (ribose, xylose, arabinose, etc.)<br>Hexoses, $C_6H_{12}O_6$ (glucose, mannose, galactose, fructose, sorbose, etc.) |
| *Disaccharides* | $C_{12}H_{22}O_{11}$ (sucrose, lactose, maltose, isomaltose, etc.) |
| *Trisaccharides* * | $C_{18}H_{32}O_{16}$ (raffinose, etc.) |
| *Polysaccharides* | $(C_6H_{10}O_5)_x$ (starch, glycogen, dextrin, cellulose, gum, mucilage, inulin, etc.) |

The monosaccharides cannot be hydrolyzed into simpler sugars. By the use of the appropriate acid or enzyme, the higher saccharides can be hydrolyzed:

$$C_{12}H_{22}O_{11} + H_2O \rightarrow C_6H_{12}O_6 + C_6H_{12}O_6$$
Sucrose $\rightarrow$ Glucose + Fructose
Maltose $\rightarrow$ Glucose + Glucose
Lactose $\rightarrow$ Glucose + Galactose

$$C_{18}H_{32}O_{16} + 2H_2O \rightarrow C_6H_{12}O_6 + C_6H_{12}O_6 + C_6H_{12}O_6$$
Raffinose $\rightarrow$ Fructose + Glucose + Galactose

A number of the polysaccharides, upon complete hydrolysis, yield glucose as the end-product (for example, glycogen, starch, dextrin, cellu-

* The term *oligosaccharides* is also used for compounds made up of two to five molecules of monosaccharides. Above this number we deal with polysaccharides.

lose); some yield fructose (for example, inulin; and some yield galactose (for example, certain gums).

## MONOSACCHARIDES

**Structure of Glucose.** The somewhat exceptional position occupied by glucose in carbohydrate metabolism (Chap. 17) and the impossibility, owing to limitations of space, of discussing the structure of each sugar individually make it desirable to describe glucose in some detail. Much of this discussion holds for other sugars.

A qualitative analysis of a purified sample of glucose shows the presence of the elements carbon and hydrogen; a quantitative analysis reveals the presence of oxygen also. The elements are in such proportion to one another that the formula ($CH_2O$) can be assigned to the compound. A molecular weight determination (by the freezing point depression method, for example) reveals that the formula assigned should be $(CH_2O)_6$, or $C_6H_{12}O_6$.

Glucose forms an oxime with hydroxylamine (p. 19) and an osazone with phenylhydrazine (p. 19), and reduces Benedict's solution (p. 17); all of these reactions point to the presence of a $\begin{matrix} | \\ C{=}O \\ | \end{matrix}$ group, and this group may represent an aldehyde or a ketone.

Glucose forms a pentaacetyl derivative with acetic anhydride, indicating the presence of five free hydroxyl groups. It is reduced by means of sodium amalgam to an alcohol, hexahydroxyhexane, $CH_2OH.(CHOH)_4.CH_2OH$, which is sorbitol; and the latter compound, when treated with hydrogen iodide, is converted to a derivative of *normal* hexane, $CH_3.(CH_2)_3.CHI.CH_3$. With hydrogen cyanide, glucose forms an addition compound which when hydrolyzed gives a straight-chain seven-carbon acid. All these facts point to a straight-chain compound, with the $\begin{matrix} | \\ C{=}O \\ | \end{matrix}$ at one end (a compound containing the aldehydic group).

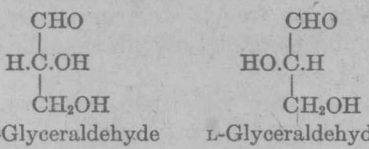
$$\begin{matrix} 1.\ CHO \\ | \\ 2.\ CHOH \\ | \\ 3.\ CHOH \\ | \\ 4.\ CHOH \\ | \\ 5.\ CHOH \\ | \\ 6.\ CH_2OH \end{matrix}$$

**Isomers of Glucose.** An examination of the formula reveals that the compound has four asymmetric carbon atoms (at positions 2, 3, 4, and 5), each carbon being attached to four different atoms or groups of atoms. For example, the carbon at position 2 may be shown thus:

$$\begin{matrix} & R & \\ & | & \\ H- & C & -OH \\ & | & \\ & R^1 & \end{matrix}$$

