METABOLIC PATHWAYS OF GLYCEROL DISSIMILATION

A Comparative Study of Two Strains of Aerobacter aerogenes[1]

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Previous studies from this laboratory have shown that *myo*-inositol is metabolized in a strain of *Aerobacter aerogenes* by a pathway which does not involve conversion to glucose and degradation according to the Embden-Meyerhof scheme (Magasanik, 1951*a*,*b*). The observation by Brooke (1951) of a positive correlation of inositol fermentation and glycerol fermentation in several hundred strains of the *Aerobacter* group suggested an investigation into possible common mechanisms in the degradation of glycerol and of inositol.

All five strains of *A. aerogenes* in this laboratory behaved like the strains previously described by Brooke. The capsulated strains fermented both glycerol and inositol, whereas the acapsulated strains fermented neither. However, the acapsulated strains oxidized glycerol but not inositol.

The degradation of glycerol was studied in one characteristic strain of each group by the method of simultaneous adaptation (Stanier, 1947), the identification and estimation of the end products of oxidation and fermentation, and of intermediate products accumulating in the presence of inhibitors.

The results of these experiments indicate the existence of different pathways for the dissimilation of glycerol in these two strains of *A. aerogenes*.

MATERIALS AND METHODS

Bacterial strains. A. aerogenes, strains 1033 and 1041, were isolated originally from patients at Boston City Hospital. The characteristic morphological and biochemical properties of the two strains are summarized in table 1. The strains were maintained by weekly transfers on tryptic digest nutrient agar slants.

Basal medium. The medium used contained Na_2HPO_4, 0.54 per cent; KH_2PO_4, 1.26 per cent; $MgSO_4 \cdot 7H_2O$, 0.02 per cent; $CaCl_2$, 0.001 per cent; $(NH_4)_2SO_4$, 0.2 per cent; pH 6.5. The solution was sterilized by autoclaving at 10 lb for 10 min. Glucose and glycerol (10 per cent solutions) were autoclaved separately and added aseptically to the basal medium to give the desired concentration, usually 0.2 per cent.

Resting cells. Suspensions of nonproliferating cells were prepared from cultures grown in 250 ml Erlenmeyer flasks in the basal medium with either glucose or glycerol (0.2 per cent) as the sole source of carbon. The medium was inoculated from 24 hour agar slants and shaken on a Camp type shaker at 37 C for 15 to 17 hours. The cells were collected by centrifugation at 5,000 rpm at 4 C, washed twice with distilled water, and then suspended in the volume of distilled water necessary to obtain the required cell concentration. The optical density was measured at a dilution of 1:100 at 590 mμ in a Coleman spectrophotometer, Model 14.

Chemicals. Dihydroxyacetone and D,L-glyceraldehyde were products of the Concord Laboratories. Their purity was confirmed by degradation with periodic acid and estimation of the formaldehyde formed: the expected ratio of one for formaldehyde formed to triose used was obtained. A solution of sodium-D,L-α-glycerophosphate was prepared by treating calcium-D,L-α-glycerophosphate (Delta Chemical Works) with the correct amount of sodium oxalate. The concentration of sodium-α-glycerophosphate in the solution was determined by the estimation of total phosphorus and of the formaldehyde formed by treatment with periodic acid. The ratio of phosphorus to formaldehyde was one, indicating the absence of β-glycerophosphate. Similarly, a solution of sodium-L-α-glycerophosphate was prepared by treating barium-L-α-glycerophosphate (kindly

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given to us by Professor Gerhard Schmidt of Tufts College Medical School) with the correct amount of sodium sulfate. In some experiments sodium glycerophosphate $5H_2O$ (Eastman Organic Chemicals) was used. The content of α-isomers in this mixture was determined as before by periodate oxidation and was found to amount to 50 per cent of the total glycerophosphates. Sodium-β-glycerophosphate (Eastman Organic Chemicals) was free of α-isomers.

Bacterial growth. The growth rate of the strains on glucose or glycerol was determined by inoculating 3.0 ml of a 17 hour minimal medium culture into 50 ml of basal medium containing 150 μg per ml of glucose or glycerol in a 250 ml Erlenmeyer flask. The flasks were shaken at 37 C, samples withdrawn at 15 minute intervals, and their optical density at 590 mμ determined.

