THE FERMENTATION REACTIONS OF CERTAIN STREPTOCOCCI *

XLII. STUDIES IN BACTERIAL METABOLISM

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The group of the streptococci comprises a very important division of bacteria, characterized fundamentally by an adherence of descendents of single spherical cells in chains of a greater or lesser length The size, and in a measure the shape, of individual units varies somewhat, and different strains or races may exhibit definite departure from the spherical morphology characteristic of the majority of members of the group.

Dynamically, the streptococci play a not unimportant part in the economy of nature; among its members are strains or races whose activities are or may be in partial opposition to those of man and the lower animals.

Some strains of streptococci appear to exist without an animal or human host. A majority live on the surface of the body or on mucous membranes of the host as "opportunists," always in communication with the exterior where escape to other hosts is readily accomplished. The normal existence of such organisms, consequently, is parasitic. The parasitic strains appear to lack the inherent power or quality of invasiveness and therefore do not ordinarily enter the tissues of the body until normal barriers which are opposed to their entrance are weakened or removed. They are "opportunists" with reference to infection. Such being the case, their association as secondary or ancillary invaders of the body in the exanthemata and other diseases is not surprising.

The type of infection induced by these "opportunists" is inflammatory in character, therefore not specific in the pathologic sense, and the portal of entry may be almost any part of the body. It is

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quite certain, furthermore, that those streptococci which do gain entrance to the underlying tissues or grow there do not succeed in escaping readily from these tissues to the surface of the body or to channels in communication with the exterior. They do not escape to other hosts in sufficient numbers, consequently, to perpetuate this infection; it is indeed doubtful whether they could penetrate the tissues of new hosts, even were escape from preceding hosts possible. In other words, parasitic strains of streptococci do not appear to be specific incitants of epidemic disease.

On the other hand, the localization of streptococci in various parts of the body in residual foci when acute symptoms have abated is an important pathologic process. It is fortunate that escape from such areas is difficult, thus preventing in a large measure the perpetuation of such strains from host to host.

Occasionally, however, strains or races of streptococci are met with which do appear to possess the power of independent invasiveness. Thus, epidemics of septic sore throat,* of streptococcus enteritis, and of streptococcus pneumonia of the epidemic type may be justly regarded as manifestations of progressive invasiveness from host to host. These instances approach most closely the fundamental requirements of a progressively pathogenic existence, namely, the multiplication of the microbe in the tissues of the host, escape from the tissues to a channel of the body in communication with the exterior, escape to the exterior, reaching a suitable portal of entry in a successive host, invasion of the tissues, multiplication within the tissues, and escape once again to a new host. In this respect, the group of the streptococci contains members of three classes: Saprophytes, which are practically without pathogenic powers; parasites, or "opportunists," and, finally, progressively pathogenic varieties.[1]

A multitude of classifications of the group streptococcus are recorded. For purposes of discussion four principal avenues of approach are clearly discernible: First, a classification based on the origin of the culture, be it with reference to the host, as Streptococcus equinus from the horse; Streptococcus lacticus from the milk of the cow; from the lesion, as Streptococcus mastitidis, Streptococcus scar-

* Secondary cases do not appear to arise from contact with primary cases; transmission appears to be through milk as a vehicle.

[1] See Kendall: Bacteriology, General, Pathological and Intestinal, for a more complete discussion of saprophytism, parasitism and pathogenism.

latinae, Streptococcus gingivae, Streptococcus erysipelatis; or from a definite secretion, as Streptococcus salivarius, Streptococcus ureae.

Secondly, a classification based on changes induced in blood pigment by various members of the group. Schottmüller[2] appears to have been the first investigator to study the problem from this angle. As a result of work in this field, the streptococci are divisible into four principal types: Those which induce hemolysis of blood, Streptococcus pyogenes or Streptococcus hemolyticus; those which produce green colonies — Streptococcus viridans; those forming viscid colonies — Streptococcus mucosus, and, finally, cultures without obvious change in the hemoglobin and which do not form viscid colonies.

Thirdly, the use of various carbohydrates, the fermentation reactions in which furnish criteria for division into several groups. Gordon,[3] and Andrews and Horder[4] have made extensive contributions to this subject.

Fourthly, a combination of the fermentation and hemolytic reactions. The works of Holman,[5] Lyall,[6] and Miss Broadhurst[7] are noteworthy in this connection. This method on the whole appears to have more nearly met the requirements of a satisfactory grouping than the others mentioned.

The work of Rosenow[8] on the transmutation of streptococci into pneumococci for a time seriously disturbed the prevailing ideas of specificity of organisms; since this work has not as yet received confirmation, final judgment as to its possible effects on attempts to classify streptococci may be withheld. The timely statement of Smith and Brown,[9] however, is reassuring: "Spontaneous changes in the structural characters of the streptococcus do not proceed rapidly enough, if they proceed at all, to interfere with current bacterial methods. Tendencies toward slow changes may be used as further valuable distinguishing characters."

