OXIDATION OF POLYOLS BY *NOCARDIA CORALLINA*

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ABSTRACT

MAURER, P. R. (University of Otago Medical School, Dunedin, New Zealand) AND R. D. BATT. Oxidation of polyols by *Nocardia corallina*. J. Bacteriol. **83**:1131–1139. 1962.—Two diphosphopyridine nucleotide-linked polyol dehydrogenases were induced in *Nocardia corallina* strain S, and grown on either mannitol or sorbitol. One enzyme was labile and was specific for the conversion of sorbitol to D-fructose.

The second enzyme, which converted D-mannitol to D-fructose and, more slowly, D-arabitol to D-xylulose, was named the D-mannitol dehydrogenase. The configurations of the hydroxyl groups at carbons 2, 3, 4, and 5 of substrates for this enzyme were stereospecific. Formation of the enzyme was induced by D-mannitol and D-sorbitol but not by D-arabitol.

Extracts of cells grown on either mannitol or sorbitol catalyzed the oxidation of the reduced form of diphosphopyridine nucleotide in the presence of D-fructose and D-xylulose.

Cell suspensions of the organism oxidized mannitol, sorbitol, glycerol, inositol, and fructose but not dulcitol, D-arabitol, D-xylulose, or L-sorbose.

The ability of *Nocardia* species to use a variety of carbon compounds for growth was studied by McClung (1954) with 43 isolates, of which no 2 used exactly the same series of compounds. The response of the organisms to polyols has been summarized in Table 1. Although most strains used mannitol and sorbitol, only 13 grew on dulcitol.

This communication describes the growth response of strain S of *N. corallina* on polyols and the nature of the enzymes concerned.

MATERIALS AND METHODS

Organism. N. corallina was originally isolated by selective culture with uracil (5 g/liter) as the main source of carbon and nitrogen in solution A (KH_2PO_4, 5 g; $MgSO_4 \cdot 7H_2O$, 2 g; $CaCl_2$, 2 g; water, 1 liter; pH 7.2). A rough variant of the organism was used in this study (Batt, 1961; Batt and Woods, 1951, 1961).

Growth tests. The basal medium to which carbon substrates were added contained $(NH_4)_2SO_4$ (2.94 g), thiamine hydrochloride (1.1 mg), phosphate buffer (900 ml, 0.1 M, pH 7.2), solu-A (50 ml), and water (to 1 liter). To each growth tube (150 by 19 mm) were added basal medium (4.5 ml) and the carbon substrate (0.5 ml containing 200 μmoles of carbon). After autoclaving (121 C for 20 min), the tubes were inoculated from an aqueous suspension of the organism taken from a yeast extract agar slope (24 hr of incubation at 30 C). The tubes were incubated sloped at 30 C and the growth turbidity measured in a special photoelectric colorimeter designed to hold tubes of 19 mm diam. All tests were carried out in duplicate and the turbidity measurements normally showed close agreement between duplicates.

Preparation of cell suspensions. The organism was grown in conical flasks (500 ml) containing 150 ml of medium of the following composition; solution A, 15 ml; glucose, mannitol, or sorbitol, 0.3 g; $(NH_4)_2SO_4$, 0.225 g; thiamine hydrochloride, 0.15 mg; phosphate buffer (pH 7.2, 0.1 M), 25 ml; and water to 150 ml. The inoculated medium (inoculum: 1.0 mg dry wt cells/flask) was shaken for 72 hr at 30 C on a slow shaker (60 oscillations/min; amplitude, 7.5 cm). The cells were reaped in a Servall Continuous Flow Centrifuge at 9,200 × *g*, washed once, with 0.1 M phosphate buffer (pH 7.2; 0.1 volume of growth medium), and resuspended in buffer to a final cell concentration equivalent to 10 mg dry wt/ml.

