rate of reaction is governed by the relative affinity of the new site for substrate or reaction products.

We wish to thank Dr B. S. Hartley for a gift of chymotrypsin. One of us (H.B.) is grateful to the Medical Research Council for a research assistantship.

REFERENCES

Baum, H., Dodgson, K. S. & Spencer, B. (1958). *Biochem. J.* **69**, 567.
Dawson, C. R. & Tarpley, W. B. (1951). In *The Enzymes*, vol. 2, part 1, p. 454. Ed. by Sumner, J. B. & Myrback, K. New York: Academic Press Inc.
Dixon, G. H. & Neurath, H. (1957). *J. biol. Chem.* **225**, 1049.
Dodgson, K. S. & Wynn, C. H. (1958). *Biochem. J.* **68**, 387.
Macdonald, C. E. & Balls, A. K. (1957). *J. biol. Chem.* **227**, 727.
Roy, A. B. (1953). *Biochem. J.* **55**, 653.
Roy, A. B. (1957). *Experientia*, **13**, 32.

Growth of *Acetobacter suboxydans* and the Oxidation of Aldoses, Related Carboxylic Acids, and Aldehydes

By J. A. FEWSTER*
Microbiology Unit, Department of Biochemistry, University of Oxford

(*Received* 23 *December* 1957)

Acetobacter suboxydans was first isolated by Kluyver & de Leeuw (1924) from beer. Referring to the oxidation of carbohydrates during aerobic growth, they described the organism as 'characterized by its slight intensity of oxidation which leads to an accumulation of intermediary products'. The discovery of the selective oxidation of polyols by growing cultures of *Acetobacter xylinium*, resulting in the accumulation of the corresponding ketoses, led to the enunciation of the well-known Bertrand rule that has been confirmed and extended by later workers studying *A. suboxydans*. Growth on aldoses as carbon source leads to the formation of the corresponding aldonic acids, and it is probable that gluconic acid is the initial product of the action of all *Acetobacter* species on glucose. More extensive oxidation of glucose to yield 5-oxogluconic acid during growth is a feature of a number of *Acetobacter* species, including *A. suboxydans*, and the concomitant formation of 2-oxogluconic acid by this organism has also been reported. These aspects have been reviewed recently by Rao (1957).

The oxidation of some carbohydrates by suspensions of *A. suboxydans* has been the subject of a number of investigations (Butlin, 1936, 1938*a*, *b*; Kluyver & Boezaardt, 1938; King & Cheldelin, 1952, 1953), and the present study, of which a preliminary communication has been made (Fewster, 1956), is designed to correlate and extend much of this work as well as to examine the effectiveness of various carbohydrates as energy sources for growth and how these, in turn, influence the oxidative activity of the harvested organisms. This first paper is concerned with growth of the organism and the oxidation of aldoses and a number of aldehydes and carboxylic acids.

* Guinness Research Fellow in Microbiological Biochemistry.

EXPERIMENTAL

Organism. The strain of *A. suboxydans* used was no. 621 of the American Type Culture Collection and was obtained from the National Collection of Type Cultures. It was maintained on malt wort (Walker & Tosic, 1946) containing chalk (2%, w/v; previously sterilized at 160° for 4 hr.) in suspension solidified with agar (2%, w/v) in sloped test tubes; the tubes were incubated at 30° for 48 hr.

Media

Media for growth tests. Semi-defined medium *B* (sterilized by autoclaving for 10 min. at 10 lb./in.^{2}) contained per litre: acid-hydrolysed casein (vitamin-free), prepared by the method of Snell & Rannefeld (1945), equivalent to 6 g. of original casein; DL-tryptophan, 100 mg.; L-cysteine hydrochloride, 100 mg.; KH_2PO_4, either 10 g. (medium B_1) or 27 g. (medium B_2); $MgSO_4,7H_2O$, 400 mg.; $FeSO_4$, $(NH_4)_2SO_4,6H_2O$, 30 mg.; $MnSO_4,4H_2O$, 20 mg.; calcium pantothenate, 2 mg.; nicotinic acid, 2 mg.; *p*-aminobenzoic acid, 27·5 μg.; Tween 80 (Honeywill and Stein Ltd., London S.W. 1), 1 ml.; the medium was adjusted to pH 6 with N-NaOH. Complex medium *C* (sterilized by autoclaving for 15 min. at 10 lb./in.^{2}) contained per litre: peptone (Evans Medical Supplies Ltd., Liverpool), 10 g.; yeast extract (Marmite Ltd., London), 10 g.; the pH of the medium was adjusted to 6 with N-NaOH.

Media for bulk growth of the organism. Two types of solid media were employed: semi-defined medium B_1 or complex medium *C* (90 ml.) was added to agar (2 g.) in a Roux bottle; the mixture was autoclaved for 15 min. at 10 lb./in.^{2} and to it was added 10 ml. of a sterile aqueous solution (50%, w/v) of the carbohydrate under investigation. To neutralize the acids produced during growth on an aldose, sterile chalk (2 g.) was added to each bottle and suspended

evenly in the medium just before solidification. The presence of a neutralizing agent was not required when the test substance was the neutral salt of an aldonic acid, a polyol or a ketose. The organism was also grown in bulk in liquid media B_1 and C containing various carbohydrates.

Growth of the organism

For testing various carbohydrates as energy sources for growth 2·5 ml. of a solution (1 or 5%, w/v) of the sugar (autoclaved for 10 min. at 10 lb./in.^{2}) was added to 2·5 ml. of sterile basal medium in glass tubes shaped like an inverted T (Monod, Cohen-Bazire & Cohn, 1951). After inoculation with 0·1 ml./tube of a 1:100 dilution of a suspension (0·12 mg. dry wt. of cells/ml.) in sterile water of organisms from a stock slope, the tubes were attached to a rocking device (see van Heyningen & Gladstone, 1953) and shaken (36 oscillations/min.) in a water bath at 30° for periods of time up to 96 hr. The extent of growth is recorded as the galvanometer reading given by an EEL photoelectric colorimeter (Evans Electroselenium Ltd., Harlow, Essex) measured directly in the T-tubes, and with a neutral-density filter and the uninoculated medium to obtain the zero setting. A culture containing 0·5 mg. dry wt. of cells/ml. gave a reading of 21; there was a linear relationship between reading and dry wt. of cells up to a reading of 45.

For bulk growth of the organism on solid media each Roux bottle of media was shown with 1·5 ml. of a suspension (0·12 mg. dry wt. of cells/ml.) of cells from a stock slope, and the inverted bottles were incubated at 30° for periods of time up to 14 days. The organisms were harvested in water and concentrated by centrifuging. For bulk growth in liquid media large T-tubes each containing 25 ml. of sterile medium B_1 or C, 25 ml. of a sterile solution (5%, w/v) of the test substance and sterile chalk (0·3 g.) if required, were each inoculated with 0·5 ml. of a suspension (EEL 5) of the organism. The T-tubes were shaken in a water bath at 30° for 72 hr. and the cells harvested by centrifuging. The organisms from all types of media were washed twice by dispersion in water (a volume equal to half that of the culture medium), followed by centrifuging, and suspended finally in buffer (0·05 M) of the desired pH at a concentration of 20 mg. dry wt./ml.

Analytical methods

Chromatography. In many cases identification of the products of metabolism was by descending chromatography on Whatman no. 1 paper at room temperature, phenol–water (4:1, w/v) and the upper phase of a mixture of butan-1-ol–acetic acid–water (4:1:5, v/v) being used as general developing solvents. The two oxogluconic acids were readily separated (Table 1) by the use of (*a*) a solution of picric acid (4%, w/v) in 2-methylpropan-2-ol–water (4:1, v/v), (*b*) *iso*butyric acid saturated with water at room temperature or (*c*) propan-1-ol–formic acid–water (6:3:1, v/v); (*a*) was also used for the separation of sugar phosphates. Reducing sugars were located by spraying the dried paper with either a 1% (w/v) solution of 3:4-dinitrobenzoic acid or 2:3:5-triphenyltetrazolium chloride in 2 N-Na_2CO_3 or, for chromatograms developed with the picric acid solvent, ethanolic 0·5 N-NaOH, followed by heating at 100° for 3–4 min. Sugar phosphates were made visible with the ammonium molybdate reagent of Hanes & Isherwood (1949); the orcinol reagent of Klevstrand & Nordal (1950) was used for the specific detection of ketoses and an ethanolic solution of bromocresol green for non-volatile acids.