The present investigation concerns the fermentative reactions of 356 cultures of streptococci, obtained chiefly from Camps Lee, Grant, Custer and Fort Sam Houston. These organisms were isolated from

[2] München. med. Wchnschr., 1903, 20, p. 849.
[3] Ann. Report Local Govt. Board, Medical Officer, 1903, p. 388.
[4] Lancet, 1906, p. 171, p. 1245.
[5] Jour. Med. Research, 1916, 34, p. 377.
[6] Ibid., 1914, 30, p. 487.
[7] Jour. Infect. Dis., 1915, 17, p. 277.
[8] Jour. Infect. Dis., 1914, 14, p. 1.
[9] Jour. Med. Research, 1914, 31, p. 508.

cases of streptococcus pneumonia, from empyemas, blood cultures and autopsies. A smaller number of organisms were obtained from the John McCormick Institute for Infectious Diseases, the Army Medical School and Museum, and the Rockefeller Institute for Medical Research. The carbohydrates used, with the exception of C. P. lactose and maltose, were obtained through the courtesy of Dr. Hudson, Chief of the Carbohydrate Laboratory of the Department of Agriculture.

The general initial treatment of the culture was to plate it twice on rabbit blood nutrient agar (10% blood) to insure purity and to obtain its hemolytic action; then to rejuvenate repeatedly in serum broth to insure vigorous growth. The actual fermentation reactions of each strain were observed in serum broth — containing 25% of beef serum —neutral to Andrade's indicator. This is the medium Holman[10] uses, and it has been found very satisfactory.[11] Duplicate, and frequently triplicate, determinations have been made throughout, usually with identical results. Rarely, a negative test has been followed by two positive tests, suggesting a lack of luxuriance in growth in the first instance. An apparent gain of fermenting power for different carbohydrates in successive trials has not been met with. Incubation at 37 C. was practiced for one week. Readings were made on the first, third, fifth and seventh days.

The carbohydrates used comprised the following:

a. Pentoses: xylose, d- and l- arabinose;[12] the alcohol d. arabite.
b. Methylpentose; rhamnose (iso-dulcite).
c. Hexoses: D-glucose, d-mannose, fructose, d-galactose; the alcohols, d-mannitol, d-dulcitol.
d. Heptoses: Alpha-gluco-heptose, manno-keto-heptose, anhydro-sedo-heptose; the alcohol, perseitol.
e. Bioses: Maltose, lactose, saccharose, trehalose (trehabiose or mycose).
f. Trisaccharid: Raffinose (meletriose).
g. Polysaccharid: Inulin.
h. Glucosids: Amygdalin, salicin, arbutin.

RESULTS

A. All strains studied gave in common the following reactions:

1. Morphology—cocci in longer or shorter chains; capsules and motility not observed; young cultures gram-positive.

[10] Jour. Med. Research, 1916, 34, p. 385.

[11] The appropriate carbohydrates are added separately as sterile aqueous solutions; one-half of 1% of each sugar has been found to be ample. The fermentation of the carbohydrate is shown both by the production of a marked red color, and by the gradual precipitation of the protein constituents of the serum (so-called acid albuminate).

[12] It should be remembered that l-arabinose, not d-arabinose, is the isomer more commonly found in nature.

2. All strains fermented d-glucose, d-mannose, fructose, d-galactose, of the hexose group; maltose, lactose, saccharose and trehalose of the biose group; salicin of the glucosid group.

3. All gave a strongly acid reaction in milk and none liquefied gelatin.

These reactions may be tentatively regarded as fundamental or group reactions for the organisms studied.

B. The following carbohydrates were not fermented by any of the strains studied:

1. Pentose group: D-arabinose, d-arabite, rhamnose.

2. Hexose group: dulcitol.

3. Heptose group: alpha-gluco-heptose, manno-keto-heptose, and perseitol, anhydro-sedo-heptose.

C. Mannitol, an alcohol with 6 carbon atoms, raffinose, a sugar containing 18 carbon atoms, and inulin are fermented by a sufficiently large proportion of strains to possess differential value. The substances themselves are important from their stereo-isomerism, and in relation to the pneumococcus group.

The glucosids, arbutin, and amygdalin, are fermented by several strains of streptococci, but the indefiniteness of knowledge of their chemical structure, together with the comparative inability of a large number of strains to utilize them, makes their importance in this connection secondary; a line of demarcation between group and individual reactions must be drawn somewhere.

D. The preliminary isolation of pure cultures from blood plates showed definitely that the action of the streptococci on the hemoglobin was a factor of considerable differential value. This is clearly indicated in Table 1.