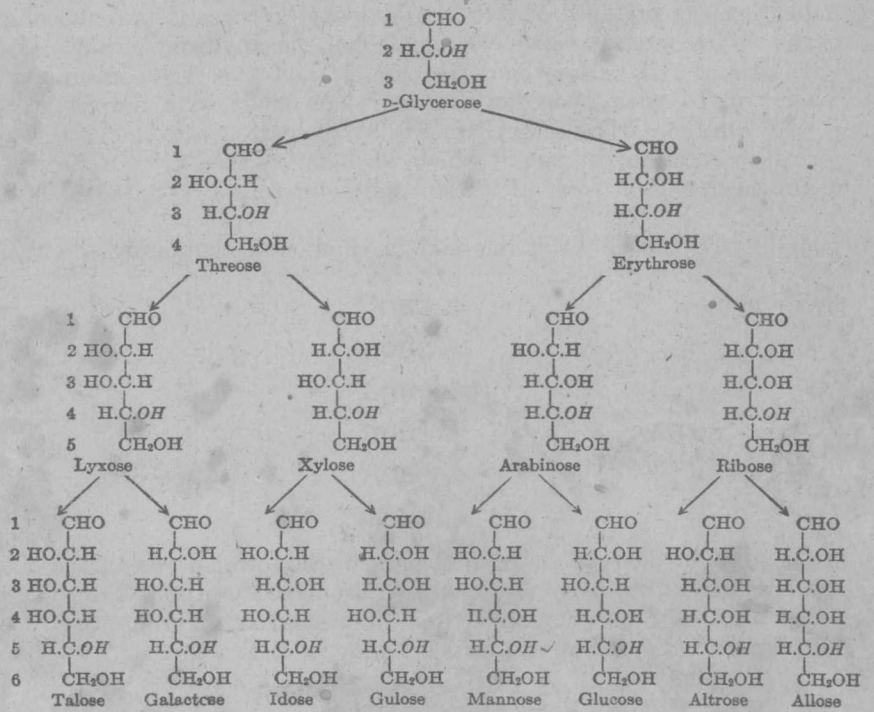
where $R$ stands for the CHO group and $R^1$ for everything below carbon 2. According to van't Hoff, the number of possible isomers is given by the formula $I = 2^n$, where $n$ represents the number of asymmetric carbon atoms. Since glucose has four such asymmetric carbon atoms, the number of isomers equals 16 ($2^4$).

**The Spatial Arrangement of the Isomers of Glucose.** The exact proof for the stereochemical configuration of each isomer of glucose is beyond the scope of this book. Taylor and also Whitmore (see references at the end of the chapter) give accounts of this phase of the work. All that can be said at this point is that the isomers are traced back to some simple compound, the constitution of which is beyond question. We may, for example, regard these isomers as being derived from glyceraldehyde:



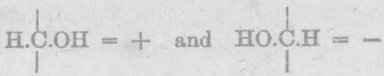

From each isomer of this aldehyde there are derived eight isomers of glucose. For example, the accompanying chart shows the derivation and configuration of eight aldohexoses:

CONFIGURATION OF THE D-ALDOSES

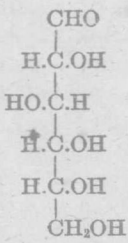



* The symbols D- and L- refer to *configuration*, whereas dextro (+) and levo (−) refer to *sign of rotation*. D- and L- are always pronounced "dee" and "ell," *never* "dextro" and "levo." See also footnote, p. 12.

Using the notation that




then D-glucose,




can be represented in shorthand form as

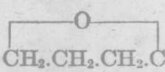

Here the two end-carbon combinations are ignored in this notation. We can represent the sixteen possible isomers as follows:

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| − + | + − | − + | + − | − + | + − | − + | + − |
| − + | − + | + − | + − | − + | − + | + − | + − |
| − + | − + | − + | − + | + − | + − | + − | + − |
| + − | + − | + − | + − | + − | + − | + − | + − |

The first of each pair represents the D-series (related to D-glucose and D-glyceraldehyde), and the second, the L-series.