The ability of the organisms to grow, ferment glucose or glycerol, and to produce acetylmethylcarbinol was determined under strictly anaerobic conditions in an atmosphere of nitrogen (partial pressure 500 mm Hg) and of CO_2 (partial pressure 1 mm Hg).

Analytical procedures. Oxygen uptake and CO_2 evolution were measured in an atmosphere of air in M/15 phosphate buffer at pH 6.0 or 7.0, or in M/15 "tris" [tris(hydroxy methyl)amino methane] buffer at pH 7.0 in the conventional Warburg apparatus by the "direct method" (Umbreit *et al.*, 1949). Anaerobic acid and gas production were measured in an atmosphere of 95 per cent N_2-5 per cent CO_2 in bicarbonate buffer at pH 7.0 (Umbreit *et al.*, 1949). All readings were corrected for endogenous respiration or fermentation.

Lactic acid was determined by the method of Barker and Summerson (1941), pyruvic acid by the method of Friedemann and Haugen (1943), and reducing sugars, after neutralization of the sample with sodium bicarbonate, by the method of Park and Johnson (1949). Glycerol was measured by oxidation with periodic acid (Reeves, 1941) followed by colorimetric estimation of the formaldehyde formed[2] (MacFayden, 1945). In this method each mole of glycerol gives rise to two moles of formaldehyde. With the same method dihydroxyacetone, glyceraldehyde, and α-glycerophosphoric acid yield one mole of formaldehyde. It was possible, however, to determine glycerol in the presence of these compounds by estimating dihydroxyacetone, and glyceraldehyde or α-glycerophosphate by other methods (reducing sugar or organic phosphorus), and then correcting the formaldehyde value accordingly. Volatile fatty acids and ethanol were distilled from 1 to 2 ml aliquots frozen in the still described by Grant (1946). Pyruvic acid was found to be somewhat volatile under these conditions. Its distillation was prevented by the addition of solid phenylhydrazine sulfate to the sample contained in the side bulb of the apparatus. The volatile acids in the distillate were estimated by potentiometric titration with 0.1 N NaOH. A Gilmont ultramicroburette (Emil Greiner Co.) and a Beckman pH meter were used. The presence or absence of formic acid could be inferred from the shape of the titration curve. After titration the reaction mixture was adjusted to a volume of 5 ml and aliquots used for the determination of formic acid (Grant, 1948) and of ethanol (Henry *et al.*, 1948). The difference between total volatile acids and formic acid was ascribed to acetic acid.

Total and inorganic phosphorus were determined by the method of King (1932).

[2] We are indebted to Dr. M. L. Karnovsky for introducing us to this method.

RESULTS

Growth on glucose and on glycerol. The characteristic properties of the two strains are given in table 1. In shaken cultures strain 1033 grows nearly twice as rapidly as strain 1041; after a period of lag, each strain grows on glycerol at about the same rate as on glucose.

The results of experiments on the effect of the addition of hydrogen acceptors to anaerobic cultures of the two strains are presented in table 2. The three naturally occurring hydrogen acceptors used failed to permit growth of strain 1041 on glycerol but markedly stimulated its growth on glucose. Strain 1033 grew on glycerol with production of acid but not of acetylmethylcarbinol. The addition of sodium fumarate resulted in increased growth and in the formation of acetylmethylcarbinol. The addition of peptone also stimulated growth but to a lesser extent. These two hydrogen acceptors similarly enhanced the growth of strain 1033 on glucose. Sodium pyruvate did not stimulate growth on glucose or on glycerol.

Oxidation and fermentation of glycerol by resting cell suspensions. Glucose grown cells of both strains oxidized and fermented glycerol initially at a very slow rate (table 3 and 4). After a lag

TABLE 1

Characteristic properties of Aerobacter aerogenes, strains 1033 and 1041

The experimental procedures are described in the text.

	STRAIN					
	1033			1041		
IMVIC*	−−++			−−++		
Capsule†	+			−		
	GLUCOSE	GLYCEROL	*m*-INOSITOL	GLUCOSE	GLYCEROL	*m*-INOSITOL
Anaerobic growth	+	+	+	+	−	−
Aerobic growth	+	+	+	+	+	−
Lag phase, min	0	30	45	0	45	
Mean generation time, min	46	51	50	102	106	

* I+ = produces indole, M+ = acid to methyl red, V+ = produces acetylmethylcarbinol, C+ = utilizes citric acid for growth.