No attempt was made to differentiate the reactivity of the cultures on hemoglobin[13] beyond the limits mentioned. Additional studies of the chemistry of the products arising from the decomposition of hemoglobin by such strains should be made before a final discussion can be satisfactorily presented.

[13] Attention is called to the loose use of the term "hemolytic." In the complement fixation reaction, the liberation of hemoglobin from the stroma of the red blood cell is termed "hemolytic." Possibly the term "stromolysis" would be more appropriate. On the contrary, "hemolytic" streptococci so alter blood pigment that it no longer gives a spectrum. Possibly the term "hemoclysis" would be more appropriate in this connection. An investigation of this subject is in progress at the present time. No adequate chemical explanation of the composition of green coloration induced in hemoglobin by Strep. viridans is available at present.

Pathogenicity and virulence for lower animals were not considered in this study, which is primarily and essentially a consideration of the ability of the organisms to utilize certain carbohydrates, together with an attempt to correlate these results with the stereo-isomerism of the substances themselves.

The accompanying table shows the results of this investigation in tabular form. For convenience and for brevity, the term "type" as used indicates the reactions possessed in common by all of the strains studied. These are:

Morphology.—Organisms typical chains of greater or lesser length, non-motile, gram-positive.

Capsules.—Not formed, lanceolate-shaped lacking.

Gelatin.—Not liquefied.

Litmus milk.—Decidedly acid, usually coagulates when heated.

Fermentation reactions. — Glucose, mannose, fructose, galactose, maltose, lactose, saccharose, trehalose, salicin, all fermented with the production of acid, but no visible gas.

TABLE 1

CULTURAL CHARACTERS OF 356 STREPTOCOCCI. (FUNDAMENTAL, OR GROUP, CHARACTERS, AS ABOVE)

Hemolytic Colonies				Green Colonies				Nonhemolytic Colonies			
Type	Mannite	Inulin	Total	Type	Mannite	Inulin	Total	Type	Mannite	Inulin	Total
234	28	9	264	28	17	9	47	32	12	8	45
Mannite and Inulin, 7				Mannite and Inulin, 7				Mannite and Inulin, 7			
Secondary Reactions *											
Arbutin			16	Arbutin			11	Arbutin			7
Amygdalin			15	Amygdalin			11	Amygdalin			6
Raffinose			23	Raffinose			12	Raffinose			5
Xylose			4	Xylose			2	Xylose			0
L-arabinose			4	L-arabinose			2	Arabinose			0

* The utilization of the carbohydrates of this group by relatively few strains suggests that they are not of noteworthy importance for purposes of general classification. On the other hand, as indicators of unusual or relatively uncommon relations between stereo-isomerisms of the substrate and adaptability of the cytoplasm of the particular strain of organism, these reactions may possess unusual significance. A discussion of this particular phase is beyond the scope of the present communication.

The terms "hemolytic" colony, green colony, and nonhemolytic colony are selfexplanatory.

The fermentation of mannite and of inulin, or both, is indicated, the respective numbers representing the strains which exhibit these characters in addition to the "type" characters.

SUMMARY OF FERMENTATION REACTIONS

Percentage

100.0 of all cultures fermented d-glucose, d-mannose, d-galactose, fructose, maltose, lactose, saccharose, salicin.
16.0 of all cultures fermented mannitol.
7.3 of all cultures fermented inulin.
5.9 of all cultures fermented both mannitol and inulin.
11.1 of all cultures fermented raffinose.
9.5 of all cultures fermented arbutin.
9.01 of all cultures fermented amygdalin.
1.7 of all cultures fermented d-xylose and l-arabinose.

DISCUSSION

The series of organisms studied cannot be regarded as wholly representative of the entire group of streptococci. The strains are wholly of human origin; hence, members of the bovine and equine types are lacking unless accidentally included. Furthermore, no cultures of pneumococci, of Streptococcus mucosus, or of Pneumococcus mucosus were recognized. It is improbable that any of these strains were present.

A clear majority of the organisms were isolated from streptococcus pneumonias, or from secondary processes arising therefrom; this may explain the total absence of nonlactose fermenting strains, a type emphasized by Holman.[14] On the other hand, inasmuch as hemolytic, nonhemolytic and green pigment producing strains were found in this series, together with a fair proportion of mannitol and inulin fermenting types, the restrictions of origin to a rather narrow type of lesion is not reflected in the comparative variety of carbohydrates utilized.*

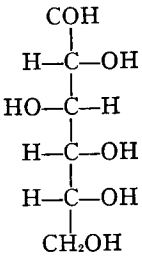
The pathogenicity of the organisms was not tested on animals. Several representative cultures of the hemolytic group were tested for hemolysin, using the procedure of Lyall.[15] In each instance the soluble hemolysin was present in eighteen to 24-hour serum broth cultures. There appears to be no tangible relation between hemolysin production and fermentability of various carbohydrates. Inasmuch, however, as the problem of carbohydrate fermentation was the primary objective of this study, the consideration of hemolysis, virulence, and questions relating thereto, were not considered intimately.