Preparation of cell-free extracts. Cells from 800 ml of medium grown as described above, for 48 instead of 72 hr, were suspended in phosphate buffer (0.1 M, pH 7.2) to give a cell concentration equivalent to approximately 40 mg dry wt cells/ml. The suspension (20 ml) was subjected to sonic oscillation for 20 to 30 min (Raytheon Sonic Oscillator, 9 kc/sec), the apparatus being

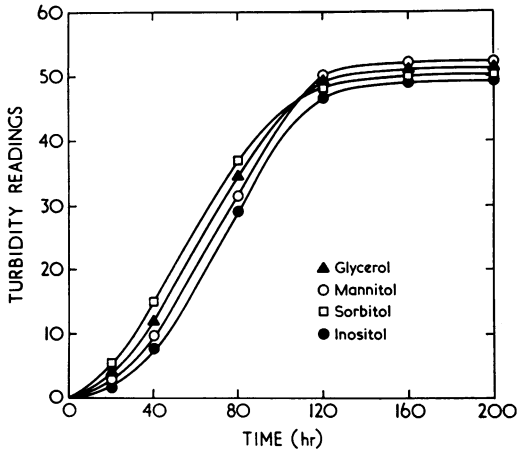

FIG. 1. *Growth response curves for Nocardia corallina. Each growth tube contained, in 5 ml of medium, the substrate in an amount equivalent to 200 μmoles of carbon. Temperature: 30 C. A turbidity reading of 50 = 1.9 mg dry wt cells/5 ml of growth medium.*

run in a cold-room at 2 to 3 C and the internal temperature of the cup kept at 0 to 1 C by the circulation of ice-cold water (1.8 liters/min). The extract was centrifuged at 20,000 × *g* for 30 min at 2 C. The opalescent reddish-brown supernatant liquids were stored in the frozen state (−10 C) and contained approximately 15 mg protein/ml as estimated by the biuret procedure (Gornall, Bardawill, and David, 1949).

TABLE 1. *Growth of Nocardia isolates* on polyols*

Substrate	Species which grew on the substrate (McClung, 1954)	Growth response of *N. corallina* ATCC 4273 (McClung, 1954)	Growth response of *N. corallina* S
	no.		
Glycerol	40	+	+
D-Mannitol	39	+	+
D-Sorbitol	40	+	+
Dulcitol	13	−	−
Inositol	35	Trace	+

* A total of 43 isolates was tested.

Analytical methods. Oxygen consumption was measured by the conventional Warburg technique (Umbreit, Burris, and Stauffer, 1957). Carbon dioxide was absorbed by 0.2 ml of 10% KOH contained in the center well. The incubation system (3.0 ml) in the manometer cup contained, unless otherwise stated, phosphate buffer (0.1 M, pH 7.2, 1.0 ml), substrate solution (0.5 ml containing 20 μmoles of carbon), and water. Chloramphenicol was added to give a final concentration of 50 μg/ml when the inhibitor was used.

Reduction of diphosphopyridine nucleotide (DPN) was followed by measuring the changes

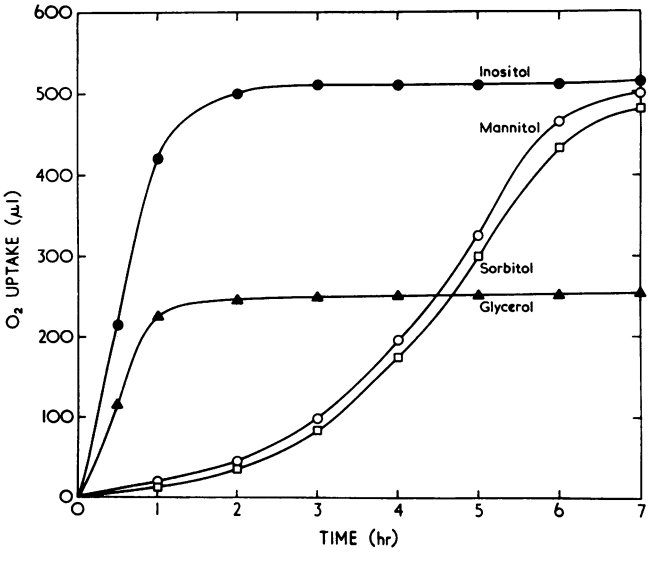

FIG. 2. *Oxygen-uptake curves obtained with cells of strain S grown on glucose and incubated with* D-*mannitol, sorbitol, glycerol, and inositol. Each manometer cup contained 10 μmoles of substrate. The values were corrected for endogenous respiration.*