† Observed in the phase microscope.

TABLE 2

The effect of hydrogen acceptors on the anaerobic growth of strains 1033 and 1041 of Aerobacter aerogenes

Each tube contained the salt base diluted 1/20. 0.5 per cent glucose or 0.5 per cent glycerol were the carbon sources. The H acceptors were 0.5 per cent sodium fumarate, 0.5 per cent sodium pyruvate, or 1.0 per cent peptone. The tubes were inoculated with 0.5 ml of a 17 hour glycerol grown culture and incubated anaerobically, as described in the text, for 18 hours at 37 C. Optical density, pH, and acetylmethylcarbinol (AMC) production then were measured. The initial optical density (at 590 mμ) of the media inoculated with strain 1033 was 0.18; of the media inoculated with strain 1041, 0.18; the initial pH of the media after the inoculation of 1033 was 7.1 and of 1041, 7.1.

CARBON SOURCE	H ACCEPTOR	STRAIN					
		1033			1041		
		OD	pH	AMC	OD	pH	AMC
—	—	0.09	6.8	0	0.09	7.1	0
Glucose	—	0.56	4.7	4+	0.26	4.7	4+
Glycerol	—	0.35	4.6	0	0.09	7.0	0
—	Peptone	0.19	7.0	0	0.12	7.1	0
Glucose	Peptone	0.78	5.3	4+	0.52	4.9	3+
Glycerol	Peptone	0.55	5.9	0	0.11	7.0	0
—	Na fumarate	0.08	7.1	0	0.09	7.2	0
Glucose	Na fumarate	0.74	5.7	4+	0.65	5.7	4+
Glycerol	Na fumarate	0.63	5.3	3+	0.15	7.0	0
—	Na pyruvate	0.36	6.0	4+	0.17	5.9	4+
Glucose	Na pyruvate	0.62	5.7	4+	0.50	5.6	4+
Glycerol	Na pyruvate	0.42	5.3	4+	0.17	5.9	4+

phase of 5 to 10 minutes in the case of strain 1033, and 20 to 30 minutes in the case of strain 1041, these cells oxidized glycerol rapidly. Under anaerobic conditions the rate of fermentation did not increase during a period of 3 hours.

On the other hand, glycerol grown cells of both strains oxidized glycerol without lag at about the same rate (table 3, and figure 1) and, in the case of strain 1033, fermented glycerol fairly rapidly (table 4).

TABLE 3

Comparison of oxidation rates of suspensions of Aerobacter aerogenes, strains 1033 and 1041, in phosphate and "tris" buffer

The Warburg vessels contained 0.5 ml of the bacterial suspension and 2.0 ml M/15 phosphate or "tris" buffer of pH 7.0 in the main compartment, 5 μM of substrate in 0.5 ml buffer in the side arm, and 0.2 ml of 20 per cent KOH in the center well. The optical density of the mixture after tipping was about 2.5. Temperature, 35 C.

SUBSTRATE	STRAIN					
	1033			1041		
	Glucose medium and phosphate buffer	Glycerol medium and phosphate buffer	Glycerol medium and "tris" buffer	Glucose medium and phosphate buffer	Glycerol medium and phosphate buffer	Glycerol medium and "tris" buffer
	*O_2 uptake, μL per hour per unit of cells**					
Glycerol	7	70	85	2	68	62
D,L-Glyceraldehyde	2	56	62	0	47	25
Dihydroxyacetone	3	68	78	0	8	7
Na D,L-α-glycerophosphate	4	20	43	0	87	77
Na β-glycerophosphate	—	0	—	—	2	—

* A unit of cells is defined as the number of cells contained in 1.0 ml of a suspension of an optical density of 1.0 at 590 mμ determined in a square cuvette (light path 13 mm) in a Coleman Universal spectrophotometer, Model 14. A unit of cells contains about 9×10^8 viable cells and 50 μg of bacterial nitrogen. The rates given are the maximal rates of the glycerol grown cells (see figure 1) and the rates measured during the first five minutes after exposure to the substrate in the case of the glucose grown cells.

The results of these experiments, together with the observations on the growth of both strains in glucose and glycerol media, indicate the adaptive nature of the enzymes active in glycerol dissimilation.

Products of glycerol oxidation and fermentation. Glycerol adapted cells of both strains oxidized glycerol with the uptake of approximately 2 moles of oxygen and the evolution of 1.5 moles of CO_2 per mole of glycerol (figures 1 and 2). In the presence of 2,4-dinitrophenol (0.0001 M) the oxygen uptake approached the theoretical value of 3.5 M/M. The incomplete oxidation in noninhibited cells probably is due to assimilation of one-half of the carbon of the glycerol molecule as CH_2O (Clifton, 1937). A similar balance of oxidation and assimilation was noted in strain 1033 with glucose or inositol as the substrate.

When sodium arsenite was used as an inhibitor in the oxidation experiments, the accumulation of pyruvic acid could be demonstrated (figures 2 and 3). With strain 1033, pyruvic acid was the main product of oxidation, whereas in strain 1041 a portion of the pyruvic acid was converted to acetylmethylcarbinol and CO_2.

The end products of glycerol fermentation in strain 1033 were ethanol and formic acid in equimolar amounts (table 5). Formic acid, rather than H_2 and CO_2, accumulated because these

TABLE 4

Fermentative rates of suspensions of Aerobacter aerogenes, strains 1033 and 1041

The Warburg vessels contained 0.5 ml of the bacterial suspension and 2.0 ml 0.01 M sodium bicarbonate in the main compartment and 5 μM of substrate in 0.5 ml sodium bicarbonate in the side arm. The gas phase was 95 per cent N_2-5 per cent CO_2. The optical density of the mixture after tipping was about 9.0. pH 7.0. Temperature, 35 C.

SUBSTRATE	STRAIN			
	1033		1041	
	Glucose	Glycerol	Glucose	Glycerol
	*CO_2 evolution, μL per hour per unit of cells**			
Glycerol	0.6	10	0.5	1.6
D,L-Glyceraldehyde	1.6	40†	2.8	3.8
Dihydroxyacetone	1.4	45†	1.1	2.0

* See footnote to table 3.

† Initial rate. After the first five minutes the rate falls to about 10. At this stage the original substrate has disappeared; about one-half of it has been converted to glycerol.

cells which had been grown with strong aeration did not contain formic hydrogenlyase (Stokes, 1949). In the presence of arsenious oxide, the rate of fermentation was greatly depressed, lactic acid was identified as an end product, and the fixation of CO_2 was observed; no other acid accumulated.

The results of these experiments indicate that the dissimilation of glycerol occurs via pyruvic acid in both strains.

is different. Glycerol grown cells oxidize α-glycerophosphate more rapidly even than glycerol. In contrast, the oxidation of dihydroxyacetone is only slightly faster in glycerol than in glucose grown cells. Glyceraldehyde occupies an intermediate position; its oxidation rate is increased by growth on glycerol but does not reach the rate at which glycerol is oxidized.

The results of these experiments implicate dihydroxyacetone and glyceraldehyde as inter-

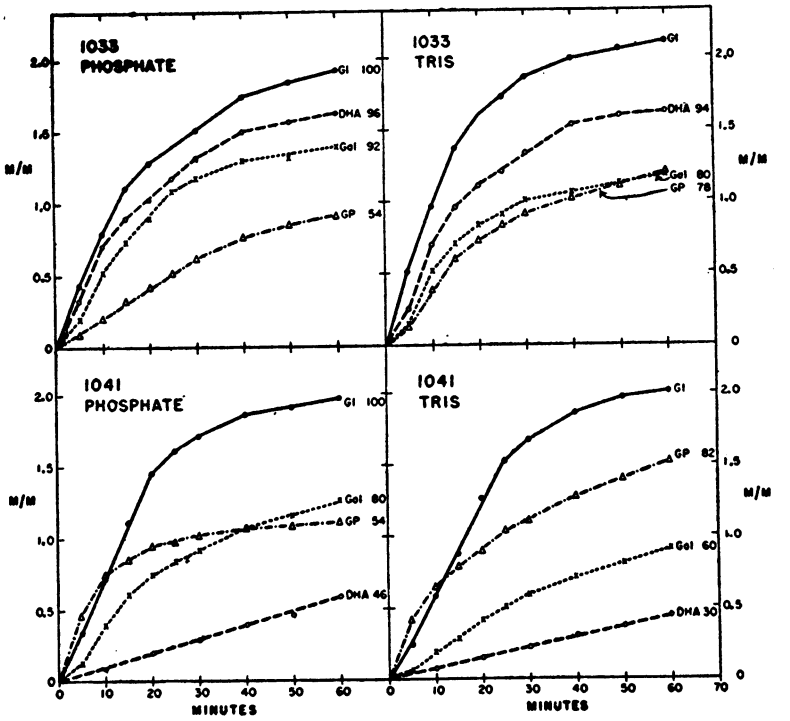

Figure 1. Oxygen uptake of glycerol grown cells of strains 1033 and 1041 in phosphate and in "tris" buffer. The experimental conditions are described in table 1. Gl = glycerol, DHA = dihydroxyacetone, Gal = D,L-glyceraldehyde, GP = Na D,L-α-glycerophosphate. The oxygen uptake is expressed in mole of oxygen taken up per mole of substrate. The figures on the right hand side of the curves give per cent of substrate consumed.

"Adaptive patterns" in glycerol degradation. In strain 1033 growth on glycerol causes an increase in the ability of the cells to oxidize dihydroxyacetone, glyceraldehyde, and α-glycerophosphate (table 3). The two first mentioned compounds are oxidized at approximately the same rate as glycerol if their higher oxidation level is taken into consideration, while α-glycerophosphate is oxidized in phosphate buffer only one-fourth as fast.

The adaptive pattern observed in strain 1041 mediates in the enzymatic degradation of glycerol in strain 1033, and α-glycerophosphate as an intermediate in strain 1041.

The oxidation and fermentation of dihydroxyacetone and of glyceraldehyde. Comparison of the oxidation of glycerol and of dihydroxyacetone by glycerol grown cells of strain 1033 in phosphate buffer and in "tris" buffer, both in the presence and absence of As_2O_3 (table 3 and figure 2), shows that dihydroxyacetone is oxidized in the same manner and at the same rate as glycerol.

The fermentation of dihydroxyacetone is initially very much more rapid than the fermentation of glycerol (tables 4 and 5) and results in the conversion of dihydroxyacetone to glycerol, acetic acid, formic acid, and ethanol. The glycerol formed then is fermented slowly to formic acid and ethanol (table 5). These observations support the hypothesis that dihydroxyacetone is an intermediate in the dissimilation of glycerol by strain 1033.

Similar considerations allow us to accept glyceraldehyde as an intermediate of glycerol dissimilation in strain 1033. The rate of oxidation of glyceraldehyde by glycerol grown cells in phosphate buffer and in "tris" buffer is sufficiently rapid (table 3 and figure 1), and the fermentation follows the same pattern as that of dihydroxyacetone (table 4). It is of interest that both optical enantiomorphs are fermented rapidly (table 4, see footnote). The decline of the oxidation rate in "tris" buffer after the uptake of about 0.7 mole of oxygen per mole of α-glyceraldehyde, however, seems to indicate the preferential utilization of the natural D-isomer under these conditions.

In strain 1041 the rate of glyceraldehyde oxidation in phosphate buffer, and even more markedly in "tris" buffer, is too slow to account for a dissimilation of glycerol via glyceraldehyde. The

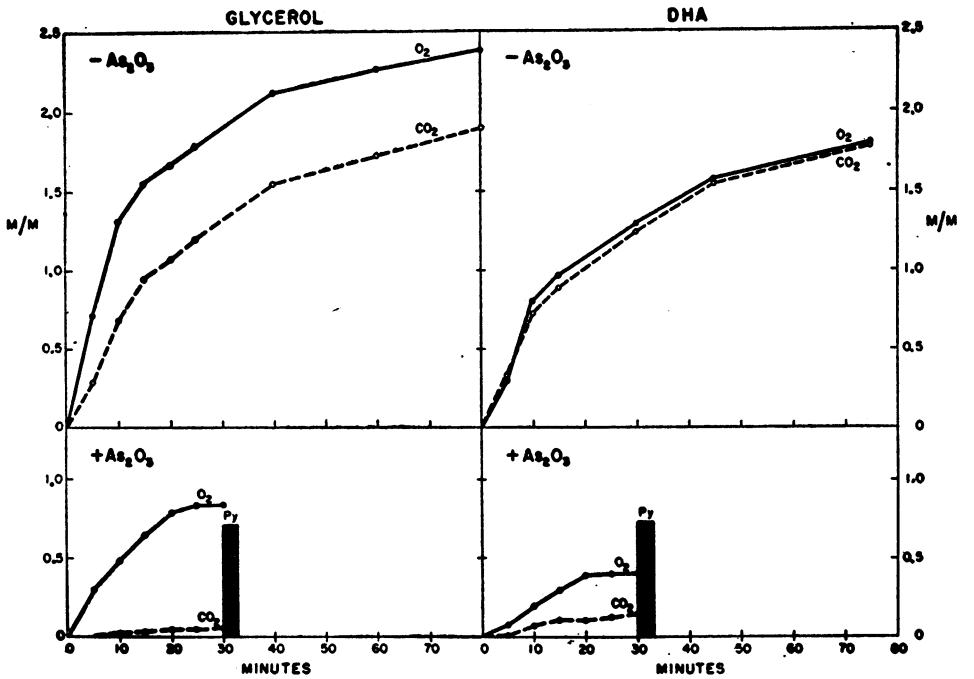

Figure 2. Oxidative dissimilation of glycerol and of dihydroxyacetone by glycerol grown cells of strain 1033 in the absence and presence of As_2O_3. Oxygen uptake, CO_2 evolution, and pyruvic acid accumulation are expressed in mole per mole of substrate. The experimental conditions differ from those described in table 3 only in that phosphate buffer of pH 6.0 was used. Each Warburg vessel contained 5 μM of As_2O_3 dissolved in the phosphate buffer when indicated.

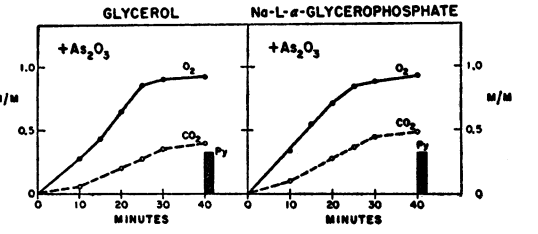

Figure 3. Oxidative dissimilation of glycerol and of Na L-α-glycerophosphate by glycerol grown cells of strain 1041 in the presence of As_2O_3. The experimental conditions are summarized in table 3 and in the legend to figure 2. About 0.2 mole of acetylmethylcarbinol is formed per mole of substrate.

TABLE 5

Metabolic products formed in the fermentation of glycerol and of dihydroxyacetone by suspensions of Aerobacter aerogenes, strain 1033, grown on glycerol

The fermentations were carried out in Warburg vessels in an atmosphere of 95 per cent N_2-5 per cent CO_2 at 35 C. The main compartment held 0.5 ml bacterial suspension and 2.0 ml 0.01 M sodium bicarbonate, which contained 5 μM As_2O_3 when indicated; the two side arms, 10 μM of substrate in 0.5 ml solution and 0.2 ml 1.5 N H_2SO_4, respectively. The optical density of the mixture after tipping of the substrate was about 9. The pH 6.9. The acid was tipped in when gas evolution had ceased. The contents of duplicate vessels were pooled, the cells removed by centrifugation at 5,000 rpm in the cold, and the supernatant fluids analyzed. All analyses were carried out at least in duplicate.

END PRODUCTS	SUBSTRATE				
	Glycerol		Dihydroxyacetone		
	No inhibitor	As_2O_3	No inhibitor*	No inhibitor†	As_2O_3
	Moles/mole of substrate				
Lactic acid	0	0.47	0	0	0.59
Pyruvic acid	0	0	0	0	0.16
Acetic acid	0.03		0.20	0.30	
Formic acid	0.81		0.32	0.56	
Ethanol	0.86		0.12	0.23	
Glycerol			0.34	0.13	0.10
CO_2	0	−0.29	0.15	0.15	0
Red. sugar‡		0.11	0.07	0.06	0.09
Total acid, manometric	0.95	0.51	0.62	0.91	0.74
Total acid, chemical	0.84	0.47	0.52	0.86	0.75
Carbon recovered, per cent	86		78	78	94
Redox index	1.02		0.99	0.94	0.98

* At the end of 5 minutes when the rate of gas evolution had decreased markedly.

† At the end of 60 minutes when gas evolution had stopped.

‡ Calculated as dihydroxyacetone.

differences in glycerol and glyceraldehyde oxidations in response to the substitution of "tris" buffer for phosphate buffer further militate against assigning to glyceraldehyde the *role* of an intermediate in glycerol dissimilation (table 4 and figure 1).

Hydrolysis and degradation of α-glycerophosphate. α-Glycerophosphate is not an intermediate in the dissimilation of glycerol by glycerol grown cells of strain 1033. The phosphate ester is hydrolyzed by the phosphatase of the cells to glycerol and inorganic phosphate. Under anaerobic conditions glycerol accumulates; in the presence of oxygen, glycerol is oxidized. The rate of α-glycerophosphate degradation depends on phosphatase activity and consequently is depressed by inorganic phosphate (table 3 and figure 1).

The rate of oxidation of α-glycerophosphate by glycerol grown cells of strain 1041 is not decreased by inorganic phosphate and exceeds the rate of oxidation of glycerol (table 3 and figure 1). The rapid uptake of oxygen ceases with one mole of oxygen taken up per mole of D,L-α-glycerophosphate; at this stage one-half of the α-glycerophosphate has disappeared (figure 1). L-α-Glycerophosphate is oxidized completely with the uptake of two moles of oxygen per mole. Both glycerol and α-glycerophosphate are affected by As_2O_3 in the same manner (figure 3). L-α-Glycerophosphate therefore may be considered as an intermediate in the degradation of glycerol by strain 1041. The slow oxidation of D-α-glycerophosphate in the absence of inorganic phosphate (figure 1) presumably is due to its hydrolysis by phosphatase, followed by oxidation of the glycerol which is formed.

DISCUSSION

The evidence presented in the preceding section allows us to formulate the pathway of glycerol dissimilation in *A. aerogenes*, strain 1033, as follows:

$$\text{glycerol} \overset{-2H}{\rightleftharpoons} \text{dihydroxyacetone} \rightleftharpoons$$

$$\text{glyceraldehyde} \xrightarrow{-2H} \text{pyruvic acid}$$

Aerobically: $4H + O_2 \rightarrow 2H_2O$

Anaerobically: pyruvic acid → acetic acid + formic acid

$4H$ + acetic acid → ethanol

Pyruvic acid can be oxidized further but accumulates when As_2O_3 is present. In the absence of oxygen, ethanol and formic acid are the final products. The dehydrogenation of glycerol to dihydroxyacetone is reversible, as shown by

the formation of glycerol in the early stages of dihydroxyacetone fermentation (table 5). This reaction has been observed previously in *Escherichia coli* grown on dihydroxyacetone (Virtanen *et al.*, 1930). Similarly, glyceraldehyde and dihydroxyacetone seem to be in equilibrium as shown by the accumulation of glycerol in the fermentation of glyceraldehyde. The conversion of glyceraldehyde to pyruvic acid was not investigated. The results of the fermentation experiments (tables 4 and 5) show that under anaerobic conditions acetic acid, or presumably "active acetate", functions as the hydrogen acceptor in the two dehydrogenation steps.

The slow anaerobic dissimilation of glycerol, in contrast to the rapid dissimilation of dihydroxyacetone, is due to the lack of a suitable hydrogen acceptor. The addition of sodium pyruvate thus would be expected to increase the rate of glycerol fermentation but did not. However, the addition of sodium fumarate stimulates glycerol fermentation and allows the accumulation of more highly oxidized end products (table 2). A recent communication (Burton and Kaplan, 1953) reporting the reversible dehydrogenation of glycerol to dihydroxyacetone with coenzyme I as hydrogen acceptor in extracts of an *A. aerogenes* strain is in good agreement with the results reported here.

The dissimilation of glycerol in strain 1041 follows a different pathway but leads to the same end products as those obtained in strain 1033.

$$\text{glycerol} + H_3PO_4 \rightarrow \text{L-}\alpha\text{-glycerophosphoric acid} \xrightarrow{-4H} \rightarrow \text{pyruvic acid} + H_3PO_4$$

$$4H + O_2 \rightarrow 2H_2O$$

Pyruvic acid is metabolized further but accumulates together with acetylmethylcarbinol and CO_2 when As_2O_3 is present. The steps between L-α-glycerophosphoric acid and pyruvic acid were not investigated but, by analogy with the pathways known in yeast and mammalian tissue, presumably consist of dehydrogenation of the phosphate ester to dihydroxyacetone phosphate, which is metabolized then according to the Embden-Meyerhof scheme. *A. aerogenes*, strain 1041, is unable to dissimilate glycerol in the absence of oxygen, even when hydrogen acceptors such as sodium pyruvate, sodium fumarate, or peptone are present. The dehydrogenase acting on L-α-glycerophosphate thus does not resemble the diphosphopyridine nucleotide linked dehydrogenase found in mammalian tissue but rather the cytochrome linked α-glycerophosphate dehydrogenase isolated by Green (1936) from rabbit muscle or the enzyme reacting directly with oxygen found in *Streptococcus faecalis* by Gunsalus and Umbreit (1945).

A pathway of glycerol dissimilation similar to that in *A. aerogenes*, strain 1041, has been observed in *Escherichia freundii* (Mickelson and Shideman, 1947).

The different behavior of strains 1033 and 1041 described in the present paper may be summarized by stating that in strain 1033 glycerol induces the synthesis of *glycerol dehydrogenase*, an enzyme catalyzing the conversion of glycerol to dihydroxyacetone, and that in strain 1041 glycerol induces the synthesis of *glycerokinase*, which catalyzes the phosphorylation of glycerol to L-α-glycerophosphate. These products in turn induce the synthesis of enzymes necessary for the conversion of the glycerol intermediates to pyruvic acid.

The correlation of glycerol and of inositol fermentation in strains of *A. aerogenes* referred to in the introduction may now be discussed.

The oxidation of glycerol to dihydroxyacetone is a well known reaction of *Acetobacter suboxydans* (Virtanen and Nordlund, 1933). This organism is unable to degrade dihydroxyacetone, which can be recovered from its culture fluids. Similarly, the oxidation of *myo*-inositol by *A. suboxydans* leads to the accumulation of 2-keto-*myo*-inositol (Posternak, 1941). Comparable enzyme systems appear to be present in *A. aerogenes*, strain 1033: glycerol is dehydrogenated to dihydroxyacetone, and *myo*-inositol to 2-keto-*myo*-inositol (Magasanik, 1951*a*,*b*). The glycerol and inositol enzyme systems are not identical since adaptation to glycerol does not adapt to *myo*-inositol and *vice versa*. In contrast to *A. suboxydans*, *A. aerogenes*, strain 1033, can dissimilate dihydroxyacetone and 2-keto-*myo*-inositol via pyruvic acid to CO_2.

The capsulated strains of *A. aerogenes*, of which 1033 is an example, possess enzymatic mechanisms which permit the dehydrogenation of glycerol and of *myo*-inositol, and perhaps other polyhydroxy compounds, without prior phosphorylation.

The acapsulated strains, such as 1041, employ the well known mechanism of phosphorylation before dehydrogenation. This mechanism does not seem to be used for *myo*-inositol degradation but can be used for the oxidative dissimilation of glycerol.

SUMMARY

The dissimilation of glycerol by a capsulated strain, 1033, and an acapsulated strain, 1041, of *Aerobacter aerogenes* has been described. In both strains exposure of the cells to glycerol evokes the production of enzymes capable of oxidizing glycerol to CO_2 and H_2O via pyruvic acid. The pathway of glycerol oxidation, however, is different in the two strains. In strain 1033 glycerol is dehydrogenated to dihydroxyacetone, while in strain 1041 glycerol is phosphorylated to L-α-glycerophosphate. In strain 1033 glycerol may be dissimilated in the absence of added hydrogen acceptors, while in strain 1041 oxygen is required for the dissimilation of glycerol. The pathways of the dissimilation of glycerol and of *myo*-inositol are compared. In both cases the first step consists in the direct dehydrogenation of a secondary hydroxyl group to a keto group. The similarity of these enzymatic reactions, in which a polyhydroxy compound is dehydrogenated without prior phosphorylation, to the oxidations carried out by *Acetobacter suboxydans* is discussed.

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