[14] Jour. Med. Research, 1916, 34, p. 377.

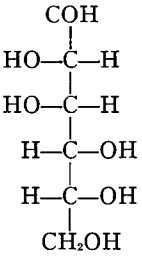
* Representatives of each type and group were isolated from the cultures originating in the Army Camps.

[15] Jour. Med. Research, 1914, 30, p. 515.

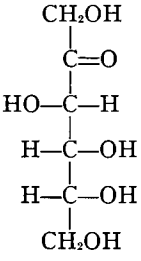
The configuration of these sugars is as follows:



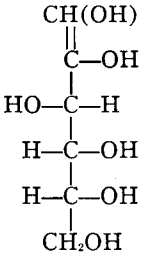
D-glucose



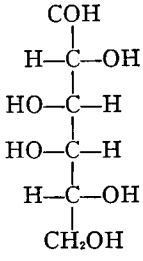
d-mannose



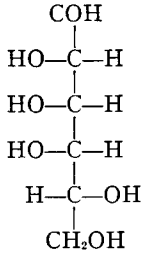
d-fructose



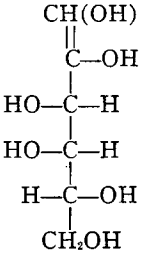
Enol form common to
d-glucose
d-mannose
d-fructose



d-galactose



d-talose



Enol form common to
d-galactose
d-talose
d-tagalose

The configuration of the H and OH groupings in the carbon atoms next the primary alcohol group (CH_2OH) is the same in the d-glucose — d-mannose — d-fructose series. In d-galactose, one OH group is reversed (the Gamma C atom) and this slight change apparently suffices to reduce the suitability of the molecule somewhat for fermentability. D-talose, which bears the same structural relation to d-galactose that d-mannose does to d-glucose — that is to say, it has the two upper OH groups arranged as in d-mannose, the lower three OH groups as in d-galactose — is ordinarily not utilized at all.[33] The utilization of d-glucose, d-mannose and fructose by an organism which can ferment any one of these is presumably related to the fact that all three hexoses form the same enol. It is assumed that an

[33] Maquenne: Les Sucres, p. 590. It is not improbable that talose may be found to be fermentable by the more vigorous carbohydrolytic bacteria.

hexose-diphosphate: $C_6H_{10}O_4(PO_4R_2)_2$) prior to the degradation of the carbohydrate with liberation of energy for yeast cells during the process of fermentation. It is a matter of no inconsiderable interest to recognize a combination of carbohydrates with phosphorus, both for the structural nucleic acid and energy phase of metabolism in the same organism. A possible relationship between the carbohydrate-phosphorus content of yeast nucleic acid and the carbohydrate-phosphorus complex in the fermentation of sugars by yeast should be borne in mind.

In this connection, the observation of Iwanoff[19] that certain molds[20] can actually derive their energy from thymus nucleic acid, liberating phosphoric acid and purin bases, while utilizing the carbohydrate thus set free, is of paramount interest. Additional studies, however, are required along these closely related and highly important lines. The information available at present is insufficient to correlate these observations with known bacterial activities, but the fundamental similarities in the metabolism of all living organisms makes it probable that similar or analagous phenomena will be met with in the bacteria.

Returning to known facts: It is a matter of common knowledge that bacteria can utilize for their energy requirements carbohydrates or carbohydrate derivatives which are not accessible to them under natural conditions.[21] It is equally well known that microbes are extremely delicate appraisers of optical antipodes which are so interwoven with the chemistry of not only carbohydrates but proteins as well. Thus, many bacteria can use dextro- but not levo-isomers of the pentose and hexose series. They will pick out and ferment very small amounts of utilizable carbohydrate from a solution containing unequal amounts of two sugars. Typhoid bacilli, for example, will ferment the small amount of glucose-like carbohydrate in milk, leaving the lactose unattacked. This reaction is much more delicate than any chemical process known at present. The classic research of Pasteur[22] on the assimilation of d-tartaric acid by Penicillium glaucum is an excellent example of the relationship existing between stereo-isomerism of an organic compound and its utilization by a micro-organism. In the experiment under consideration it was found that Penicillium glaucum assimilated the dextro component of racemic acid, leaving the

[19] Ztschr. f. physiol. Chem., 1903, 39, p. 31.
[20] Aspergillus niger and Penicillium glaucum.
[21] Harden: Nature of Enzyme Action, p. 152.
[22] Compt. rend., 1858, 46, p. 615; 1860, 51, p. 298.

levo component practically untouched. More recent observations indicate that the levo component is, or may be, slowly decomposed after the dextro acid has disappeared, but the principle involved holds in either case.[23] According to Fischer and Abderhalden,[24] trypsin will hydrolize alanyl-glycine ($CH_3.CHNH_2.CO.NH.CH_2.COOH$), but will not cleave glycyl-alanine ($NH_2.CH_2.CO.NH.CH.CH_3.COOH$).