TABLE 2. *Oxidation of polyols and carbohydrates by cell suspensions of Nocardia corallina**

Compound added (containing 0.24 mg C)	Theoretical O_2 uptake for complete combustion/mole substrate	Theoretical O_2 uptake/sample taken	Observed O_2 uptake	Per cent of theoretical	Q_{O_2}
	moles				
Glucose-grown cells					
Dihydroxyacetone	3.0	448	168	38	5.0
Glyceraldehyde	3.0	448	115	26	6.5
D-Glucose	6.0	448	180	40	31
D-Mannose	6.0	448	125† (4)	—	3.2†
D-Fructose	6.0	448	155	35	20.5
Sucrose	12.0	448	155	35	9.5
Glycerol	3.5	523	194	37	33
D-Arabitol	5.5	493	30† (5)	—	—
Inositol	6.0	448	162	36	42
Sorbitol	6.5	485	197	41	15 (lag)‡
Mannitol	6.5	485	187	39	18 (lag)‡
Sorbitol-grown cells					
Fructose	6.0	448	184	41	40
Sorbitol	6.5	485	252	52	35
Mannitol	6.5	485	202	42	37
D-Arabitol	5.5	493	30† (5)	—	—
Mannitol-grown cells					
Glucose	6.0	448	168	37	45
Sorbitol	6.5	485	257	53	54
Mannitol	6.5	485	215	44	57
D-Arabitol	5.5	493	30† (5)	—	—

* All oxygen uptake values (expressed as μliters/20 μmoles of carbon) are corrected for endogenous respiration; Q_{O_2} = μliters per mg dry wt bacteria per hr at the maximal rate of oxygen uptake. Compounds not oxidized by the cell suspensions were (glucose-grown cells): *Meso-erythritol, ribitol, xylitol, L-arabitol, dulcitol,* D-talitol, volemitol, *perseitol,* 1-deoxy-D-gulitol, D-erythrose, D-ribose, L-arabinose, D-arabinose, D-xylose, L-fucose, D-galactose, rhamnose, lactose, maltose, *D-ribulose, D-xylulose, L-sorbose,* and *D-tagatose*; (*sorbitol- and mannitol cells*): the compounds in italics, together with L-iditol.

† Signifies that the oxidation was still going at the time (hr) indicated in the parentheses.

‡ See Fig. 2.

in optical density at 340 mμ in a Beckman spectrophotometer. Extracts usually showed high DPNH (reduced form of DPN)-oxidase activity, and it was therefore necessary to add 0.1 M KCN (neutralized to pH 7.0 with 1 N HCl) to test systems, giving a final concentration of 0.005 M. The inhibition by cyanide was more effective at pH 9.0 than at 7.0. Cuvettes contained in (2 ml): tris(hydroxymethyl)aminomethane (tris)-HCl buffer (pH 9.0, 0.2 M), 1.0 ml; substrate, 60 μmoles; DPN, 0.15 mg; and extract, 0.1 ml.

Identification of ketoses from the action on polyols of DPN-linked dehydrogenases. Incubation systems contained polyol (20 μmoles), tris-HCl buffer (1.0 ml, 0.1 M, pH 9.0), DPN (1.5 mg), and extract (1 ml) obtained from cells grown on

TABLE 3. *Oxidation of polyols by extracts from cells grown on mannitol, sorbitol, and glucose**

Growth substrate	Oxygen uptake (μliters/120 min) with			
	No addition	Mannitol	Sorbitol	Arabitol
Glucose	8	8	5	10
Mannitol	8	52	37	37
Sorbitol	3	49	20	31

* Each Warburg flask contained: 0.5 ml of extract (from cells grown on either glucose, mannitol, or sorbitol), 1.0 ml of phosphate buffer (pH 7.2; 0.1 M), 10 μmoles of substrate (added from the side-arm after equilibration), and 0.75 mg of DPN. Total volume: 3.0 ml; gas phase: air; temp: 30 C; time: 120 min.