Absorption spectra. The wavelengths of the absorption bands of the cytochromes (reduced with sodium dithionite) of suspensions of the organism were measured with a Hartridge reversion spectroscope (R. and J. Beck Ltd., London W. 1), both at room temperature and after immersion of the suspensions in liquid air.

Manometry. The conventional Warburg technique was used. Each vessel contained 1 ml. of a suspension of organisms in 0·05 M-buffer (20 mg. dry wt./ml. in phosphate, pH 6, except where otherwise stated), buffer to a final concentration of 0·05 M and substrate (10–20 μmoles for measurement of the extent of the oxidation; 100 μmoles for rate determinations) was added from the side bulb after equilibration; the final volume was 3 ml. When measuring O_2 consumption the centre well of each flask contained 20% (w/v) NaOH (0·2 ml.) and a pleated filter paper; CO_2 evolution (corrected for retention by the buffer) was determined by the 'direct' method. Incubation was in air at 30°. The results of the measurements of the extent of oxidation of various substrates are expressed as moles of O_2 consumed or moles of CO_2 evolved/mole of substrate, blank values, which were very low (0·5–1·0 μl. of O_2/mg. dry wt. of cells/hr.), having been deducted.

Chemicals

Calcium 5-oxogluconate was obtained in good yield by growth of *A. suboxydans* in a yeast-extract medium containing glucose and chalk (Kulka & Walker, 1954) and removed by filtration. The crude salt was purified by dissolving in the minimum volume of 3 N-HCl at 50°, the solution filtered and the filtrate adjusted to pH 6·9 with aq. 3 N-NH_3 soln.; the precipitated calcium salt was removed by filtration and twice recrystallized from hot water to give pure $(C_6H_9O_7)_2Ca,2\frac{1}{2}H_2O$. A solution of 0·05 M-sodium 5-oxogluconate was prepared in the following

Table 1. *Chromatography of oxogluconic acids on paper*

Figures given are R_G values, i.e. ratio of the distance moved by the compound to the distance moved by glucose.

	Developing solvent		
Compound	Picric acid (4%) in 2-methylpropan-2-ol–water (4:1)	*iso*Butyric acid satd. with water	Propan-1-ol–formic acid–water (6:3:1)
2-Oxogluconic acid	1·13	0·86	1·06
5-Oxogluconic acid	1·49	1·04	1·30

manner: calcium salt (117 mg.) was dissolved in 0·2N-HCl (5 ml.) and 0·25M-oxalic acid (1 ml.) added; the solution was adjusted to pH 6·5 with 0·4N-NaOH, centrifuged, the precipitate washed with a little water, the washings added to the supernatant and the volume made up to 10 ml. with water. Solutions of sodium 2-oxogluconate were prepared from calcium 2-oxogluconate, $(C_6H_9O_7)_2Ca,3\frac{1}{2}H_2O$, in a similar manner; these solutions were stored at −20°. Solutions of D-glucosone were prepared by acid hydrolysis of 1:2-2:3-5:6-tri-*O*-*iso*propylidene-(2-hydroxy-D-*arabino*-hexose) (Bayne & Fewster, 1956). 3:6-Anhydro-D-glucose (m.p. 120°, uncorrected) was synthesized by the method of Ohle, Vargha & Erlbach (1928). αα-Trehalose was isolated from yeast. Grateful acknowledgement is made of the following gifts: 6-*O*-methyl-D-glucose (Dr D. J. Bell), 2-deoxy-D-glucose (Dr S. Bayne), D-glucosamine hydrochloride (2-amino-2-deoxy-D-glucose hydrochloride), *N*-acetyl-D-glucosamine and D-glucosaminic acid (Dr P. W. Kent), α- and β-methyl-D-glucopyranoside (Dr N. W. Taylor), sodium glyoxylate (Dr H. L. Kornberg) and methyl phenazonium methosulphate (Professor F. Dickens, F.R.S.).

Other compounds were commercial products; the sodium salts of carboxylic acids and sugar phosphates were used. Furfuraldehyde was purified by redistillation under reduced pressure; acetaldehyde solutions were standardized by titration with bisulphite. The sodium salt of 2:4-dinitrophenol was used for studies of inhibition.

RESULTS

Growth of the organism on various energy sources

Table 2 shows the results of growth in liquid semi-defined medium at two levels of buffering capacity (medium B_1, 0·03M-phosphate, and B_2, 0·1M-phosphate, both at pH 6) and in liquid complex medium *C*, each containing various carbohydrates as energy sources at two concentrations (0·5 and

Table 2. *Effectiveness of various carbohydrates as energy sources for growth in liquid media*

Incubated in rocked T-tubes at 30°; all media pH 6. Tr., Indicates 'trace' growth.

Energy source	Final concn. (%, w/v)	Medium	Growth (mg. dry wt. of organisms/100 ml.) 24 hr.	48 hr.	72 hr.	pH after incubation for 72 hr.
(*a*) D-Glucose	0·5	B_1	7	10	12	4·0
		B_2	12	35	40	5·8
		C	15	20	25	3·5
	2·5	B_1	5	10	10	3·0
		B_2	5	10	15	3·0
		C	35	40	45	3·0
(*b*) Sodium D-gluconate	0·5	*C*	2	7	20	7·0
	2·5	*C*	Tr.	Tr.	5	6·7
(*c*) D-Mannitol	0·5	B_1	Tr.	50	55	5·1
		B_2	Tr.	65	70	6·0
		C	40	55	55	4·9
	2·5	B_1	Tr.	80	95	4·7
		B_2	5	85	105	5·0
		C	80	100	105	4·8
(*d*) D-Fructose	0·5	B_1	Tr.	20	65	5·0
		B_2	Tr.	25	70	6·0
		C	Tr.	20	60	5·0
	2·5	B_1	Tr.	15	70	4·7
		B_2	Tr.	10	70	5·1
		C	Tr.	30	60	4·8
(*e*) D-Sorbitol	0·5	B_1	7	50	60	5·3
		B_2	7	65	70	5·9
		C	25	50	55	5·0
	2·5	B_1	60	90	100	5·0
		B_2	55	105	105	5·3
		C	75	95	100	5·1
(*f*) L-Sorbose	2·5	*C*	Tr.	7	12	5·4
(*g*) Glycerol	0·5	B_1	5	40	65	5·1
		B_2	5	45	70	5·9
		C	35	45	50	5·3
	2·5	B_1	10	85	85	5·1
		B_2	15	90	90	5·5
		C	60	85	95	4·8

2·5%, w/v, final concentration). In the absence of added carbohydrate no growth was obtained in the semi-defined medium and 'trace' growth in the complex medium.

Except in medium B_2 with low sugar concentrations, growth on glucose in semi-defined medium was rapidly inhibited, as compared with that on glucose in the complex medium, by the fall in pH due to gluconic acid produced overcoming the buffering capacity of the medium. This acid arises not only as the product of the utilization of glucose during growth but also as the product of the oxidation of glucose by non-proliferating organisms, a reaction which occurs at a pH as low as 3 (see later), whereas the optimum pH for growth is 5–6. Growth on the complex medium was more rapid than on the defined medium; although after incubation for 72 hr. the pH of the medium had also fallen to 3, the overall growth was much greater. In Fig. 1 the progress of growth after a large inoculum in the liquid complex medium containing glucose is plotted together with the fall in pH, illustrating the inhibition of growth by acid production. Maximal growth with glucose occurred in media containing chalk as a neutralizing agent (Fig. 1). The results obtained with this modification in semi-defined medium B_1 and complex medium *C*, given in Table 3, were very similar and should be compared with the widely different results obtained for the same two media in the absence of chalk, also recorded in Table 3; the amount of chalk used per tube was such as to be completely consumed after incubation for 72 hr., which was also the time for maximum growth.

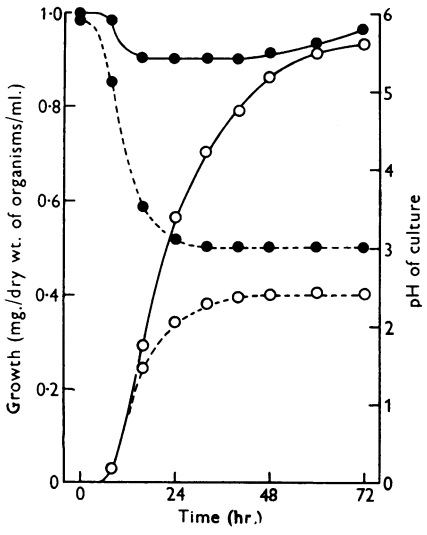

Fig. 1. Growth in liquid complex medium *C* containing glucose (2·5%, w/v)±chalk (0·6%, w/v); growth (○—○) and pH (●—●) of culture in the presence of chalk; growth (○---○) and pH (●---●) of culture in the absence of chalk. Excess of chalk and precipitated calcium salts were dissolved by the addition of HCl before measuring growth in the first experiment.