Similarly, maltase (alpha glucase) will hydrolyze alpha-methyl d-glucosid, but not beta-methyl d-glucosid, while emulsin (beta glucose) will hydrolyze the beta but not the alpha glucosid.[25] The corresponding alpha and beta xylosids, containing each one less carbon atom, but having otherwise the same stereo-configuration, are unattacked by these enzymes. Observations[26] are recorded which would indicate that maltase does hydrolyze beta glucosids, but much more slowly than the alpha isomer.

The utilization of carbohydrates for energy by bacteria is reducible to three quite distinct phases:

(*a*) The preparation of the carbohydrate (if it be a glucoside, biose or polysaccharid) for assimilation. This is usually an hydrolytic cleavage, resulting finally in a hexose, as one of the final products, for example, the formation of dextrose and galactose from the hydrolysis of lactose, of dextrose from starch, of dextrose and saligenin from salicin.

(*b*) The assimilation of the product or products of hydrolysis.

(*c*) The intracellular utilization of the assimilated product, which implies, of course, that the stereo-configuration of the assimilated molecule is compatible in structure with the requirements of the microbe. Of the three phases, the last is of vital importance to the organism.

The mechanism of hydrolytic cleave of bioses, trioses and polysaccharids is not well known in so far as it relates to bacterial action. The supposition is that some bacteria produce soluble enzymes which are comparable to the soluble amylo- and saccharolytic enzymes found in the intestinal tract of man. If such be the case the interesting question arises, Is there a separate and distinct enzyme for each biose, for example, as a sucrase, a lactase and a trehalose, or does one enzyme

[23] Literature and discussion in Landoldt, Optical Rotating Power, translated by Long, p. 117.

[24] Ztschr. f. physiol. Chem., 1905, 46, p. 52.

[25] Armstrong: Simple Carbohydrates and Glucosides, p. 79.

[26] See Fajan's; Ztschr. f. physiol. Chem., 1910, 73, p. 25; 75, p. 232, for discussion.

fit several bioses, as a master key fits several locks? Proteolytic enzymes, as pepsin and trypsin, will hydrolyze a multitude of proteins, and it is by no means impossible to conceive of bacterial enzymes which exhibit varying degrees of hydrolytic versatility.[27]

The fact that bacteria can break down and utilize carbohydrates to which they cannot have access in nature is suggestive. For example, trehalose is fermented by streptococci, as is salicin, yet few streptococci are ever thrown into contact with these substances.[28] Thus far, however, attempts to demonstrate soluble saccharolytic enzymes in streptococcus cultures have been unsuccessful.

The utilization of the products of hydrolysis of bioses and saccharids — chiefly hexoses — by bacteria and by yeasts has opened a wide field of investigation. The first extensive investigations of the relations between different sugars and their fermentability were made by Emil Fischer and his associates.[29] From their studies it was shown that d-glucose, d-mannose, d-fructose[30] and d-galactose were fermented by several varieties of yeasts, while the l-components of the same sugars were unattacked. Furthermore, d-glucose and d-mannose were readily utilizable; d-galactose, which is quite close in its configuration to d-glucose, is less readily fermented. D-talose, which bears the same stereo-isomeric relation to d-galactose that d-mannose bears to d-glucose, was not attacked by any yeast. This relationship between fermentability and configuration is most clearly shown by the yeasts, Saccharomyces apiculatus[31] and productivus,[32] which do not ferment d-galactose, although they do ferment the glucose-mannose-fructose series readily.

[27] It is not intended to suggest that the cleavage of protein by exo-proteolytic enzymes as pepsin and trypsin, finds its analogy in the carbohydrate-cleaving enzymes. The proteins are composed of complexes of the same set of amino acids, while carbohydrates of the biose and polysaccharid groups may be resolved into hexoses of the same empirical formula, but of considerable differences in their space arrangement. In this connection, a considerable difference may be recognized between exoproteolytic enzymes, as trypsin, which appear to be without action on living cholera vibrios in the intestinal tract, and specific immune enzymes, as cholera lysin, which will dissolve cholera vibrios in vivo and in vitro. An analogy among carbohydrates does not appear to have been demonstrated, unless the development of a specific sucrase in the blood serum of animals following subcutaneous injections of sucrose be so regarded.

[28] In the case of salicin, the glucose component alone is utilized, so far as is known, and cleavage would appear to be necessary for the liberation of the glucose molecule.