either sorbitol or mannitol. After 6 hr at 30 C, trichloroacetic acid was added (1.5 ml, 10%) and the precipitate removed by centrifugation. The supernatant liquid was neutralized (1 N NaOH) and eluted through a column (1.0 by 10 cm) of Amberlite IRC 120 (H^+). The eluate liquid was evaporated to dryness under vacuum (14 mm Hg pressure) at a bath temperature between 35 and 40 C. The residue was dissolved in water (0.5 ml) and chromatographed on Whatman no. 1 paper using phenol-water solvent (80 g:20 ml). The paper was developed by dipping in a solution of orcinol in trichloroacetic acid and acetone and heating at 100 C for 3 min. Ketoses showed as brown spots and were readily localized by the fluorescence of the areas in a beam of ultraviolet light (Bevenue and Williams, 1951).

TABLE 4. *Relative rates of oxidation of substrates by extracts of cells grown on sorbitol and mannitol**

Substrate	Increase in optical density at 340 mμ (× 10^3/min)	Rates relative to mannitol rate = 100
Extract of mannitol-grown cells		
D-Mannitol	82	100
D-Arabitol	56	68
Sorbitol	15	18
Extract of mannitol-grown cells (stored for 2 months)		
D-Mannitol	30	100
D-Arabitol	23	77
Sorbitol	0	0
Extract of sorbitol-grown cells		
D-Mannitol	160	100
D-Arabitol	120	75
Sorbitol	80	50
Extract of sorbitol-grown cells (stored for 2 months)		
D-Mannitol	50	100
D-Arabitol	34	68
Sorbitol	0	0

* The rates of oxidation were determined spectrophotometrically by measuring the speed of reduction of DPN in incubation systems containing KCN (0.005 M final concentration).

Substrates. L-Iditol, D-xylulose, D-ribulose, and D-tagatose were gifts from D. R. D. Shaw. The sources of D-arabitol, D-talitol, perseitol, volemitol, and 1-deoxy-D-gulitol have been given by McCorkindale and Edson (1954). Other substances used in the investigation were obtained from commercial sources.

RESULTS AND DISCUSSION

Growth of the organism on polyols. N. corallina (strain S) grew on glycerol, mannitol, sorbitol, and inositol as the main source of carbon; the growth responses are shown in Fig. 1. The following polyols did not support growth: ethylene glycol, mesoerythritol, ribitol, xylitol, D-arabitol, L-arabitol, L-iditol, and dulcitol.

The growth responses of *Nocardia* species to glycerol, sorbitol, mannitol, dulcitol, and inositol (McClung, 1954) are compared in Table 1 with results obtained for strain S. Of the 40 strains

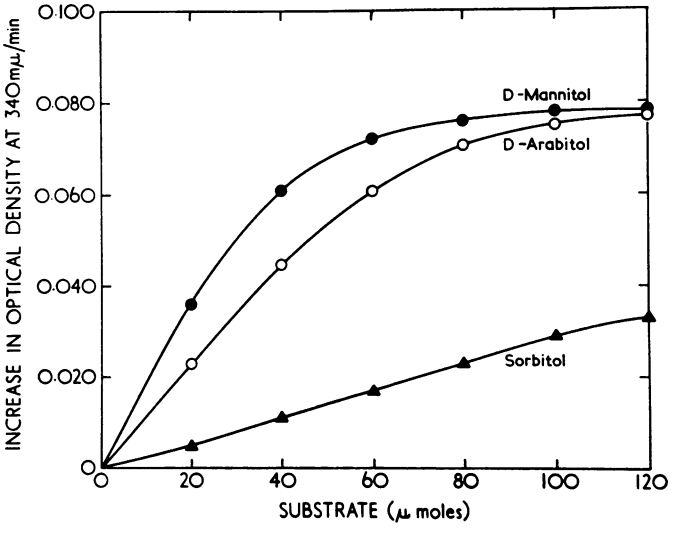

FIG. 3. *Effect of substrate concentration on the rate of reduction of DPN with an extract of cells grown on mannitol.*

studied by McClung which grew on mannitol or sorbitol, 36 used both. However, no evidence has been presented to indicate if a single enzyme system initiated the catabolism of the two polyols.