The effect of 2:4-dinitrophenol on growth with glucose was tested (Table 3); at a concentration of mM growth was inhibited by up to 90% and at this same concentration the oxidation of glucose by suspensions of the washed organisms was inhibited by 74% and 2- and 5-oxogluconic acids accumulated (see later, and Fewster, 1956). Considerable inhibition (65%) of growth also occurred in the presence of 0·1 mM-dinitrophenol.

Sodium gluconate did not serve as an energy source in the semi-defined media, and poor growth was obtained in the complex medium when a rise in the pH of the medium was observed (see Table 2); the better growth at the lower concentration of sodium gluconate was inhibited by a concentration of Na^+ ions (provided by sodium chloride) equivalent to that present in the medium at the higher concentration of sodium gluconate. Fair growth was obtained with calcium gluconate (Table 3); calcium 5-oxogluconate was precipitated and was dissolved by addition of N-HCl before measurement of the extent of growth. The pH of the culture did not rise above 6·5.

Best growth of the organism was obtained on the polyols D-mannitol and D-sorbitol and was similar for all types of medium in extent, although the rate of growth on mannitol in the semi-defined media was slower than that on sorbitol; at the higher concentration good growth was also obtained on glycerol (Table 2). In all liquid media slow but good growth was obtained with D-fructose, the first product of mannitol metabolism; in contrast, growth on L-sorbose, which accumulated in good yield during growth on sorbitol, resulted only at the higher concentration and in complex medium. In neither semi-defined nor complex media was growth on mannitol significantly inhibited by 0·1 mM-dinitrophenol; a 50% inhibition occurred with 0·2 mM-dinitrophenol (Table 3).

Growth on fructose was 60% inhibited by 0·1 mM-dinitrophenol (i.e. very similar to the inhibition in a medium containing glucose; Table 3).

The figures for growth on solidified media containing various energy sources are given in Table 4. Again, good growth was obtained on the polyols and on glucose in the presence of chalk and was maximum after 72 hr. The reduction of growth on glucose in the absence of the neutralizing agent was again shown and was particularly apparent on the semi-defined medium B_1. Only on the complex medium *C* was growth on sodium gluconate obtained; with the calcium salt very variable growth was obtained on the two media. Under the conditions of this type of experiment (where a

Table 3. *Bulk growth in liquid media*

Basal medium was diluted 1:1; chalk, where included (+), 0·6% (w/v).

Energy source	Final concn. (%, w/v)	Basal medium	Chalk	DNP (final concn., mM)	Growth (mg. dry wt. of organisms/ 100 ml. in 72 hr.)	Inhibition (%)
(*a*) D-Glucose	2·5	B_1	+	.	80	.
		B_1	−	.	15	.
		B_1	−	0·1	5	65
		C	+	.	95	.
		C	+	0·1	33	65
		C	+	1·0	14	85
		C	−	.	40	.
		C	−	0·1	15	63
		C	−	1·0	4	90
(*b*) Sodium D-gluconate	0·5	B_1	−	.	0	.
		C	−	.	20	.
	2·5	B_1	−	.	0	.
		C	−	.	8	.
(*c*) Calcium D-gluconate	2·5	B_1	−	.	32	.
		C	−	.	36	.
(*d*) D-Mannitol	2·5	B_1	−	.	110	.
		B_1	−	0·1	95	14
		B_1	−	0·2	50	55
		C	−	.	120	.
		C	−	0·1	120	0
		C	−	0·2	60	50
(*e*) D-Fructose	2·5	B_1	−	.	65	.
		B_1	−	0·1	29	55
		C	−	.	55	.
		C	−	0·1	22	60

Table 4. *Bulk growth on solid media*

Energy source (5%, w/v)	Basal medium	Chalk (2%, w/v)	Growth (mg. dry wt. of organisms/ 100 ml. of medium in 72 hr.)
(*a*) D-Glucose	B_1	+	125
	B_1	−	15
	C	+	175
	C	−	50
(*b*) Sodium D-gluconate	B_1	−	0
	C	−	20–40
(*c*) Calcium D-gluconate	B_1	−	40–75
	C	−	50–100
(*d*) D-Mannitol	B_1	−	190
	C	−	240
(*e*) D-Fructose	B_1	−	40
	C	−	55
(*f*) D-Sorbitol	B_1	−	200
	C	−	215
(*g*) L-Sorbose	B_1	−	5
	C	−	30
(*h*) Glycerol	B_1	−	115
	C	−	140
(*i*) D-Galactose	C	+	20
(*j*) D-Mannose	C	+	10
(*k*) D-Xylose	C	+	20
(*l*) L-Arabinose	C	+	10
(*m*) Sucrose	C	+	5

large inoculum was used) some growth was obtained on sorbose as well as on fructose although growth on the latter was poor compared with that resulting in liquid media. Very poor growth occurred on D-galactose, D-xylose and L-arabinose; D-mannose and sucrose did not support growth. Growth on solidified media was the principal method used for the production of organisms for the testing of their oxidative capabilities in washed suspension.

Oxidation of glucose and gluconic acids by washed organisms

The results presented in this section were obtained with organisms harvested after growth for 72 hr. on solidified complex medium *C* containing glucose and chalk, and suspended in phosphate buffer, pH 6, at 30°. Glucose was extensively oxidized with an uptake of 3·75 moles of O_2 (see Fig. 2) and an output of up to 4·0 moles of CO_2/mole of glucose; these figures were constant over a wide range (1–20 μmoles of glucose/20 mg. dry wt. of cells) of substrate concentration and represent approx. 60% complete oxidation of the molecule.

Gluconic acid, either in the form of glucono-δ-lactone or the calcium or sodium salt, was oxidized at a rate and to an extent with regard to both O_2 consumption and CO_2 evolution consistent with it being the primary product of the oxidation of glucose (Fig. 2). Chromatographic examination on paper of the contents of manometer vessels 5 min. after the addition of glucose to the suspension of organisms showed the presence of 2- and 5-oxogluconic acid as well as gluconic acid, i.e. the same products as accumulate during growth of the organism on a medium containing glucose; the formation of sugar phosphates could not be demonstrated. Under the conditions of the experiments with washed organisms, namely, low concentration of substrate, high concentration of organisms and vigorous aeration, the keto acids were rapidly further metabolized. Fresh solutions of sodium 5-oxogluconate were not oxidized by suspensions of well-washed organisms. With solutions that had been stored for several days at room temperature, or suspensions of organisms contaminated with traces of oxidizable material from the growth medium, figures for O_2 uptake of up to 2 moles and for CO_2 output of 2 moles/mole of keto acid were recorded; the latter type of oxidation was abolished by incubation of the suspension for 1 hr. at 30° in phosphate buffer, pH 6, before use. Oxidation of fresh solutions of 5-oxogluconate was not initiated by addition of di- or tri-phosphopyridine nucleotide (DPN or TPN) even at substrate concentration, adenosine triphosphate (ATP) and Mg^{2+} ions, or methyl phenazonium methosulphate or by previous incubation of the suspension with small amounts of readily oxidized substrates. However, either simultaneous addition of the keto acid and a readily oxidized substrate (such as glucose, gluconate, mannitol, fructose or pyruvate), or addition of the 5-oxogluconate during the oxidation of the other substrate, resulted in steady oxidation of the acid (Fig. 2 and Table 5); thus in 4 hr. and in the presence of 5 μmoles of glucose an O_2 uptake of 3·0 moles and a CO_2 output of 3·5 moles/mole of 5-oxogluconate (10 μmoles) was observed, O_2 consumption ceasing at a figure of 3·5 moles/mole after 5–6 hr. (corresponding values for the added glucose are subtracted). Relatively small amounts of added substrate were required to effect the oxidation of a given amount of the acid; however, a minimal requirement in terms of absolute uptake of O_2 by the added substrate for maximal oxidation of 10 μmoles of the keto acid could be measured as shown in Table 5, in which the effectiveness of glucose (oxidized to the extent of 3·75 moles of O_2/mole) is compared with that of pyruvate (0·5 mole/mole).

Sodium 2-oxogluconate was oxidized to the extent of 0·5 mole of O_2/mole (Fig. 2), with an accompanying evolution of 0·5 mole of CO_2. This degree of oxidation was not increased by addition

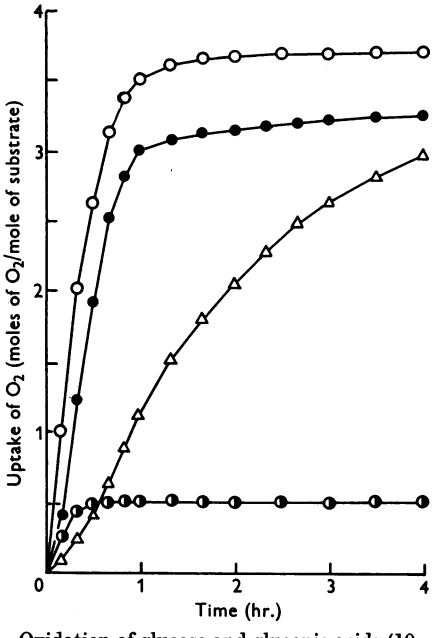

Fig. 2. Oxidation of glucose and gluconic acids (10 μmoles) by washed organisms (20 mg. dry wt. of cells) harvested after growth for 72 hr. on solidified complex medium *C* containing glucose and chalk; ○, glucose; ●, sodium gluconate; ◑, sodium 2-oxogluconate; △, sodium 5-oxogluconate plus glucose (5 μmoles), value for glucose subtracted.

of DPN, TPN, ATP and Mg^{2+} ions, or readily oxidized substrates. The non-reducing acidic product of this oxidative decarboxylation (the intermediate formation of D-arabinose could not be demonstrated) was identified chromatographically as D-arabonic acid. No evidence was obtained for the formation of 2:5-dioxogluconate shown by Katznelson, Tanenbaum & Tatum (1953) to be the product of the oxidation of 2-oxogluconate by 'aged' preparations of *Acetobacter melanogenum*.

Table 5. *Oxidation of 5-oxogluconate in the presence of added substrates*

5-Oxogluconate (10 μmoles) was added to a suspension in 0·05 M-phosphate buffer, pH 6, of washed organisms (20 mg. dry wt.) harvested after growth for 72 hr. on solidified complex medium *C* containing glucose and chalk; supplementary substrates were added simultaneously; 20% (w/v) NaOH (0·2 ml.) and pleated filter paper were placed in each centre well; total volume 3 ml.; incubated in air at 30° for 4 hr.

Added substrate (μmoles)		Uptake of O_2 by added substrate (μl.)	Uptake of O_2 by 5-oxogluconate (μl.)
None		—	0–20
D-Glucose,	5	415	670
	2	165	650
	1	83	630
	0·5	42	630
	0·1	8	170–425
Pyruvate,	10	110	630
	5	58	615
	1	10	10

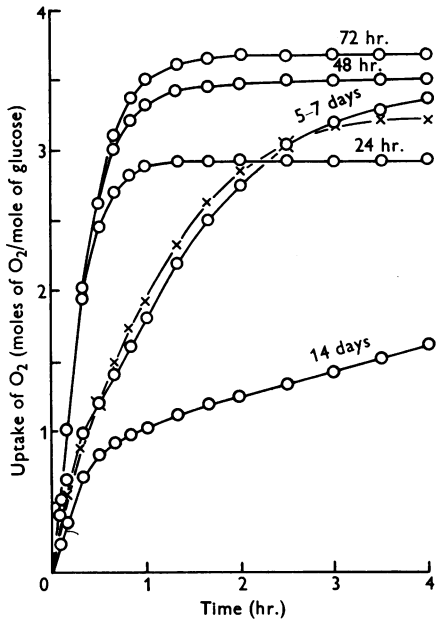

Fig. 3. Oxidation of glucose by washed organisms harvested from solidified complex medium *C* containing glucose and chalk after various incubation times; ○, incubation times as indicated; ×, oxidation of glucose by organisms harvested after incubation for 3 days followed by storage at 0–4° in sterile saline for 48 hr.

Factors affecting the oxidation of glucose

Age of organisms and pH *of growth medium.* Maximum oxidation of glucose was obtained with organisms harvested after 72 hr. from solidified complex medium *C* containing glucose and chalk; for this medium such an incubation time also gave the maximum yield of organisms (Table 4). Organisms harvested after 48 hr. were slightly less active, and very young cells (24 hr.) attacked glucose at a similar rate but to a more limited extent (Fig. 3). Periods of incubation longer than 72 hr. caused a decrease in rate (5–7 days) and later (14 days) both rate and extent of oxidation (Fig. 3). The ageing of organisms harvested after 72 hr. by storage in sterile saline for 48 hr. at 0–4° (Fig. 3) or 24 hr. in phosphate buffer, pH 6, at 30° (Fig. 5), led to a small loss of activity; rigorous washing of the organisms (dispersion in water followed by centrifuging, repeated six times) or pre-incubation in phosphate buffer, pH 6, for 2 hr. at 30° caused a slight decrease in the rate but not in the extent of oxidation.

The absence of chalk from the growth medium caused a rapid fall in pH to 3 after 72 hr. (as compared with a figure of 5·5 when the neutralizing agent was present). Under these conditions there was a progressive loss of oxidizing power of the organisms towards glucose (Fig. 4). Thus organisms harvested after incubation for 24 hr. oxidized glucose at a rate and to an extent similar to those measured for organisms grown for the same time period on a medium containing chalk; after incubation for 48 hr. the development of acidity resulted in a very considerable decrease in activity. A 72 hr. culture oxidized glucose to gluconic acid quantitatively with an uptake of 0·5 mole of O_2/mole and no CO_2 evolution; gluconic acid was not attacked. The only other substrate of the many tested that was oxidized at a comparable rate (95%) was 2-deoxy-D-glucose, to give what was tentatively identified as 2-deoxy-D-gluconic acid (Fig. 4). D-Mannose, D-xylose and L-arabinose were oxidized at 1–2% of the oxidation rate of glucose and after 4 hr. the O_2 consumed was approx. 0·05 mole of O_2/mole; all other compounds listed in Table 8 as well as D-mannitol, D-fructose, D-sorbitol, L-sorbose, D-arabitol, ribitol, glycerol and ethanol were inactive as substrates. Similar losses in oxidizing activity occurred when organisms harvested from medium containing glucose and chalk were incubated in phthalate buffer, pH 3, at

30° (Fig. 5). These results confirm the work of Butlin (1936, 1938*a*, *b*), who first demonstrated the limited oxidizing power of *A. suboxydans* towards glucose when grown under conditions where production of acid was not controlled or when normal organisms (which in Butlin's work were much less active than those that have been described in the present study) were treated with acid. In agreement with Butlin it was noted that fully active organisms were pink in colour, whereas those grown in the absence of chalk were 'dirty white'. The active pink organisms, harvested after growth for 24, 48, or 72 hr., were rich in a cytochrome component having an α-band at 552 mμ and a β-band at 524 mμ when measured after immersion in liquid air. This 'single' cytochrome was described by Smith (1954*a*, *b*) as being characteristic of *A. suboxydans* but has recently been shown to contain at least three components (Keilin & Smith, 1957). With regard to cytochrome content and appearance as well as oxidative activity, organisms harvested after growth for 24 hr. on the medium containing glucose and no chalk were indistinguishable from those grown in the presence of chalk; after growth for 48 hr. in the absence of the neutralizing agent there was a large decrease in content of the 'characteristic' cytochrome, whereas after incubation for 72 hr. the organisms contained as the only cytochrome-like constituent a small amount of material exhibiting absorption bands at 559 and 530 mμ, and were themselves very pale cream and shrunken in appearance. Thus the decrease in oxidizing capacity towards glucose with increase in incubation time in the absence of chalk, i.e. increase in acidity of the growth medium, was paralleled by either a loss or a non-production of cytochrome. These results are in contrast with those obtained when organisms grown on glucose in the presence of chalk were incubated at pH 3; under these conditions, although there was a loss of oxidizing activity (Fig. 5) and bleaching of the organisms, the presence of the characteristic cytochrome after reduction with dithionite was demonstrated in apparently undiminished amount and shown to be oxidized by O_2; a reduced cytochrome band at 559 mμ was not detected.

The activity towards glucose of organisms grown on glucose in the absence of chalk for 72 hr. could not be increased in either rate or extent by adding DPN, TPN, coenzyme A, cytochrome *c*, ATP and Mg^{2+} ions, methyl phenazonium methosulphate or

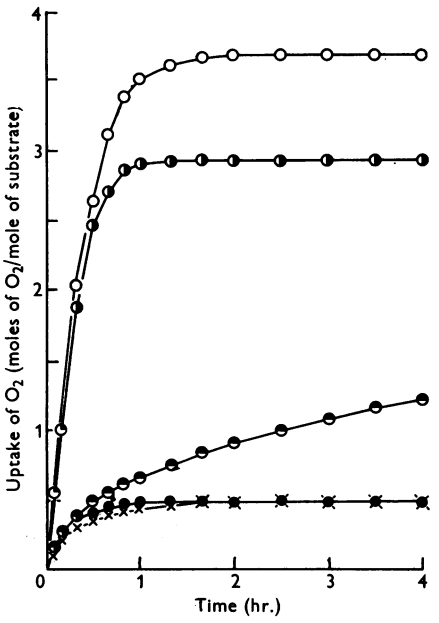

Fig. 4. Oxidation of substrates by washed organisms harvested from solidified complex medium *C* containing glucose but no chalk after various incubation times; ◑, glucose, 24 hr.; ◓, glucose, 48 hr.; ●, glucose, 72 hr.; ×, 2-deoxy-glucose, 72 hr.; ○, maximum oxidation of glucose, i.e. by organisms harvested after growth for 72 hr. on medium *C* containing glucose and chalk.

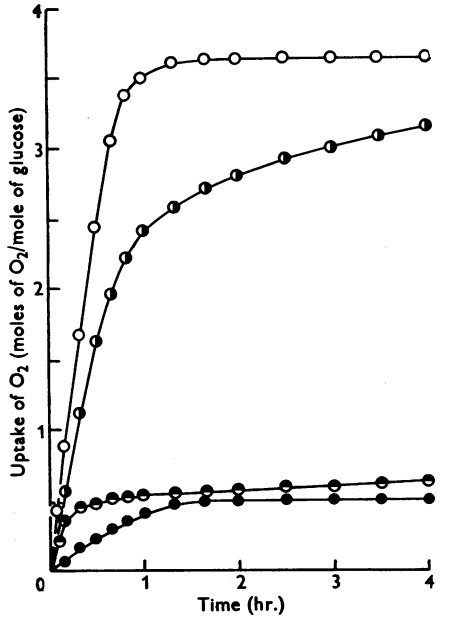

Fig. 5. Effect on the oxidation of glucose of pre-incubating organisms harvested after growth for 72 hr. on solidified complex medium *C* containing glucose and chalk; after pre-incubation the organisms were washed and resuspended in 0·05 M-phosphate buffer, pH 6; ○, control, no pre-incubation; ◑, after pre-incubation in phosphate buffer, pH 6, for 24 hr. at 30°; ◓, after pre-incubation in phthalate buffer, pH 3, for 3 hr. at 30°; ●, after pre-incubation at pH 3 for 24 hr. at 30°.

a hot-water extract of organisms harvested from a medium containing glucose and chalk.

With the reagent of Hestrin (1949) it was shown that the immediate product of the oxidation of glucose by the cytochrome-deficient organisms formed an acylhydroxamic acid; with bicarbonate buffer and a gas phase of 100% CO_2 the presence in suspensions of the organisms of an enzyme which slowly delactonized D-glucono-δ-lactone was demonstrated manometrically. Such an enzyme was also present in much higher concentration in organisms grown in the presence of chalk. Both types of organism were also shown to be rich in catalase, but with those grown in the absence of chalk no evidence was obtained for the formation of hydrogen peroxide when glucose was oxidized to gluconic acid. Thus the O_2 uptake of 0·5 mole/mole of glucose was not increased in extent by either mM-KCN or 20 mM-hydroxylamine, both potent inhibitors of catalase; in fact the rate of oxidation was 65 and 95% inhibited by 1 and 5 mM-KCN respectively. In addition, during the oxidation of glucose no coupled oxidation of ethanol could be demonstrated, a reaction shown to occur with notatin in the presence of catalase by Keilin & Hartree (1948*b*). Phenolindo-2:6-dichlorophenol served as a hydrogen acceptor from glucose under anaerobic conditions (demonstrated by the Thunberg technique), whereas methylene blue and 2:3:5-triphenyltetrazolium chloride were inactive.

pH *of suspension medium.* The optimum pH for the extensive oxidation of glucose by organisms harvested from a medium containing glucose and chalk was 6·0–6·5; above and below this range the extent of O_2 consumption rapidly decreased, although the oxidation of glucose to gluconic acid occurred at such extremes of pH as 3 and 9.

Growth medium with particular reference to energy source. The oxidation of glucose by washed organisms harvested after growth for 72 hr. on the same solidified complex medium *C* but containing D-galactose plus chalk, D-mannitol, D-sorbitol, D-fructose or L-sorbose instead of glucose plus chalk was similar, in both extent and rate of O_2 uptake. However, yields of organisms obtained for the various sugars differed considerably (Table 4). Organisms grown on solidified medium *C* containing sodium gluconate, or L-arabinose and chalk, oxidized glucose at a similar rate but to a decreased extent. The use of glycerol as an energy source in the same basal medium produced organisms which oxidized glucose to a greatly decreased extent (Fig. 6); gluconate was not oxidized in a manner compatible with it being the sole primary product of glucose utilization, and the extent of oxidation of the keto acids was much decreased compared with that by organisms grown in the presence of glucose (Table 6). A significant decrease in the extent of oxidation of glucose also occurred when organisms were grown on solidified semi-defined medium B_1 containing glucose and chalk (Fig. 6) or sorbitol as energy source, but this was not observed for mannitol or fructose; no growth was obtained on gluconate in this medium, and growth on sorbose was very poor (see Table 4). Maximum activity was obtained with organisms harvested from liquid complex medium *C* containing either glucose and chalk or mannitol; a decrease in activity resulted

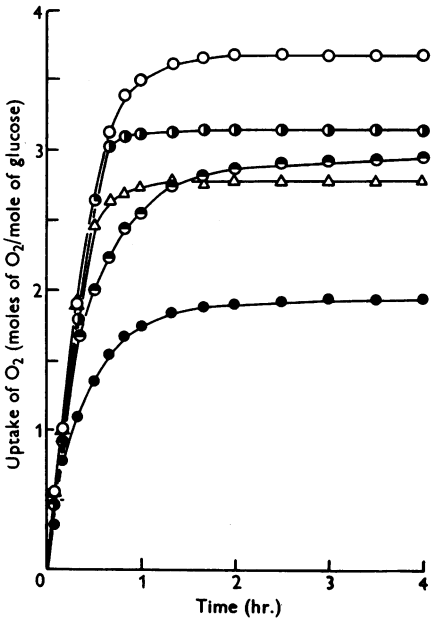

Fig. 6. Oxidation of glucose by washed organisms harvested after growth for 72 hr. on solidified media containing various energy sources; ○, organisms harvested from complex medium *C* containing glucose and chalk; ◓, from semi-defined medium B_1 containing glucose and chalk; ◑, from medium *C* containing sodium gluconate; ●, from medium *C* containing glycerol; △, from medium *C* containing L-arabinose and chalk.

Table 6. *Oxidative activity of organisms grown in the presence of glycerol*

Substrates (10 μmoles) were added to washed organisms (20 mg. dry wt.) harvested after growth for 72 hr. on solidified complex medium *C* containing glycerol (5%, w/v) and suspended in 0·05 M-phosphate buffer, pH 6; incubated in air at 30° for 4 hr.

Substrate	Uptake of O_2 (mole of O_2/mole)	Output of CO_2 (mole of CO_2/mole)
Glucose	1·95	1·35
Gluconate	0·85	0·2
2-Oxogluconate	0·06	—
5-Oxogluconate	0·02	—
5-Oxogluconate + glucose (2 μmoles)	0·63	—

Table 7. *Effect of various energy sources for growth on the extent of oxidation of glucose by the harvested organisms*

Energy source was at 5% (w/v) and chalk, where included (+), at 2% (w/v) in solidified media; energy source was at 2·5% (w/v) and chalk, where included, at 0·6% (w/v) in liquid media; organisms were harvested after incubation for 72 hr.; glucose (10 μmoles) was added to washed organisms (20 mg. dry wt.) in 0·05 M-phosphate buffer, pH 6, at 30°; figures in parentheses are times (hr.) for completion of O_2 uptake.

		Uptake of O_2 (μmoles/mole of glucose)			
Basal growth medium		*C*	B_1	*C*	B_1
Energy source	Chalk	(solidified)	(solidified)	(liquid)	(liquid)
D-Glucose	−	0·5 (1)	0·5 (1)	0·5 (1)	0·5 (1)
D-Glucose	+	3·75 (1·5)	3·0 (3)	3·5 (1·5)	3·0 (3)
Sodium D-gluconate	−	3·1 (1·5)	.	.	.
D-Galactose	+	3·7 (1·5)	.	.	.
L-Arabinose	+	2·8 (1·5)	.	.	.
D-Mannitol	−	3·55 (1·5)	3·6 (1·5)	3·6 (1·5)	3·1 (2)
D-Fructose	−	3·75 (1·5)	3·7 (1·5)	.	.
D-Sorbitol	−	3·7 (1·5)	3·1 (2)	.	.
L-Sorbose	−	3·75 (1·5)	.	.	.
Glycerol	−	1·95 (2·5)	.	.	.

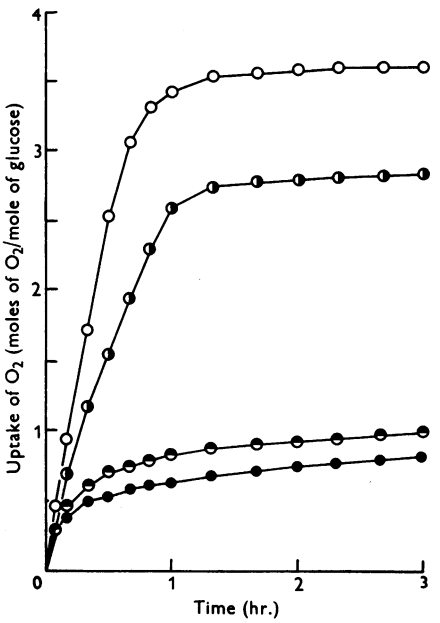

Fig. 7. Inhibition by dinitrophenol (DNP) of the oxidation of glucose at pH 6 by washed organisms harvested after growth for 72 hr. on solidified complex medium *C* containing glucose and chalk; ○, ±0·01 mM-DNP; ◑, +0·1 mM-DNP; ◓, +mM-DNP; ●, +10 mM-DNP.

with these two energy sources in liquid semi-defined medium B_1. Organisms grown in liquid complex medium *C* containing mannitol and 0·2 mM-dinitrophenol (the latter reagent caused 50% inhibition of growth—see Table 3) possessed normal activity for the oxidation of glucose. In no case was the activity towards glucose reduced by pre-incubation of the organisms in phosphate buffer, pH 6, for 1 hr. at 30°. These results are summarized in Table 7.

Chemical inhibitors. Organisms harvested after incubation for 72 hr. from solidified complex medium *C* containing glucose and chalk, and suspended in phosphate buffer, pH 6, were used; inhibitors were added to the suspension of organisms. Oxidation of glucose was 80% inhibited and that of 2-oxogluconate and 5-oxogluconate oxidation abolished by mM-KCN and 50 mM-KF; mM-arsenite caused up to 20% inhibition of the extent of glucose oxidation. The effect of various concentrations of dinitrophenol, an uncoupler of oxidative phosphorylation, is illustrated in Fig. 7; mM-dinitrophenol caused the accumulation of 2- and 5-oxogluconic acids (identified chromatographically), in approximately equal proportions, as the only detectable products of the oxidation of glucose or gluconate. A similar inhibition (73%) and accumulation was given by 0·2 mM-phenylmercury acetate, a general inhibitor of enzymes depending for their activity on the presence of free sulphydryl groups. With 0·1 mM-dinitrophenol, which inhibited growth in media containing glucose by 65%, the extent of glucose oxidation was inhibited by up to 20% (Fig. 7).

Oxidation of derivatives of D-*glucose and other aldoses*

Slight modification in the glucose molecule led to a great reduction in the degree of oxidation by organisms harvested from solified complex medium *C* containing glucose and chalk. Thus with 2-deoxy-D-glucose and 6-*O*-methyl-D-glucose O_2 was taken up rapidly to a level corresponding to the formation of aldonic acids; thereafter the uptake was very slow and ceased at a value approaching

Table 8. *Oxidation of various aldoses, disaccharides, aldehydes and carboxylic acids*

Substrates (10–20 μmoles) were added to washed organisms (20 mg. dry wt.), harvested after growth for 72 hr. on solidified complex medium *C* containing glucose and chalk, suspended in 0·05 M-phosphate buffer, pH 6; incubated in air at 30°. The following were not oxidized to a significant extent: α-methyl-D-glucopyranoside, *N*-acetyl-D-glucosamine, D-glucurone, D-glucose 6-phosphate, L-rhamnose, D-arabonate, αα-trehalose, lactose, maltose, acetate, succinate, citrate, 2-oxoglutarate, oxalate, D-tartrate, L-tartrate, *meso*tartrate, dihydroxytartrate, tartronate and glyoxylate.

Substrate	Uptake of O_2 (moles of O_2/mole of substrate) 1 hr.	4 hr.
D-Glucose	3·5	3·75
D-Gluconate	3·05	3·2
2-Oxo-D-gluconate	0·5	0·5
5-Oxo-D-gluconate (+D-glucose)	1·15	3·0
2-Deoxy-D-glucose	0·65	1·02
3-*O*-Methyl-D-glucose	0·33	0·69
6-*O*-Methyl-D-glucose	0·64 (0·85)*	0·75 (1·00)
3:6-Anhydro-D-glucose	0·03	0·15
β-Methyl-D-glucopyranoside	0·14	0·35
D-Glucosamine	0·18	0·61
D-Glucosaminate	0·06	0·18
D-Glucosone	0·41	0·83
D-Mannose	0·35	0·95
D-Galactose	0·45	0·51
D-Arabinose	0·18	0·47
L-Arabinose	0·51	0·52
D-Xylose	0·48	0·50
L-Xylose	0·11	0·25
D-Ribose	0·10 (0·55)	0·49 (1·00)
D-Lyxose	0·42	0·61
Sucrose	0·38	1·75
Acetaldehyde	0·48	0·49
Glycollaldehyde	0·48	0·51
Furfuraldehyde	0·51	0·52
Lactate	0·56	1·00
Pyruvate	0·49	0·51
Glycollate	0·05	0·09

* Figures in parentheses were obtained with organisms harvested from solidified medium *C* containing mannitol.

Table 9. *Rates of oxidation of aldoses and of gluconate*

Substrates (100 μmoles) were added to washed organisms (1·5 mg. dry wt.) harvested after growth for 72 hr. on solidified complex medium *C* containing glucose and chalk, suspended in 0·05 M-phosphate buffer, pH 6; incubated in air at 30° for 1 hr.; Q_{O_2} values (μl. of O_2 taken up/mg. dry wt. of organisms/hr.) were calculated from O_2 uptake measured during period 10–40 min. after addition of each substrate.

Substrate	Q_{O_2}
D-Glucose	750
Sodium D-gluconate	80
D-Galactose	65
D-Mannose	50
L-Arabinose	70
D-Xylose	160

1 mole of O_2/mole. D-Glucosamine and D-glucosaminic acid were slowly oxidized; *N*-acetyl-D-glucosamine was not attacked. β-Methyl-D-glucopyranoside, but not the α-isomer, was oxidized slowly and steadily. 3-*O*-Methyl-D-glucose and D-glucosone were slowly oxidized with an uptake approaching 1 mole of O_2/mole in 4 hr. 3:6-Anhydro-D-glucose was metabolized very slowly, whereas D-glucurone and D-glucose 6-phosphate were inert. Similar results were obtained with organisms harvested from solidified complex medium *C* containing mannitol, except that the oxidation of 6-*O*-methyl-D-glucose was appreciably faster (see Table 8).

D-Mannose was oxidized slowly with an uptake of 1 mole of O_2/mole and D-galactose was oxidized to D-galactonic acid, a degree of oxidation not exceeded even by organisms harvested from solidified complex medium *C* containing D-galactose and chalk. Of the aldopentoses tested, L-arabinose, D-xylose and D-lyxose were oxidized rapidly, with the consumption of 0·5 mole of O_2/mole, whereas D-arabinose, L-xylose and D-ribose were slowly oxidized to the same extent; L-rhamnose (6-deoxy-L-mannose) was not oxidized. Similar results were obtained with organisms harvested from growth medium containing mannitol, except that D-ribose was steadily oxidized with an uptake of 1 mole of O_2/mole.

D-Glucose was the most rapidly as well as the most extensively oxidized of the aldoses tested; Q_{O_2} values of a number of aldoses and of gluconate, recorded in Table 9, were determined with a concentration of organisms equal to that present in a liquid culture at half-growth (0·5 mg. dry wt./ml.).

Sucrose was the only disaccharide of those tested to be oxidized; the steady evolution of CO_2 which accompanied consumption of O_2 suggested that the sucrose was slowly hydrolysed and the resulting glucose and fructose were oxidized normally.

Oxidation of aldehydes and carboxylic acids

Organisms were harvested from complex medium *C* containing glucose and chalk; the use of mannitol as an energy source for growth did not affect the activity towards these substrates. Acetaldehyde, glycollaldehyde and furfuraldehyde were oxidized with the uptake of 0·5 mole of O_2/mole and the production of one equivalent of acid. An uptake of 1 mole of O_2/mole of lactate accompanied by an output of 1 mole of CO_2 was observed; the corresponding figures for pyruvate were 0·5 mole of O_2 and 1·0 mole of CO_2. King & Cheldelin (1954) have demonstrated the presence of a yeast-type pyruvic carboxylase in cell-free extracts of *A. suboxydans*. In agreement with King & Cheldelin (1953) it was observed that the ultimate product of pyruvate oxidation, acetate, was not oxidized and nor were a number of intermediates of the tricarboxylic acid cycle, namely, succinate, citrate and 2-oxoglutarate. L-Tartrate, oxalate and glycollate, reported to be products of the oxidation of 5-oxogluconate by various *Acetobacter* species and *Pseudomonas fluorescens* (Jackson, Koepsell, Lockwood, Nelson & Stodola, 1949), were not oxidized by washed organisms; D-tartrate, *meso*tartrate, dihydroxytartrate, tartronate and glyoxylate were also not attacked.

These results are summarized in Table 8, together with those obtained for the various aldoses.

DISCUSSION

The growth experiments show clearly that a number of polyols were satisfactory as energy sources for growth of the organism, but of the aldoses tested only glucose, in the presence of an adequate neutralizing agent, allowed good growth. The primary products of oxidation by the organism of these substances also varied considerably in their ability themselves to support growth.

The poor growth on gluconate supports the view of Stubbs, Lockwood, Roe, Tabenkin & Ward (1940) that the energy for growth on glucose is derived mainly from the oxidation to gluconic acid, the further oxidation of this acid to a mixture of keto acids being carried out by non-proliferating organisms. Fair but slow growth was obtained with fructose, the primary product of the oxidation of mannitol. Dinitrophenol at a concentration of 0·1 mM inhibited growth on glucose and on fructose by 60%, suggesting that energy-transfer mechanisms common to growth on both these sugars are inhibited. The same concentration of reagent did not significantly diminish growth on mannitol. Similar lack of inhibition of growth on sorbitol and glycerol was reported by King & Cheldelin (1953), but their study of the effect of this inhibitor was, of necessity, incomplete since, like the present author, they found sorbose (and also dihydroxyacetone) to be valueless as a promoter of growth.

All the compounds that served as good energy sources for growth were extensively oxidized by washed organisms. However, the converse was not true; of the poor promoters of growth galactose, xylose and arabinose were not oxidized beyond the stage of the corresponding aldonic acids, but gluconate and sorbose were apparently extensively oxidized as intermediates in the oxidation of glucose and sorbitol respectively. The low rate of oxidation of gluconate compared with that of glucose by washed organisms may explain the inadequacy of this compound as a growth promoter; it also provides a reason for the accumulation of primary products during growth on glucose, the rate of oxidation of gluconate being the limiting factor in the rate of formation of 2- and 5-oxogluconate. The extent of oxidation of sorbose as such (2–3 moles of oxygen/mole) by washed organisms was more limited than that of fructose (up to 4·5 moles of oxygen/mole); was greatly decreased by previous starvation of the washed organisms (unlike that of fructose); and was very much more sensitive to dinitrophenol than was that of fructose (J. A. Fewster, unpublished work).

The so-called 'energy source' may be called upon to meet requirements not only for energy but also for part at least of the available carbon for biosynthetic purposes; it is likely therefore that (*a*) the compound must be readily oxidized and (*b*) either it or one of the primary products of its oxidation may be able to enter metabolic pathways leading to biosynthesis only in a phosphorylated form. The high yields of primary products accumulating during growth of the organism suggest that in (*a*) the rate of oxidation may be more important than the extent, that the oxidative reaction is the predominant one, and that the quantitative requirement for (*b*) is small. Glucose and fructose have been shown to be readily phosphorylated by ultrasonic extracts (Fewster, 1957*b*). No evidence has been obtained for the phosphorylation of mannitol, sorbitol or sorbose; however, the first is readily oxidized to fructose and there is indirect

evidence (Arcus & Edson, 1956; Cummins, King & Cheldelin, 1957; J. A. Fewster, unpublished work) that some fructose, presumably sufficient to satisfy the need for phosphorylated intermediates, is formed from sorbitol during growth. The inadequacy of sorbose as a growth promoter may be ascribed to non-phosphorylation or a low rate of oxidation or both; these same explanations also apply to galactose, xylose and arabinose. Mannose, although phosphorylated by extracts (Fewster, 1957*b*), was also oxidized very slowly by washed organisms; an alternative explanation is that the product of mannose phosphorylation is not further utilizable by the organism. The limited growth obtained in complex medium in the presence of these sugars was probably due to the 'sparking' action of traces of a utilizable carbon source in the yeast extract. Thus any carbon compound suitable for the growth of *A. suboxydans* (e.g. glucose, glycerol or sorbitol) has been shown to be capable at the 0·1% (w/v) level of 'sparking' growth in a medium containing ethanol, erythritol or inositol, themselves unable to support growth (Fulmer & Underkofler, 1947; Rao & Stokes, 1953).

Just as glucose was the only aldose to act as an adequate substrate for growth, so was it the only aldose to be extensively oxidized by washed organisms. The first stages of the oxidation of glucose to gluconate and thence to 2- and 5-oxogluconate are considered unlikely to involve phosphorylation of the substrates. Thus the formation of the keto acids by washed organisms was insensitive to high concentrations of fluoride and to a concentration of phenylmercury acetate which abolished the phosphorylation of glucose by the soluble fraction of ultrasonic extracts (Fewster, 1957*b*); the rates of aerobic oxidation of glucose and gluconate by a particulate fraction rich in cytochrome of such extracts were not dependent upon the addition of ATP and the two keto acids were the only products detected (Fewster, 1957*a*). Similar observations have been made concerning the oxidation of glucose and gluconate to 2-oxogluconate by *Pseudomonas aeruginosa* (Stokes & Campbell, 1951) and *Pseudomonas fluorescens* (Wood & Schwerdt, 1953).

D-Arabonate, formed from 2-oxogluconate by the action of washed organisms, is considered to be an end product; the existence of an alternative pathway for 2-oxogluconate breakdown is indicated by the demonstration of the presence of a 2-oxogluconokinase in ultrasonic extracts (Fewster, 1957*b*). In addition, evidence has been presented by Kovachevich & Wood (1955) for the operation of the Entner–Doudoroff pathway in *A. suboxydans*. There is a requirement for the presence of any one of a number of readily oxidized substrates for the initiation of the oxidation of 5-oxogluconate (with small amounts of added substrates their oxidation was complete long before that of 5-oxogluconate ceased). This suggests that energy is required for the uptake of the keto acid by the organisms. Hauge, King & Cheldelin (1955) showed that pre-incubation of washed suspensions of *A. suboxydans* with a readily oxidized carbohydrate resulted in the organisms acquiring the ability to oxidize dihydroxyacetone rapidly. Tosic (1946) was able to reduce the lag period of acetate oxidation by suspensions of *Acetobacter turbidans* by the addition of small amounts of a variety of compounds, and suggested that these compounds caused the activation of one of the enzymes essential for acetate oxidation. The problem of the pathway of oxidation of 5-oxogluconate is worthy of attention.

With the exception of glucose 6-phosphate, D-glucurone and L-rhamnose all aldoses tested were oxidized, at various rates, to an extent corresponding to the formation of aldonic acids; only 2-deoxy-, 3-*O*-methyl- and 6-*O*-methyl-D-glucose and D-mannose were further oxidized, with negligible evolution of carbon dioxide, to give reducing acids. The non-oxidation of glucose 6-phosphate may be explained in terms of impermeability, since it has been shown to be oxidized by ultrasonic extracts (Fewster, 1957*a*). The presence of a complete pentose cycle in cell-free extracts of *A. suboxydans* has been demonstrated by Hauge *et al.* (1955); the non-utilization of the various aldoses other than glucose beyond preliminary stages indicates the absence of phosphorylating or isomerizing mechanisms or both enabling these compounds to enter the main metabolic pathway.

The specificity of the oxidative enzyme system of the cytochrome-deficient organisms resulting from growth in media containing glucose in the absence of chalk resembles that of the flavoprotein-glucose oxidase or notatin of *Penicillium notatum* (Keilin & Hartree, 1948*a*, 1952); this latter enzyme has recently been shown by Sols & de la Fuente (1957) to oxidize 2-deoxy-D-glucose, although at only 25% the rate of the oxidation of glucose. Notatin can also utilize phenolindo-2:6-dichlorophenol as a hydrogen acceptor (Keilin & Hartree, 1948*a*). However, no evidence was obtained for the formation of hydrogen peroxide in the present study and, unlike notatin, the rate of oxidation of glucose was sensitive to cyanide under aerobic conditions and triphenyltetrazolium chloride did not serve as a hydrogen acceptor under anaerobic conditions. The properties of the enzyme also differed from those of a cyanide-sensitive oxidase found in a solubilized particulate fraction of *A. suboxydans* by King & Cheldelin (1957), which was shown to be cytochrome-dependent and to oxidize galactose as well as glucose.

SUMMARY

1. The effectiveness of various carbohydrates as energy sources for the growth of *Acetobacter suboxydans* in complex and semi-defined media is described; maximum growth was obtained with D-mannitol and D-sorbitol, good growth with glycerol, D-glucose in the presence of chalk, and D-fructose, and poor growth with calcium D-gluconate; negligible growth resulted with sodium D-gluconate, L-sorbose, D-mannose, D-galactose, D-xylose, L-arabinose and sucrose.

2. The extent of oxidation of glucose by washed organisms was influenced by the age and pH of the culture and the energy source for growth, and was sensitive to cyanide, fluoride, 2:4-dinitrophenol and phenylmercury acetate.

3. Maximum oxidation of glucose by washed organisms occurred with an uptake of 3·75 moles of oxygen and an output of up to 4·0 moles of carbon dioxide/mole of glucose; gluconate and 2- and 5-oxogluconate were shown to be intermediates and are considered to be formed without the participation of phosphate esters.

4. By the action of washed organisms 2-oxogluconate underwent oxidation and decarboxylation to give arabonate; 5-oxogluconate, in the presence of added readily oxidized substrate, was extensively oxidized with an uptake of oxygen of up to 3·5 moles and an output of carbon dioxide of 4·0 moles/mole.

5. Glucose was the only aldose to be extensively oxidized by washed organisms; some derivatives of glucose, other hexoses, pentoses and aldehydes were oxidized to a limited extent. A number of intermediates of the tricarboxylic acid cycle and acids formed during the growth of *Acetobacter* and *Pseudomonas* species in media containing glucose were not oxidized.

6. Organisms grown on a medium containing glucose and under conditions where acid production was allowed to proceed unchecked were shown to lack the characteristic cytochrome spectrum of *A. suboxydans* and to oxidize only glucose and 2-deoxy-D-glucose to the corresponding aldonic acids.

The author is greatly indebted to Professor D. D. Woods, F.R.S., for constant advice and encouragement, and to the members of the Microbiology Unit, Oxford, for helpful criticism.

REFERENCES

Arcus, A. C. & Edson, N. L. (1956). *Biochem. J.* **64**, 385.

Bayne, S. & Fewster, J. A. (1956). *Advanc. Carbohyd. Chem.* **11**, 43.

Butlin, K. R. (1936). *Biochem. J.* **30**, 1870.

Butlin, K. R. (1938*a*). *Biochem. J.* **32**, 508.

Butlin, K. R. (1938*b*). *Biochem. J.* **32**, 1185.

Cummins, J. T., King, T. E. & Cheldelin, V. H. (1957). *J. biol. Chem.* **224**, 323.

Fewster, J. A. (1956). *Biochem. J.* **63**, 26P.

Fewster, J. A. (1957*a*). *Biochem. J.* **65**, 14P.

Fewster, J. A. (1957*b*). *Biochem. J.* **66**, 9P.

Fulmer, E. I. & Underkofler, L. A. (1947). *Iowa St. Coll. J. Sci.* **21**, 251.

Hanes, C. S. & Isherwood, F. A. (1949). *Nature, Lond.*, **164**, 1107.

Hauge, J. G., King, T. E. & Cheldelin, V. H. (1955). *J. biol. Chem.* **214**, 11.

Hestrin, S. (1949). *J. biol. Chem.* **180**, 249.

Jackson, R. W., Koepsell, H. J., Lockwood, L. B., Nelson, G. E. N. & Stodola, F. H. (1949). *Abstr. 1st Int. Congr. Biochem., Cambridge*, p. 536.

Katznelson, H., Tanenbaum, S. W. & Tatum, E. L. (1953). *J. biol. Chem.* **204**, 43.

Keilin, D. & Hartree, E. F. (1948*a*). *Biochem. J.* **42**, 221.

Keilin, D. & Hartree, E. F. (1948*b*). *Biochem. J.* **42**, 230.

Keilin, D. & Hartree, E. F. (1952). *Biochem. J.* **50**, 331.

Keilin, D. & Smith, L. (1957). Unpublished work. Quoted by King, T. E. & Cheldelin, V. H. (1957). *J. biol. Chem.* **224**, 579.

King, T. E. & Cheldelin, V. H. (1952). *J. biol. Chem.* **198**, 127, 135.

King, T. E. & Cheldelin, V. H. (1953). *J. Bact.* **66**, 581.

King, T. E. & Cheldelin, V. H. (1954). *J. biol. Chem.* **208**, 821.

King, T. E. & Cheldelin, V. H. (1957). *J. biol. Chem.* **224**, 579.

Klevstrand, R. & Nordal, A. (1950). *Acta chem. scand.* **4**, 1320.

Kluyver, A. J. & Boezaardt, A. G. J. (1938). *Rec. Trav. chim Pays-Bas*, **57**, 609.

Kluyver, A. J. & de Leeuw, F. J. G. (1924). *Tijdschr. vergelijk. Geneesk.* **10**, 170.

Kovachevich, R. & Wood, W. A. (1955). *J. biol. Chem.* **213**, 737.

Kulka, D. & Walker, T. K. (1954). *Arch. Biochem. Biophys.* **50**, 169.

Monod, J., Cohen-Bazire, G. & Cohn, M. (1951). *Biochim. biophys. Acta*, **7**, 585.

Ohle, H., Vargha, L. von & Erlbach, H. (1928). *Ber. dtsch. chem. Ges.* **61**, 1214.

Rao, M. R. R. (1957). *Annu. Rev. Microbiol.* **11**, 317.

Rao, M. R. R. & Stokes, J. L. (1953). *J. Bact.* **66**, 634.

Smith, L. (1954*a*). *Arch. Biochem. Biophys.* **50**, 299.

Smith, L. (1954*b*). *Bact. Rev.* **18**, 106.

Snell, E. E. & Rannefeld, A. W. (1945). *J. biol. Chem.* **157**, 475.

Sols, A. & de la Fuente, G. (1957). *Biochim. biophys. Acta*, **24**, 206.

Stokes, F. N. & Campbell, J. J. R. (1951). *Arch. Biochem. Biophys.* **30**, 121.

Stubbs, J. J., Lockwood, L. B., Roe, E. T., Tabenkin, B. & Ward, G. E. (1940). *Industr. Engng Chem. (Industr.)*, **32**, 1626.

Tosic, J. (1946). *Biochem. J.* **40**, 209.

van Heyningen, W. E. & Gladstone, G. P. (1953). *Brit. J. exp. Path.* **34**, 221.

Walker, T. K. & Tosic, J. (1946). *J. Soc. chem. Ind., Lond.*, **65**, 104.

Wood, W. A. & Schwerdt, R. F. (1953). *J. biol. Chem.* **201**, 501.