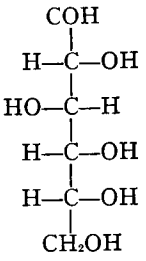
[29] Ber. d. deutsch. chem. Gesellsch., 1890, 23, pp. 2621, 382 and 389; 1894, 27, p. 2031; Ztschr. f. physiol. Chem., 1898, 26, pp. 60 and 89.

[30] Stereo-isomerism of d-group, but left rotating.

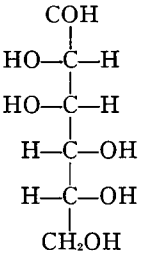
[31] Slator: Trans. Chem. Soc., 1906, 89, p. 128; 90, p. 217.

[32] Cremer: Ztschr. f. Biol., 29, p. 525.

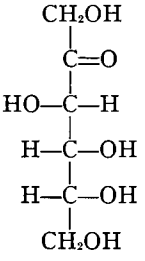
The configuration of these sugars is as follows:



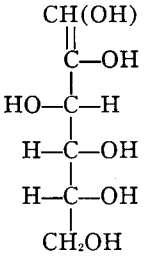
D-glucose



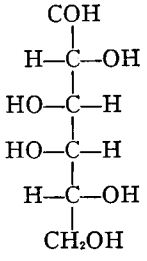
d-mannose



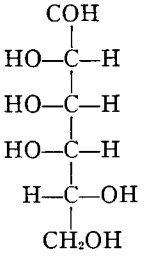
d-fructose



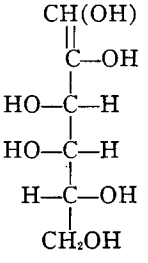
Enol form common to
d-glucose
d-mannose
d-fructose



d-galactose



d-talose



Enol form common to
d-galactose
d-talose
d-tagalose

The configuration of the H and OH groupings in the carbon atoms next the primary alcohol group (CH_2OH) is the same in the d-glucose — d-mannose — d-fructose series. In d-galactose, one OH group is reversed (the Gamma C atom) and this slight change apparently suffices to reduce the suitability of the molecule somewhat for fermentability. D-talose, which bears the same structural relation to d-galactose that d-mannose does to d-glucose — that is to say, it has the two upper OH groups arranged as in d-mannose, the lower three OH groups as in d-galactose — is ordinarily not utilized at all.[33] The utilization of d-glucose, d-mannose and fructose by an organism which can ferment any one of these is presumably related to the fact that all three hexoses form the same enol. It is assumed that an

[33] Maquenne: Les Sucres, p. 590. It is not improbable that talose may be found to be fermentable by the more vigorous carbohydrolytic bacteria.

enzyme first converts the sugar, be it glucose, mannose or fructose, into the enol and the subsequent decomposition has its origin at the double bond of the enol. It is worthy of note in this connection that Wohl[34] has shown that glucose, mannose or fructose in alkaline solution gradually reaches a state of equilibrium through the formation of enol in which proportionate amounts of all three sugars are finally present in solution. If this be so, and available evidence suggests strongly that it is, then it is useless to make individual tests for fermentation of d-glucose, d-mannose and fructose, respectively. One test will settle the question.

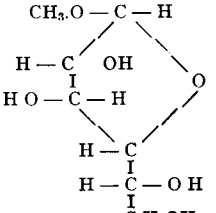
D-galactose does not form the same enol as the d-glucose series. Hence, a second test with this sugar is essential. A complication presents itself here, however. Talose and tagalose should form an enol in common with d-galactose. The few observations available suggest that talose is not fermented by any organism hitherto reported. According to the enol theory, the gradual transformation of talose to the enol form should be associated with a gradual decomposition of the enol, or possibly of d-galactose, should the enol prove refractory. It is of course possible that the products of fermentation, which are acid, may present enolization so soon that the process is overlooked. In any event, it is theoretically possible to test the entire theory of enolization and tautomerism by biological means. Experiments with the galactose — talose — tagalose series are needed to elucidate this important question.

It is significant that glucosids are not fermented by many yeasts that ferment d-glucose readily;[35] the terminal carbons of the glucose in the

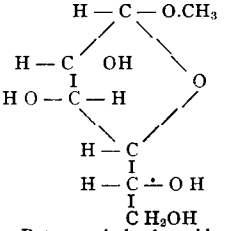
[34] Ber., 1893, 26, p. 730; 1897, 30, p. 3101.

[35] The ordinary formula for d-glucose fails to indicate certain peculiarities of behavior of glucose solutions not accounted for satisfactorily by the simple aldehyd formula. Tollens (Kurzes Handb. d. Kohlenhydrate) has proposed a solution involving the formation of two possible aldehydrols of glucose, which is now generally accepted.

The two isomers, consequently, are known as alpha and beta glucose. The methyl glucosids have the formulas:



Alpha methyl glucosid



Beta methyl glucosid

It will be seen that enol formation is not possible in these compounds, both of which, however, are derived from d-glucose.

former case cannot undergo enolization, probably, therefore, explaining in a logical manner the inability of the organism to utilize the glucosid prior to its cleavage by another enzyme.

According to Fischer's studies,[24] enzymes which cleave carbohydrates exhibit similar specificity for the alpha and beta components of the glucosids, respectively; thus, emulsin splits the beta methyl dextro glucosid, but not the alpha methyl glucosid, and maltase splits the alpha methyl dextro glucosid, but not the beta methyl dextro glucosid. Neither enzyme splits the corresponding levo-glucosids. This is in harmony with the fact that levo components of fermentable hexoses are not decomposed by enzymes which cleave the dextro components.

It would appear then that the asymmetrical active agent of yeast cells attacks and ferments only those sugars which are dextro rotatory[36] and whose stereo-isomerism closely approaches that of d-glucose; similarly, the enzymes mentioned attack glucosids whose molecules approach the dextro glucosid configuration (either alpha or beta) but fail to attack the levo glucosids.

The observations above recorded deal exclusively with yeasts. In recent years much information of the catalogue type has accumulated concerning fermentation reactions by bacteria. Systematic efforts, using various stereo-isomeric series as substrates, are lacking, but one striking feature has appeared, namely, that the alcohols of the d-glucose series are less readily fermented, generally speaking, than the aldoses corresponding. Contrary to expectation, furthermore, d-mannitol, the alcohol of d-mannose, is somewhat more readily utilized than d-sorbitol, the alcohol of d-glucose. Dulcitol (inactive from internal compensatory symmetry of structure) is practically unattacked by a majority of bacteria. An explanation of this phenomenon is not available at present, but there appears to be a somewhat definite decrease in utilizability of members, otherwise the same, in the series R.COOH; R.CHO; $R.CH_2OH$, in the order mentioned. Thus, tartaric acid is quite generally fermented by bacteria while the corresponding alcohol, erythritol, is unattacked by ordinary bacteria.[37]

It should be noted that this difference does not necessarily hold with bioses; for example, maltose (glucose alpha glucosid) and lactose (glucose beta galactosid) which possess potentially free aldehyd groups,

[36] Dextro-configuration; not necessarily dextro rotatory, however.

[37] Bertrand (Compt. rend. 126, p. 762) states that the sorbose bacterium, Bact. xylinum, transforms erythritol to erythrose.

are on the whole not much more readily fermented than saccharose and trehalose, which do not reduce Fehling's solution and have no free aldehyd group. Terminal primary alcohol radicals are certainly present in their molecules. Neither of these latter sugars is inverted by any known enzymes which will hydrolize alpha or beta glucosids.

It is a matter of common observation that the end products of the utilization of carbohydrate are almost invariably acid in character, and frequently CO_2 (action of a carboxylase) is formed as well.[38]

Turning to the fermentation reactions exhibited by the organisms studied in this series, all of the cultures examined utilized the carbohydrates of the d-glucose series, which form a common enol, namely, d-glucose, d-mannose and fructose. So far as these observations go, the significance of this phenomenon is two-fold, namely, that a single test, using any member of the tautomeric series, will indicate what may confidently be expected from the others. Secondly, it is very probable that in general an organism which ferments one tautomerid of the dextro glucose series will utilize the other members. The mechanism of this process is not definitely known but two possibilities present themselves: Either the organism possesses an enzyme which incites enol formation prior to fermentation, or the configuration of the three members is compatible with the stereo-requirements of the cell.[39]

Of the galactose series, only d-galactose was tried. Fermentation was distinctly slower in galactose mediums than in the glucose series, but all of the strains studied acted on this carbohydrate.[40]

D-mannitol was fermented by several but by no means all the organisms. A comparison with d-sorbitol would have been interesting, because some strains of streptococci are known to utilize this alcohol.[41] Inasmuch as enol formation is not possible in the alcohol series corresponding to the hexose series, a closer relation should exist between the space relations of the alcohol and the cytoplasm of the bacterium to insure fermentability than in the hexose series where a common enol furnishes a plausible reason for the mutual utilizability of the tautomerids. For this reason, the alcohols of the hexose series

[38] The amount of CO_2 formed is not necessarily large, and it may escape detection in ordinary procedures. It may be demonstrated readily with Van Slyke's carbon dioxid apparatus, however.

[39] The possibility of a reestablishment of equilibrium of three tautomerids in alkaline solution, from a single member, is not strictly a part of this phase of the discussion.

[40] It would have been very important to have d-tagalose and d-talose to complete the series of galactose tautomerids. These hexoses were not obtainable.

[41] Smith and Brown: Jour. Med. Research, 1914, 31, p. 508.

appear to be of unusual interest and importance, as indicators of the versatility of the bacterial cytoplasm in relation to stereo-isomerism of the carbohydrates.

Dulcitol, the alcohol of galactose, was not fermented by any of the strains studied. In general, it is quite resistant to bacterial attack.[42]

The bioses, maltose (glucose alpha glucosid) and lactose (glucose beta galactosid), representing, respectively, an alpha and beta compound, are readily utilizable by all the streptococci studied. Holman[14] and others have found strains, chiefly from nonhuman sources, which cannot ferment the beta galactosid, however. Trehalose, a glucose derivative of glucose but without aldehyd and ketone groupings, is readily fermented, as is saccharose. Inasmuch as hydrolytic enzymes were not demonstrated in cultures of these organisms, the mere fact that the substances are utilized by the bacteria is all that can be stated definitely. Salicin, a natural glucosid, composed of glucose and ortho-oxybenzyl alcohol, was fermented by each strain studied. Arbutin, a glucose-hydroquinon compound, and amygdalin, composed of two glucose molecules with d-mandelonitrile, were utilized by a much smaller group of organisms. It is not known whether these glucosids are of the alpha or beta type; hence, nothing of a structural nature can be stated at present.

Generally speaking, those streptococci which did not produce clear areas of hemolysis around individual colonies were somewhat less versatile in the variety of carbohydrates fermented than those of the hemolytic group; those cultures which produced green colonies were on the whole the most active chemically.

The most striking feature, from the viewpoint of origin of the culture, however, is the lack of uniformity in fermentation reactions among a considerable number of strains isolated from lesions of apparently nearly identical types. This is in striking contrast, for example, with a corresponding group of typhoid bacilli where practical identity of reactions would be expected. In other words, in spite of the epidemic tendency of these streptococcus pneumonias, the organisms isolated from them failed to exhibit a corresponding uniformity in chemical reactions on carbohydrate.[43]

[42] Members of the coli and paracolon groups appear to utilize d-dulcitol, fermenting it with the production of gas and acid.

[43] It is suggestive that typhoid bacilli incite a definite clinical syndrome with a well marked pathology. Streptococci, as a rule, do not incite such definite unified syndromata and usually give rise to inflammatory reactions not restricted to definite organs or tissues.

Inasmuch as the products formed from the intracellular utilization of any and all of the carbohydrates are qualitatively similar, however, this means comparatively little from the standpoint of pathogenesis. The products arising from the action of the organisms on protein, on the contrary, are almost certainly of paramount importance in relation to the clinical disease and pathologic lesions, and the specificity of pathogenesis of the streptococci, therefore, is rather to be sought for in the domain of protein than carbohydrate chemistry.

SUMMARY

Carbohydrates are important in bacterial nutrition chiefly as sources of energy. They spare or protect nitrogenous constituents in so far as energy requirements are concerned, but they do not and cannot substitute for nitrogenous substances, which are indispensable for the structural requirements of the organisms. It is probable that certain members of the group also become integral parts of structural units, as for example, components of nucleins.

Carbohydrates are biochemically of extreme interest in bacteriology through the relations which exist between the stereo-isomerism of members of the group having the same empiric formula, and their utilizability by various types of bacteria.

Carbohydrates possess value in the classification of bacteria through the fact that a definite relationship apparently exists between the stereo-configuration of various definite groups of these substances and the ability of the organisms to ferment them. In other words, the "fermentation reactions" of many kinds of bacteria are a means of their identification.

The significance of a classification of bacteria based on fermentation reactions, therefore, depends on a recognition of the relation between the space arrangement of the carbohydrate and a fundamental capacity of the cytoplasm of the microbe to dissociate it with the liberation of energy for the cell.

The unerring specificity of these reactions possess theoretical interest in that a careful selection of bacteria as reagents of extraordinary delicacy opens a way for the testing of important theories relating to carbohydrates, as, for example, the formation of enols, and of tautomerism.

The products of fermentation produced by a great majority of bacteria are qualitatively very similar, irrespective of the carbohydrate

fermented and of the organism inciting the process. Thus, diphtheria, typhoid, colon bacilli, streptococci and staphylococci produce varying amounts of organic acid, chiefly lactic, from a great variety of sugars.[44] The specificity of action of these bacteria, therefore, would appear to depend principally on the products arising from the utilization of protein, not only for structure but for energy as well.[45]

The classification of bacteria, including the streptococci, therefore, on the basis of fermentation reactions makes it possible to separate them into convenient and distinct groups which have divisional value.

There is no clearly discernible relationship, however, between cultural grouping based on carbohydrolysis and pathogenesis.

[44] In other words, they form the essential products of "sour milk" in utilizable carbohydrate-protein media.

[45] Kendall: Am. Jour. Med. Sc., 1918, 156, p. 157.