THE DEHYDROGENATION OF ALCOHOLS BY STREPTOCOCCI OF GROUP B

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Received for publication, March 12, 1942

The most characteristic metabolic activity of streptococci is the fermentation of carbohydrates to lactic acid. Although this process accounts almost quantitatively for the carbohydrate fermented, Friedemann (1938, 1939) has shown that traces of ethyl alcohol and volatile acids occur when growing cultures ferment glucose, thus indicating that the predominant metabolic process of these organisms is not their only fermentative process.

Using glucose as a substrate for dehydrogenation studies with methylene blue as hydrogen acceptor, Wood and Gunsalus (1942) have established conditions for the production of resting cell suspensions of streptococci with strong dehydrogenase activity toward this substrate. In a search for other substrates which could be activated as hydrogen donors it was found that ethyl alcohol was even more active than glucose, while organic acids were inactive or very weak hydrogen donors. This result is opposite to that found by Quastel and Whetham (1925) in their studies of *Escherichia coli*. Although *Escherichia coli* produces considerable quantities of ethyl alcohol among its fermentation products, it has only very weak dehydrogenase activity toward alcohols.

This strong dehydrogenase activity of streptococci for ethyl alcohol led us to make the following study of alcohols in general with group B streptococci.

EXPERIMENTAL

Five strains of group B streptococci from the departmental culture collection were used. Three strains (Nos. 68c, 70b, and 83a) were originally isolated from milk. All had originally fermented lactose but at the time these experiments were performed two (68c and 70b) had lost the power of fermenting lactose. Two strains (Nos. G1, 44P1) were from human sources; 44P1 isolated from human feces, and G1 from a kidney infection. Strain G1 did not ferment lactose when isolated. All strains were members of Lancefield's group B hemolytic streptococci and had the usual physiological characteristics of this group of organisms (Sherman 1937). One strain of *Escherichia coli* (71) was carried in the experiments for comparative purposes.

The cell suspensions were obtained as suggested by Wood and Gunsalus (1942). Cells grown 12 hours at 37°C. in a 1 per cent tryptone, 1 per cent yeast extract, 0.5 per cent K_2HPO_4, 0.1 per cent glucose medium were collected by centrifugation, washed once with $\frac{1}{4}$ growth volume of M/30 phosphate buffer, pH 7.4, and resuspended in $\frac{1}{10}$ growth volume of the same buffer. The ability of the organism to activate various substrates as hydrogen donors was deter-

mined by observing the reduction of methylene blue in Thunberg tubes containing the following:

In side arm: 1 ml. 1:4000 methylene blue.

In tube: 2 ml. M/15 phosphate buffer pH 7.2, 1 ml. substrate (glucose M/20, alcohols 1%), 1 ml. cell suspension in M/30 phosphate buffer.

After evacuation, the tubes were allowed to come to the temperature of the bath, the methylene blue tipped into the tube, and the time required for 90 per cent reduction recorded.

It should, of course, be realized that not all compounds activated by organisms need donate hydrogen to methylene blue under these conditions. Positive data so obtained, however, are useful. All the alcohols employed as substrates were Eastman Kodak products used without further purification; appreciable quantities of impurities in them would lead to erroneous results as only a very small

TABLE 1

Effect of substrate concentration on reduction rate

S. mastitidis (70b), (0.23 mg. bacterial nitrogen/tube)·7.2 40°C

PER CENT ALCOHOL	MINUTES FOR 90% REDUCTION OF 1:20,000 METHYLENE BLUE		
	Ethyl	Isopropyl	Propyl
20	42	>60	
15	6		
10	2.4		>60
8	2.0		2
5	2.0	1.3	1.5
2	2.0	1.2	1.5
1	2.0		1.5
0.5	2.0	1.2	1.5
0.1	3.2	1.3	1.5
0.05	5.4		2.8
0.02	9.4	2.5	4.5

amount of methylene blue is present (1.6×10^{-4} molar). Quantitative methods to determine the products formed would be indicated once it is known that a compound is activated by the organisms.