In this D-series, the hydroxyl group next to the primary alcohol group is written to the right; whereas the reverse is true with the L-series.

**The Cyclic Structure for Glucose.** However, the above formula for glucose still does not explain all the facts. To begin with, glucose, which is pictured as an aldehyde, falls somewhat short of certain common aldehydic properties. For example, glucose fails to give a Schiff's test (the formation of a reddish-violet color with magenta solution which has been decolorized with $SO_2$), nor does it form a stable addition compound with sodium bisulfite. Hydroxy acids of the $\gamma$- or $\delta$-variety, similar in general structure to glucose, form lactones very readily, and these are cyclic in structure; for example, $\gamma$-hydroxybutyric acid, $CH_2OH.CH_2.CH_2.COOH$, is changed to $\gamma$-butyrolactone,




**Mutarotation.** But even more important is the problem of mutarotation or "change of rotation." A freshly crystallized sample of glucose has a specific rotation (in water) of $+111°$ ($[\alpha]_D + 111°$); upon standing, this changes to $+52°$. Since the specific rotation of any compound is, as a rule, characteristic of the optically active compound in question

(just as melting and boiling points are usually characteristic criteria), a change in rotation suggests a change in the structure of the substance. This appears all the more probable when it is shown that the compound with the rotation +111° (now known as α-glucose) can be dissolved in boiling pyridine and crystallized from this solvent to give an isomer with the specific rotation of +19° (now known as β-glucose), which, upon standing, also changes slowly to +52°. The β-form is the more stable one at temperatures around 100° C.

Another fact has to be recorded now. When an aldehyde is treated with methyl alcohol (using an acid to catalyze the reaction), an acetal is formed.

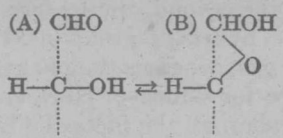
$$R.CHO + 2CH_3OH \rightarrow R.CH(OCH_3)_2 + H_2O$$

Here two molecules of methyl alcohol are used for one molecule of the aldehyde. When, however, glucose is treated similarly, the sugar combines with but *one* molecule of methyl alcohol:

$$C_6H_{12}O_6 + CH_3OH \rightarrow C_7H_{14}O_6 + H_2O$$

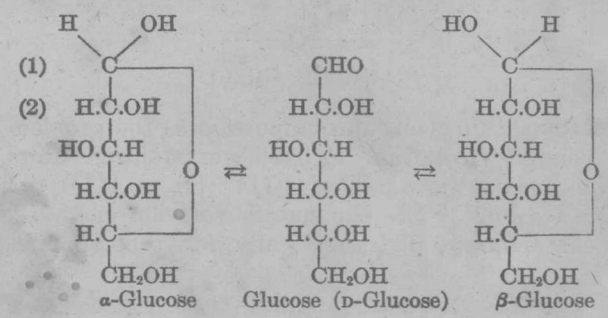
The methyl glucoside which is formed can be resolved into two modifications: an α-methyl glucoside (rotation +159°) and a β-methyl glucoside (rotation −34°). The enzyme maltase hydrolyzes the former initially to α-glucose, and the enzyme emulsin hydrolyzes the latter initially to β-glucose (followed by mutarotation in each case).

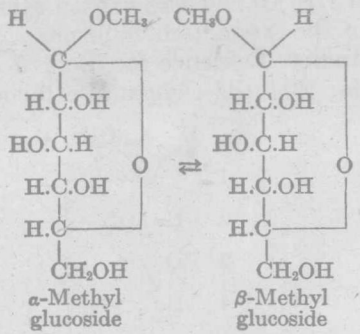
The two modifications each of D-glucose and the corresponding methyl glucoside suggest the presence of an additional asymmetric carbon atom in the molecule, which can be shown if we assume a cyclic structure:



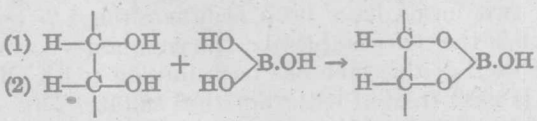

Here the carbon atom at (A), part of an ordinary aldehydic group, has been converted into an asymmetric carbon atom (B); so that now in glucose we have the possibility not of 16 (= $2^4$), but of 32 (= $2^5$) isomeric aldohexoses.