Oxidation of polyols by cell suspensions. Cells grown on glucose oxidized glycerol and inositol at a maximal rate from the time of adding the substrate, and oxidized mannitol and sorbitol after time lags (Fig. 2). The over-all oxygen uptake figures were approximately proportional to the number of μmoles of carbon added; i.e., the O_2 uptake amounts for mannitol, sorbitol, and inositol (60 μmoles carbon added) were similar and almost twice the over-all value for glycerol (30 μmoles carbon added).

The oxygen uptake figures were equivalent to approximately 40% of the theoretical values for the complete oxidation of the substances to carbon dioxide and water (Table 2). Presumably, oxidative assimilation occurred concurrently with the breakdown of the substrates (Midwinter and Batt, 1960).

The oxidations by glucose-grown cells of a variety of alcohols and carbohydrates were followed, and the results are given in Table 2. The oxidation of mannitol and sorbitol was inhibited by chloramphenicol; the results suggested that these polyols were oxidized by induced enzymes. This possibility was supported by the findings that cells of strain S, grown on either mannitol or sorbitol, oxidized both polyols rapidly from zero time, and that the oxidations were insensitive to chloramphenicol. Several polyols not oxidized by glucose-grown cells were tested and found to be not oxidized by cells grown on mannitol or sorbitol (Table 2). Suspensions of mannitol- and sorbitol-grown cells showed slight activity with D-arabitol.

The induction by sorbitol or mannitol of enzymes for sorbitol and mannitol oxidation suggested that each polyol induced a nonspecific enzyme which would oxidize both polyols. However, instances have been reported in the literature (Monod and Cohn, 1952; Batt, 1961) where an enzyme inducer is not a substrate.

Oxidation of polyols in cell-free extracts of the organism. Extracts of cells grown on sorbitol or mannitol oxidized mannitol, sorbitol, and D-arabitol (but not xylitol) in Warburg manometers (Table 3). The absence of activity in glucose-grown cell extracts supported the evidence with intact cells, i.e., that the enzymes for the oxidation of mannitol and sorbitol were induced by these compounds. The relatively high activity of extracts with D-arabitol was surprising, since whole cells grown on polyols showed only low oxidative ability with this polyol (Table 2).

TABLE 5. *Oxidation of mixtures of polyols by cell-free extracts**

Substrates	Increase in optical density at 340 mμ (× 10^3/min) with extracts of cells grown on	
	Mannitol	Sorbitol
Sorbitol	35	35
D-Mannitol	80	105
D-Arabitol	60	80
Sorbitol + D-mannitol	40	135
Sorbitol + D-arabitol	48	115
D-Mannitol + D-arabitol	64	80

* Incubation systems contained 70 μmoles of each substrate.

Coenzyme requirements. The oxidation of mannitol, sorbitol, and D-arabitol by extracts of the organism grown on mannitol or sorbitol could be coupled to DPN but not triphosphopyridine nucleotide (TPN) reduction. The extract rapidly oxidized DPNH (but not the reduced form of TPN) at pH 7.0, and the oxidation was inhibited by cyanide. The coupled oxidations of the polyols were therefore carried out in the presence of cyanide and at pH 9.0 where the inhibition effect was maximal. The comparative rates of reduction of DPN by extracts from cells grown on mannitol and sorbitol are given in Table 4. Storage of the extracts at 0 C for 2 months resulted in a complete loss of enzyme reactivity with sorbitol, while the activities with arabitol and mannitol were reduced (Table 4). The ratio of mannitol to arabitol activity was of the same order in all extracts. The results suggested that at least two enzymes, each DPN-dependent, were responsible for the oxidation of polyols in the extracts, namely, a labile sorbitol dehydrogenase and a more stable enzyme for mannitol and D-arabitol oxidation.

The rates of oxidation of the three polyols were followed with increasing amounts of substrate and a constant sample of the enzyme preparation. The effect on the maximal rate for each substrate of adding another polyol was also determined. The results (Fig. 3, Table 5) showed

that the relationships between substrate concentration and reaction velocity for mannitol and arabitol were similar to each other for both extracts, and were different from the relationship for sorbitol. With an extract of cells grown on sorbitol, the addition of sorbitol, with mannitol or arabitol, to the preparation resulted in an increased rate of oxidation to that observed as maximal for mannitol or arabitol alone with the extract. However, the mixing of mannitol and arabitol to an extract, each at a concentration high enough to give the maximal rate if added alone, resulted in no increase over the rate for mannitol. In fact, the rate was usually inter-

TABLE 6. *Paper chromatography of ketoses produced from sorbitol, D-mannitol, and D-arabitol by extracts**

Type of cells used to prepare the extract	Substrate	Distances moved by ketoses (cm)				
		Product	L-Sorbose	D-Fructose	D-Xylulose	D-Ribulose
Mannitol-grown	D-Mannitol	20.9		20.6		
	Sorbitol	27.8	22.6	28.0		
	D-Arabitol	24.9			25.6	30.0
Sorbitol-grown	D-Mannitol	21.0		20.5		
	Sorbitol	27.2	22.5	27.0		
	D-Arabitol	25.3			25.5	30.0

* Solvent: phenol-water. The chromatograms were equilibrated, run for 48 hr, and developed specifically for ketoses using the orcinol-trichloroacetic acid reagent.

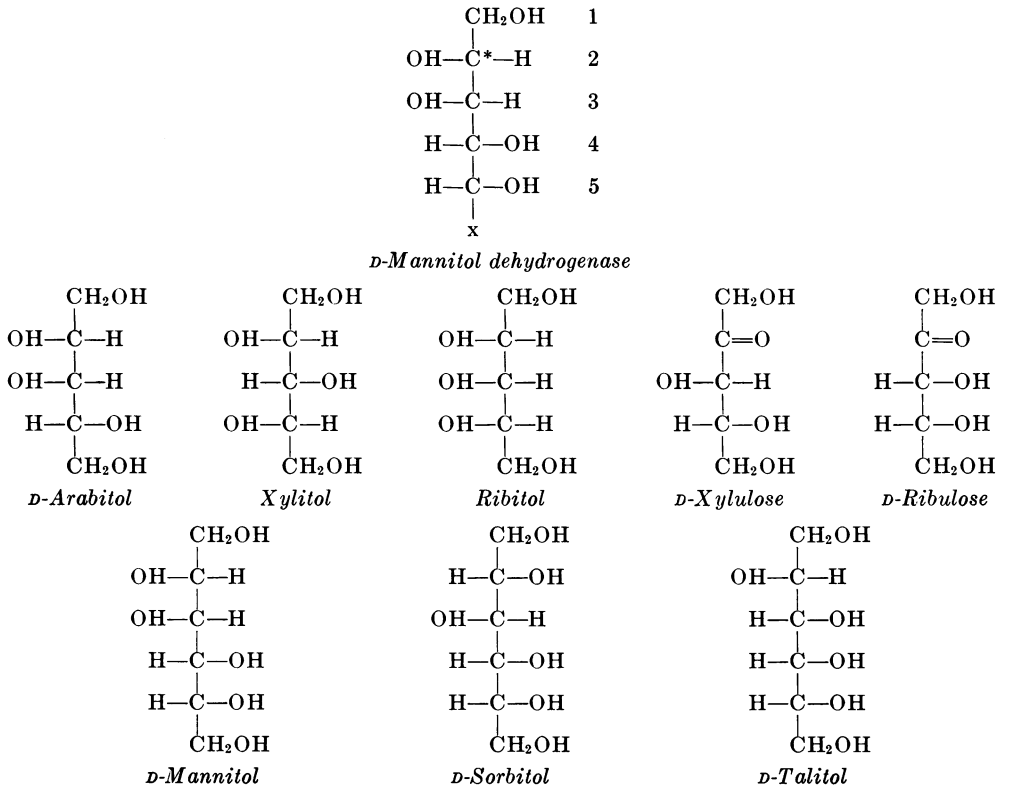

FIG. 4. *Substrate configuration for D-mannitol dehydrogenase. The asterisk denotes the site of oxidation. The x equals —H or —CH₂OH (i.e., D-arabitol or D-mannitol). Structures of ketoses and polyols related to the enzyme substrates are given for comparison.*

mediate between the individual rates for mannitol and arabitol (Table 5). When similar experiments were carried out with extracts of cells grown on mannitol, the addition of sorbitol with mannitol or arabitol resulted in an inhibition of activity for mannitol and arabitol. Mixing mannitol with arabitol and the extract gave rates similar to those observed for arabitol alone. Although the results support the proposition that polyols are oxidized by two enzymes (sorbitol dehydrogenase and mannitol-arabitol dehydrogenase), it appeared that sorbitol may act as an inhibitor of the mannitol-arabitol enzyme when induced by mannitol but not when induced by sorbitol.

Identification of the products of the reaction. The products obtained from the incubation of D-mannitol, sorbitol, or D-arabitol with extracts of cells grown on polyols were identified by paper chromatography. The distances run by the products are compared in Table 6 with the values for L-sorbose, D-fructose, D-xylulose, and D-ribulose. The results showed that both D-mannitol and sorbitol gave fructose, and D-arabitol was oxidized to xylulose.

Intact cells of strain S grown on mannitol, sorbitol, or glucose oxidized fructose without a lag, but had no action on L-sorbose, D-xylulose, D-ribulose, or D-tagatose (Table 2).

Enzymatic reduction of ketoses. When D-fructose or D-xylulose (50 μmoles) was added to an extract of sorbitol-grown cells in a cuvette with DPNH (0.1 mg) in the presence of cyanide (0.1 ml, 0.1 M), the coenzyme was reoxidized. The rates of oxidation were higher for D-xylulose than for D-fructose; the decreases in optical density at 340 mμ/min were: for xylulose, 0.115, and for D-fructose, 0.042. L-Sorbose, D-ribulose, and D-tagatose were not reduced in this system. Extracts of cells grown on mannitol showed similar reactions with D-xylulose and D-fructose, and were more reactive, in this respect, than the extracts of sorbitol-grown cells.

Specificity of the enzymes for polyol metabolism. Two DPN-dependent enzymes were present in extracts of strain S irrespective of whether the cells were grown on sorbitol or mannitol. One enzyme appeared to be specific for sorbitol (oxidized to D-fructose), having no detectable action on xylitol, and was more labile than the enzyme which oxidized D-mannitol (to fructose) and D-arabitol (to xylulose).

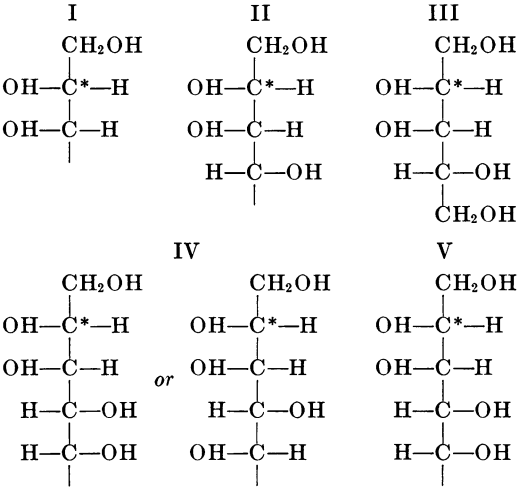

FIG. 5. *Essential configurations of substrates for bacterial enzymes which oxidize* D-*mannitol and* D-*arabitol or both (see Touster and Shaw, 1962).* (I) *Cytochrome-linked* D-*mannitol dehydrogenase of Acetobacter suboxydans (Arcus and Edson, 1956).* (II) *TPN-linked* D-*mannitol dehydrogenase of A. suboxydans (Bygrave and Shaw, 1961).* (III) D-*Arabitol dehydrogenase of Aerobacter aerogenes; oxidizes only* D-*arabitol (Wood, McDonough, and Jacobs, 1961).* (IV) D-*Arabitol dehydrogenase of A. aerogenes; oxidizes* D-*mannitol,* D-*arabitol, and (slowly) sorbitol (Lin, 1961).* (V) D-*Mannitol dehydrogenase of Azotobacter agilis, (Marcus and Marr, 1961) and Nocardia corallina. The asterisk in each structure denotes the site of oxidation. In formula IV, the optional configurations at C-5 lent preference for the* D *configuration.*

The necessary spatial configuration of substrates for the mannitol-arabitol enzyme is similar to that defined for the D-arabitol dehydrogenase of *Aerobacter aerogenes* (Lin, 1961), shown in Fig. 4. The enzyme in *N. corallina* was more active with mannitol than with D-arabitol; the enzyme in *A. aerogenes* was approximately twice as active with *D*-arabitol. Since the enzyme from *A. aerogenes* has been named the D-arabitol dehydrogenase, on the basis of D-arabitol being the substrate most rapidly oxidized, the enzyme from strain S should correspondingly be named the D-mannitol dehydrogenase. The requirement for DPN distinguishes this enzyme from the less specific TPN-linked D-mannitol dehydrogenase of *Acetobacter suboxydans* studied by Bygrave and Shaw (1961), which oxidized D-mannitol (to fructose), D-arabitol (to xylulose), and sorbitol (to L-sorbose).

The specificity of the substrate configuration for the D-mannitol dehydrogenase in strain S (Fig. 4) was deduced from the inability of cell-free extracts to oxidize several polyols related in structure to D-arabitol and D-mannitol. Substrates could not have the alternative configuration at carbon 2, since neither sorbitol nor xylitol were attacked. If the hydroxyl on carbon 3 could be on the right side of the carbon chain, then D-arabitol would give ribulose as the product and D-talitol would be oxidized; in fact, D-arabitol was oxidized to xylulose and D-talitol was not attacked. The alternative configuration at carbon 4 would include ribitol and D-talitol as possible substrates, but neither polyol was oxidized. If the hydroxyl group at carbon 5 could be on either side of the carbon chain, sorbitol should be a substrate for the enzyme yielding L-sorbose; but under conditions where sorbitol activity was found in fresh extracts, the only detectable product was D-fructose.

Other compounds tested and found to be inactive with extracts were meso-erythritol, L-iditol, dulcitol, and L-arabitol. Perseitol may have been oxidized slightly but the results obtained were of the same order as the experimental error for the method of estimation. Activity with this polyol would be in accordance with the configuration shown in Fig. 4 where X = —CHOH · CH_2OH.

The specificity of the D-mannitol dehydrogenase of strain S is compared in Fig. 5 with the specificities of other bacterial enzymes which have been reported to oxidize D-mannitol or D-arabitol or both. With the enzyme described by Marcus and Marr (1961) in *Azotobacter agilis*, the D-mannitol dehydrogenase of strain S shows a high degree of specificity for its substrates based on a defined stereochemical arrangement of hydroxyls on four adjacent carbons in the polyol structure.

Specificity of inducers for polyol dehydrogenases. Both enzymes for polyol oxidation in strain S were induced by mannitol and sorbitol; D-arabitol was ineffective as an inducer under the conditions tested.

The sorbitol dehydrogenase induced by either mannitol or sorbitol oxidized only sorbitol; the spatial configuration of the hydroxyl on carbon 2 is therefore nonspecific for the inducer but specific for the substrate.

The DPN-linked D-mannitol dehydrogenase was induced by both mannitol and sorbitol but was inhibited by sorbitol when acting on D-mannitol or D-arabitol. The induction pattern for this enzyme differs from that for the inducible D-arabitol dehydrogenase of *A. aerogenes* (Lin, 1961), which was induced by D-arabitol but not by D-mannitol. Although the failure of D-arabitol to actively induce D-mannitol dehydrogenase in strain S may be owing to the requirement for a highly specific inducer, it is possible that permeation systems for the polyols may be induced only by mannitol and sorbitol for these two polyols.

The induction of enzymes for polyol oxidation has been studied in pseudomonads by Sebek and Randles (1952), who concluded that mannitol and sorbitol are normally oxidized by different induced enzymes. The ability of cells to oxidize both mannitol and sorbitol was present irrespective of which polyol was used as inducer.

ACKNOWLEDGMENTS

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