Substrate concentration

With glucose as substrate a final concentration of M/100 in the tube proved satisfactory. With alcohols any concentration added would be decreased by volatilization during evacuation, and, on the other hand, too high a concentration would be inhibitory. Table 1 indicates the range within which the concentration of alcohol is not a factor in the reduction rate. As can be seen from the table, propyl and isopropyl alcohols are toxic at lower concentrations than is ethyl. However, any concentration from 0.5 to 5 per cent is satisfactory for these alcohols. On the basis of these data a concentration of 1 per cent alcohol (0.1–0.2 molar) was selected as satisfactory for dehydrogenase studies with

these streptococci. Quastel and Whetham (1925) found the inhibitive action of alcohols toward dehydrogenase systems of *Escherichia coli* increasing with the length of the carbon chain. In their studies the lactic and succinic dehydrogenase systems were inhibited by ethyl alcohol at about 25 per cent and propyl at about 10 per cent concentration.

pH optimum

In order to compare the rates of dehydrogenation of alcohols with that of glucose it would be desirable to work as near to the optimum of each as possible. As can be seen from table 2, the optimum range for glucose is slightly more acid than the optimum for the alcohols. A fairly broad optimum, half of a pH unit, exists for glucose and an even broader optimum for the alcohols. In order not to decrease the rate for glucose and still work near the optimum for the alcohols, a pH of 7.2 was used in subsequent experiments.

TABLE 2

Effect of pH on rate of dehydrogenation

S. mastitidis (70b), (0.22 mg. bacterial nitrogen/tube.) 40°C

pH IN THUNBURG	MINUTES FOR 90% REDUCTION OF 1:20,000 METHYLENE BLUE		
	M/100 glucose	2% ethyl alcohol	2% isopropyl alcohol
5.8	5.5		
6.1	4.5	3.5	2.1
6.4	3.5	3.0	1.8
6.8	3.3	2.5	1.5
7.1	3.2	2.1	1.2
7.3	3.1	1.9	1.2
7.7	7.5	2.0	2.3
8.0	11.3	3.3	3.5

Alcohols dehydrogenated

In order to determine whether other alcohols were dehydrogenated by these streptococci 5 strains were tested on a number of alcohols and glycols. The rates of dehydrogenation of the various alcohols, based on the time required for 90 per cent reduction of 1:20,000 methylene blue compared to the rate with glucose taken as 100, are given in table 3. Methyl alcohol was not activated by any of the streptococci, nor by *Escherichia coli*. The other normal alcohols from ethyl to amyl were all activated and were equal to, or more active than, glucose as hydrogen donors. Propyl alcohol was the most active of the normal alcohols, although only slightly more active than ethyl. Butyl and amyl alcohols ranged from activity equal to ethyl (two cases) to about half the activity of ethyl, or approximately equal to that of glucose. Among the secondary and tertiary alcohols, isopropyl, secondary butyl, and tertiary amyl were found to be very active hydrogen donors under the conditions studied. In fact, all three were more active than the normal alcohols, the activity ranging up to 6 times that of glucose in the case of isopropyl and secondary butyl. The high reactivity

of tertiary amyl is rather surprising. In view of the lack of hydrogen in the carbon atom to which the hydroxyl group is linked, it is difficult to write a reaction for the dehydrogenation, as an aldehyde or ketone could not be formed without breaking the carbon chain. Quantitative work with this substrate should yield interesting results. The oxidation products of the two highly-reactive secondary alcohols, isopropyl and secondary butyl, would not be as difficult to predict as would the tertiary amyl. The next secondary alcohol, 2-pentanol, while having the same spacial arrangement of the alcohol group as

TABLE 3

Dehydrogenation of various alcohols by S. mastitidis

(Rates compared to glucose = 100)

Culture	70b	68c	83a	44P1	Gl	E. coli 71
Glucose Minutes	4.5	2.75	11.0	3.0	3.25	0.50
Glucose	100	100	100	100	100	100
Control	<2	<2	<2	<2	<2	<1
Alcohols:						
Methyl	<2	<2	<2	<2	<2	<1
Ethyl	140	170	130	200	185	2.2
Propyl	165	220	200	240	200	2.0
Butyl	80	180	105	150	90	2.2
Amyl	110	170	90	230	70	<1
Iso-propyl	260	240	625	400	220	1.4
Sec. butyl	225	275	625	225	220	1.5
2-Pentanol	110	110		50	40	1.5
Methyl-isopropyl carbinol	30	60	40	4	10	
Iso-butyl	11	20	30	30	18	<1
Tert. butyl	10	<5	<5	<2	<2	<1
Tert. amyl	225	275	290	120	130	1.4
Glycols:						
Ethylene	<2	<2	<5	<2	<2	10
Trimethylene	180	45	25	65	20	9
2,3 butylene	53	220	730	330	190	5
Gylcerol	<2	7	<5	<2	<2	10
Mannitol	<2	<2	<5	<2	<2	25

the 3 and 4 carbon secondary alcohols, has only about half the reactivity under the conditions used. Its reaction rate, however, is still equal to that of glucose. The other butyl and amyl alcohols tried were less active, ranging from half the activity of glucose with methyl isopropyl carbinol to no activity with tertiary butyl. *Escherichia coli* had only slight activity toward the alcohols.

Among the poly-hydroxy alcohols these streptococci did not activate ethylene glycol nor mannitol, as hydrogen donors. The latter result agrees with fermentation data, mannitol not being fermented by group B streptococci. Tri-

methylene glycol was active as a hydrogen donor as was 2,3 butylene glycol. With 2 of the strains of streptococci the latter compound was the most reactive of those studied. There is similarity in structure between 2,3 butylene glycol and the other secondary alcohols which were highly reactive, namely, isopropyl and secondary butyl. In contrast to the glycols, glycerol was only slightly reactive as a hydrogen donor toward one strain of group B streptococci. That this is not a true picture of the ability of the organism to attack glycerol will be discussed later under adaptation. *Escherichia coli* attacked mannitol as well as glycerol and the glycols. These reactions were very slow in comparison to rates with glucose and organic acids as hydrogen donors. The results with *Escherichia coli* are in agreement with those found by Quastel and Whetham (1925).

Adaptation to substrates

If the organisms to be used for the preparation of resting cell suspensions are grown in the presence of the substrates upon which they are to be tested instead of on glucose, it should be possible to study the formation of adaptive enzymes

TABLE 4

Formation of adaptive enzymes for galactose

Streptococcus mastitidis (68c) pH 7.2, 40°C. 90% reduction 1/20,000 methylene blue

GROWN ON	MG. BACT. N/ML. OF SUSPENSION	REDUCTION TIME IN MINUTES ON			
		Glucose	Fructose	Mannose	Galactose
Glucose	0.24	3.8	3.8	3.8	45.0
Fructose	0.22	5.0	4.5	6.5	43.0
Mannose	0.21	3.2	2.8	3.0	50.0
Galactose	0.24	2.5	2.0	2.8	2.

by the methylene blue technique. With sugars which serve as readily-available energy sources for streptococci, the method is satisfactory as indicated by the example in table 4. Growing the culture in galactose broth increases the rate of dehydrogenation on galactose by about 25 times over the rate when the cells are grown in the presence of glucose, fructose, or mannose. The rate of dehydrogenation on the other hexoses does not appear to depend upon the growth substrate, i.e., the catalysts are constitutive enzymes (Dubos, 1940). The adaptation of this strain to galactose is analogous to the results of Hegarty (1939) with *Streptococcus lactis*. All of the cultures of *Streptococcus mastitidis* do not show so great adaptation to galactose as does the 68c strain.

The results obtained with glycerol, which is not reported to be fermented by these organisms, are more complex. As recorded in table 3, when grown in glucose only one of the 5 strains of *Streptococcus mastitidis* was capable of dehydrogenating glycerol, and this one slowly. After the cultures had been transferred serially upon glycerol broth, all dehydrogenated glycerol, some at a rate approaching that for glucose. At first this appeared to be an adaptation of the

type reported above for galactose, but further experiments have shown that factors other than cultivation in the presence of glycerol affect the dehydrogenation of this compound with methylene blue as hydrogen acceptor. Some streptococci dehydrogenate glycerol rapidly when harvested from a growth medium to which no glycerol has been added.

Barron and Jacobs (1938) have reported that certain hemolytic streptococci of human origin oxidize glycerol in manometric experiments with oxygen as the hydrogen acceptor instead of methylene blue. They indicate that the rate of glycerol oxidation is variable but do not relate it to adaptive enzyme formation. These authors also report that those cultures which oxidize glycerol oxidize ethyl, propyl and butyl alcohols in manometric experiments.

SUMMARY

Resting cell suspensions of *Streptococcus mastitidis*, Lancefield group B, have been found to oxidize a number of alcohols, as indicated by methylene blue reduction in Thunberg tubes. Several of the alcohols, including the normal alcohols C_2 to C_5, secondary alcohols from C_3 to C_5, tertiary amyl alcohol, and 2,3 butylene glycol are more active as hydrogen donors to methylene blue than is glucose. Methyl alcohol is not activated by these organisms. Glycerol is dehydrogenated rapidly by some strains, but the factors contributing to its dehydrogenation are not completely elucidated. An adaptive mechanism is present in some strains for the dehydrogenation of certain sugars, as for example, galactose.

A strain of *Escherichia coli*, studied for comparative purposes, was found to dehydrogenate alcohols very slowly. This observation is in agreement with the results of Quastel and Whetham.

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