In the accompanying chart the structures and relationships of the compounds just discussed are given (the evidence for the oxygen bridge in the 1:5 position will be taken up presently).



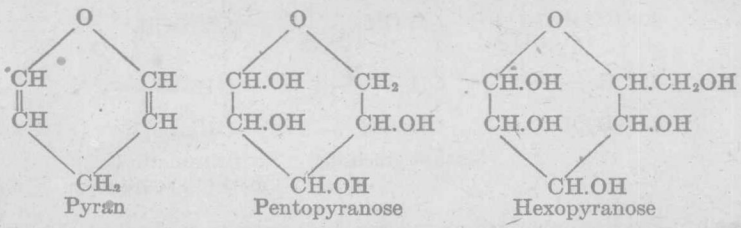



It might be pointed out that there are reasons for writing the H and OH positions at carbon (1) in α- and β-glucose as shown. For example, α-glucose combines very readily with boric acid:



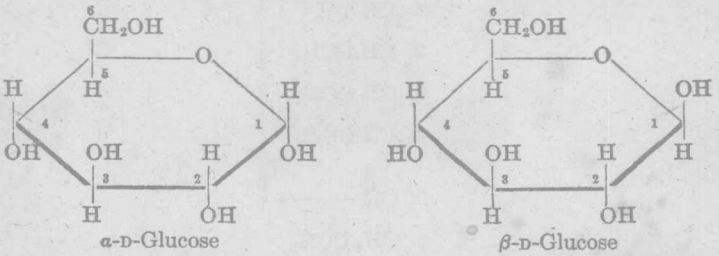

suggesting that the two OH groups in (1) and (2) are on the right. The β-form, however, does not combine so readily with boric acid until mutarotation has given some α-form.

Assuming, for the time being, a 1:5 oxygen bridge, glucose may be pictured as a derivative of a pyran, the 5-carbon sugar form for which would be a pentopyranose, and the 6-carbon sugar a hexopyranose:




One such hexopyranose, glucose, can therefore be called glucopyranose.

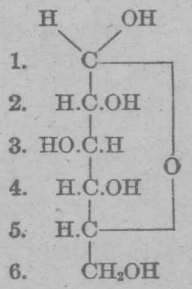
Haworth shows this model in perspective, with the H and the OH groups above or below the plane of the ring:




The ring is at right angles to the plane of the paper. The thin bonds of the ring are behind the plane of the paper, and the thick bonds in

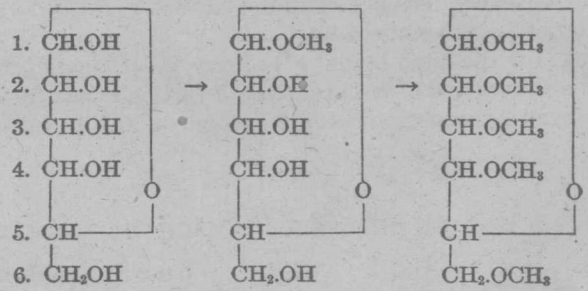
front of it. (H and OH attached to carbon atoms 1, 2, 3, 4, which are on the *right* side of the straight-chain formula, are here on the *bottom*.)

**Methylation Studies. Evidence for the 1:5 Oxygen Bridge.** It has been assumed so far that the oxygen is attached to carbons 1 and 5.




There are obviously several other possible attachments. As a matter of fact, but two forms have been isolated: the 1:5 and the 1:4. Of these, the 1:5 is the more stable and the commoner form.

After the methyl glucoside has been formed (with $CH_3OH + HCl$), the product is next treated with dimethyl sulfate. This results in complete methylation of the OH groups. Without using the stereochemical formulas, and still assuming the 1:5 oxygen bridge, the reactions are:




The glucosidic methoxyl group can be easily hydrolyzed, whereas the other methoxyls cannot—behaving, indeed, as true ethers. If, then, we hydrolyze the completely methylated compound, we get tetramethyl glucopyranose: