The Regulation of Transport of Glucose, Gluconate and 2-Oxogluconate and of Glucose Catabolism in *Pseudomonas aeruginosa*

By PAUL H. WHITING,* MELVIN MIDGLEY and EDWIN A. DAWES

Department of Biochemistry, University of Hull, Hull HU6 7RX, U.K.

(Received 17 September 1975)

1. The induction by glucose and gluconate of the transport systems and catabolic enzymes for glucose, gluconate and 2-oxogluconate was studied with *Pseudomonas aeruginosa* PAO1 growing in a chemostat under conditions of nitrogen limitation with citrate as the major carbon source. 2. In the presence of a residual concentration of 30 mM-citrate an inflowing glucose concentration of 6–8 mM was required to induce the glucose-transport system and associated catabolic enzymes. When the glucose concentration was raised to 20 mM the glucose-transport system was repressed, but the transport system for gluconate, and at higher glucose concentrations, that for 2-oxogluconate, were induced. No repression of the glucose-catabolizing enzymes occurred at the higher inflowing glucose concentrations. 3. In the presence of 30 mM-citrate no marked threshold concentration was required for the induction of the gluconate-transport system by added gluconate. 4. In the presence of 30 mM-citrate and various concentrations of added glucose and gluconate, the activity of the glucose-transport system accorded with the proposal that a major factor concerned in the repression of this system was the concentration of gluconate, produced extracellularly by glucose dehydrogenase. 5. This proposal was supported by chemostat experiments with mutants defective in glucose dehydrogenase. Such mutants showed no repression of the glucose-transport system by high inflowing concentrations, but with a mutant apparently defective only in glucose dehydrogenase, the addition of gluconate caused repression of the glucose-transport system. 6. Studies with the mutants showed that both glucose and gluconate can induce the enzymes of the Entner–Doudoroff system, whereas for the induction of the gluconate-transport system glucose must be converted into gluconate.

The complexity of glucose metabolism in *Pseudomonas aeruginosa* was revealed by the work of Stokes & Campbell (1951), Claridge & Werkman (1953, 1954) and Wang *et al.* (1959). Glucose may be oxidized directly to gluconate and 2-oxogluconate before phosphorylation, or may be phosphorylated directly and then oxidized. Our demonstration of a kinase for 2-oxogluconate and a reductase for 2-oxogluconate 6-phosphate (Roberts *et al.*, 1973) meant that all these pathways converge on 6-phosphogluconate, which is then further metabolized by the Entner–Doudoroff and pentose phosphate pathways in the approximate ratio 7:3 in glucose-grown organisms (Wang *et al.*, 1959). Terminal oxidation occurs via the tricarboxylic acid cycle. There is not a functional glycolytic sequence, since *Ps. aeruginosa* lacks phosphofructokinase, and the presence of fructose 1,6-diphosphate aldolase is controversial (Lessie & Neidhardt, 1967; Tiwari & Campbell, 1969).

We have previously shown that when *Ps. aeruginosa* is grown on an organic acid, such as succinate or citrate, the principal enzymes of glucose metabolism

are severely repressed (Hamilton & Dawes, 1960; Hamlin *et al.*, 1967). Chemostat studies with nitrogen-limited cultures grown on 75 mM-citrate to which increasing concentrations of glucose were introduced disclosed that significant induction of the enzymes of glucose catabolism did not occur until a threshold concentration of 6–8 mM-glucose was exceeded (Hamlin *et al.*, 1967; Ng & Dawes, 1973). The existence of such a threshold concentration suggested the presence of an inducible transport system, which might be playing an important regulatory role in glucose metabolism. Midgley & Dawes (1973) demonstrated the presence of high and low affinity uptake systems. The high-affinity system ($K_m = 8 \mu\text{M}$) was found to be a glucose-transport system of rather broad specificity, whereas the low-affinity system ($K_m \approx 1 \text{ mM}$) was due to the activity of a transport system for gluconate, which was produced by the action of glucose dehydrogenase. Midgley & Dawes (1973) showed that this enzyme was oriented in the cytoplasmic membrane in such a manner that it oxidized its substrate extracellularly, i.e. in the periplasmic space (Fig. 1). Roberts *et al.* (1973) then showed that

* Present address: Department of Biochemistry, University of Nottingham, Nottingham NG7 2RD, U.K.

Fig. 1. Extracellular and intracellular pathways of glucose catabolism and related transport systems in *Ps. aeruginosa*

gluconate dehydrogenase was similarly oriented and thus they were able to explain the apparent complexity of glucose catabolism in *Ps. aeruginosa* in terms of the direct oxidative pathway and the phosphorylative pathway occurring in different compartments of the organism, i.e. extracellularly and intracellularly respectively (Fig. 1). The extracellular and intracellular pathways are linked by inducible transport systems and specific, independently regulated, uptake mechanisms for gluconate and 2-oxogluconate were discovered (Midgley & Dawes, 1973; Roberts *et al.*, 1973); all three uptake systems were completely repressed by growth on citrate.

We report here a chemostat study of the regulation of these transport systems by citrate and also present evidence that gluconate, produced by the extracellular activity of glucose dehydrogenase, plays a role in the control of glucose catabolism in *Ps. aeruginosa*. A preliminary account of some of this work has been presented at the Sixth International Conference on Continuous Culture (Dawes *et al.*, 1975).

Experimental

Organisms

The organisms used were *Pseudomonas aeruginosa* PAO1, kindly provided by Professor B. W. Holloway, Department of Genetics, Monash University, Clayton, Victoria 3168, Australia, and two strains derived from PAO1, designated PAOL6 and PAOFDL6. The former strain was produced by mutagenesis and subsequent screening on indicator plates as described by Midgley & Dawes (1973). PAOFDL6 was a spontaneous mutant isolated after freeze-drying strain PAOL6. Strain PAOL6 lacked glucose dehydrogenase activity, as judged by the same criteria that Midgley & Dawes (1973) used for previous isolates, and strain PAOFDL6 additionally lacked gluconate dehydrogenase, gluconate kinase and gluconate transport. Thus the latter organism was unable to grow on gluconate.

Growth and maintenance of cultures

Routine maintenance, batch growth of the organism, harvesting procedures and the preparation of bacterial extracts were as previously described (Ng & Dawes, 1973; Midgley & Dawes, 1973). For chemostat inocula the organism was subcultured at least ten times in the appropriate medium.

The chemostat used was identical with that described by Ng & Dawes (1973). The dilution rate used is specified for each experiment but was approx. 0.4 h^{-1} throughout. The preparation and composition of nitrogen-limited medium was as described by Whiting *et al.* (1976). The concentrations of the various carbon sources used are specified in the Results section.

Transport studies

These were performed as described by Midgley & Dawes (1973) by using the substrate concentrations and specific radioactivities specified by Whiting *et al.* (1976). The radioactivity present in each sample was determined as described by Midgley & Dawes (1973).

Enzyme assays

The enzymes of glucose and 2-oxogluconate catabolism were assayed under the conditions described by Ng & Dawes (1973), except that glucose dehydrogenase was assayed in the presence of phenazine methosulphate (2 mM). The combined activity of 2-oxogluconate kinase and 2-oxo-6-phosphogluconate reductase was assayed by the method of Whiting *et al.* (1976).

Analyses

These were performed as described by Whiting *et al.* (1976) and Ng & Dawes (1973).

no effect on the activities of hexokinase and glucose 6-phosphate dehydrogenase. However, higher glucose concentrations induced gluconate kinase as well as greatly increasing the activities of glucose dehydrogenase and gluconate dehydrogenase. In a medium containing 45 mM-glucose alone, the enzymes of 2-oxogluconate catabolism were induced. Some maintenance of the transport of glucose, gluconate and 2-oxogluconate and of activities of gluconate dehydrogenase and glucose dehydrogenase was observed when the steady state was changed from 45 mM-glucose to a medium containing 75 mM-citrate plus 4 mM-glucose. The utilization of glucose reflects the changes in specific activity observed (Table 1). At all glucose concentrations studied a significant proportion of the residual carbon in the chemostat was present as gluconate and 2-oxogluconate, produced via the extracellular action of glucose dehydrogenase and gluconate dehydrogenase (Table 1).

It is well established that the activities of intracellular enzymes are subject to regulation and it was thus of interest to determine whether the same is true of the activity of the enzymes of the extracellular pathway, since their location presumably presents problems not encountered in the regulation of cytoplasmic intracellular enzymes. Such control was demonstrated in an experiment in which washed bacterial suspensions, from the steady state with 75 mM-citrate plus 20 mM-glucose metabolized 2-deoxyglucose [via glucose dehydrogenase (Midgley & Dawes, 1973)] at a rate of 2.6 $\mu\text{mol/h}$ per g dry wt. in the absence of citrate, whereas in the presence of

20 mM-citrate the rate fell to 0.3 $\mu\text{mol/h}$ per g dry wt. That the activity of the extracellular pathway is indeed controlled in some way by citrate is also indicated by an experiment in which the production of gluconate from glucose was inhibited by the presence of citrate (Fig. 5).

Fig. 4. Effect of various concentrations of citrate and glucose in the inflowing medium on the transport systems for glucose, gluconate and 2-oxogluconate in NH_4^+ -limited *Ps. aeruginosa* PAO1

Transport systems for glucose, $\circ$; gluconate, $\bullet$; 2-oxogluconate, $\square$.

Table 1. Effect of various concentrations of citrate and glucose in the inflowing medium on the steady-state utilization of citrate and glucose and on the residual concentrations of these substrates, gluconate and 2-oxogluconate with chemostat-grown *Ps. aeruginosa* PAO1

The values are means of duplicate determinations, which did not differ by more than 5%. The dilution rate was 0.38 h^{-1} .

Citrate:glucose ratio in inflowing medium (mm)	Rate of utilization (mmol/h per g dry wt.)		Residual concentrations (mm)			
	Citrate	Glucose	Glucose	Citrate	Gluconate	2-Oxogluconate
75:0	9.8	0	0	26.0	0	0
75:2	9.7	0.4	0.2	27.0	1.0	0.25
75:4	9.2	0.8	0.2	28.5	1.3	0.3
75:6	8.5	1.1	0.5	32.2	1.2	0.6
75:8	9.1	1.6	0.3	29.3	2.6	0.9
75:10	7.9	1.7	1.5	30.5	0.8	0.8
75:15	8.2	2.2	4.1	33.8	1.8	1.0
75:20	7.5	2.7	6.7	37.5	2.0	1.3
75:30	6.4	4.4	8.3	42.6	1.1	1.0
75:45	6.3	7.2	9.2	42.3	1.2	2.5
45:45	1.4	7.2	9.0	38.3	2.4	5.1
0:45	0	7.3	8.4	0	1.7	0.6
75:4	9.4	0.8	0.1	28.2	1.0	0.3

Fig. 5. Inhibition of glucose dehydrogenase by citrate

A washed suspension (1.7mg dry wt./ml) of *Ps. aeruginosa* PAO1, grown under conditions of nitrogen limitation on 75mM-citrate plus 20mM-glucose as carbon source, was incubated with 20mM-glucose in the presence (○) and absence (●) of 75mM-citrate and gluconate production was measured.

Fig. 7. Effect of various concentrations of citrate and gluconate in the inflowing medium on the enzymes of glucose catabolism in NH_4^+ -limited *Ps. aeruginosa* PAO1

Glucose dehydrogenase, Δ ; gluconate dehydrogenase, $\blacktriangle$; hexokinase, $\circ$; glucose 6-phosphate dehydrogenase, $\bullet$; gluconate kinase, $\square$; 2-oxogluconate enzymes, $\blacksquare$; Entner-Doudoroff enzymes, ∇ .

Fig. 6. Effect of various concentrations of citrate and gluconate in the inflowing medium on the transport systems for gluconate and 2-oxogluconate in NH_4^+ -limited *Ps. aeruginosa* PAO1

Transport of gluconate, $\bullet$; 2-oxogluconate, $\square$.

Effect of increasing gluconate concentrations on the steady-state specific activities of citrate-grown organisms

As gluconate is produced extracellularly by the action of glucose dehydrogenase on glucose it was important to examine the effect of increasing concentrations of gluconate in the presence of citrate. In particular, the response of the Entner-Doudoroff enzymes to gluconate in comparison with glucose and, in a subsequent experiment, the effect of added

gluconate on the induction of the glucose-transport system by glucose were of interest. The results of the former experiment, analogous to that carried out with glucose and citrate, are shown in Figs. 6 and 7.

The activity of the gluconate-transport system increased with increasing gluconate concentrations to 5.0mM, above which there was no further effect on this system, but the 2-oxogluconate-transport system was induced (Fig. 6). A decrease of the citrate concentration in the inflowing medium did not affect either transport system. On reverting from a medium containing 45mM-gluconate to one containing 75mM-citrate plus 2.5mM-gluconate, severe repression of transport systems for both gluconate and 2-oxogluconate occurred, but some activity of the latter transport system was maintained.

The glucose-catabolizing enzymes were induced with increasing gluconate concentration (Fig. 7). A decrease in citrate concentration almost doubled the activity of gluconate dehydrogenase. Increasing gluconate concentrations also induced the enzymes of 2-oxogluconate metabolism in the presence of citrate.

A significant proportion of gluconate in the medium was converted into 2-oxogluconate by the extracellular action of gluconate dehydrogenase (Table 2). The rate of gluconate utilization increased with increasing gluconate concentration and concomitant enzyme induction, whereas that of citrate decreased (Table 2). Glucose-transport activity could not be detected in any of these steady states of growth on gluconate and gluconate/citrate media, thus

Table 2. Effect of various concentrations of citrate and gluconate in the inflowing medium on the steady-state utilization of these substrates and their residual concentrations and that of 2-oxogluconate with chemostat-grown *Ps. aeruginosa* PAO1

The values are means of duplicate determinations, which did not differ by more than 5%. The dilution rate was 0.40 h^{-1} .

Citrate:gluconate ratio in inflowing medium (mM)	Rate of utilization (mmol/h per g dry wt.)		Residual concentration (mM)		
	Citrate	Gluconate	Citrate	Gluconate	2-Oxogluconate
75:0	9.7	0	26.7	0	0
75:1	9.6	0.1	27.2	0.5	0.2
75:2.5	9.6	0.4	27.2	0.7	1.4
75:3.75	9.4	0.7	27.7	0.7	1.0
75:5	9.5	0.9	28.2	0.8	1.2
75:7.5	8.3	1.2	33.7	1.0	1.6
75:10	8.3	1.8	33.5	0.9	1.6
75:15	8.2	2.2	34.0	4.0	2.9
75:20	7.6	3.8	36.9	0.8	1.8
75:30	7.1	5.7	39.5	1.5	5.8
75:45	4.8	7.3	51.0	8.5	6.8
37.5:45	1.5	7.8	30.0	6.1	7.1
0:45	0	7.8	0	5.9	7.5
75:2.5	9.6	0.3	26.7	0.8	1.3

Table 3. Activities of glucose and gluconate transport in mutant and wild-type *Ps. aeruginosa* PAO1 grown on glucose or gluconate in batch culture

Initial rates were measured in duplicate over the initial 75 s of transport. The glucose concentration was $5\text{ }\mu\text{M}$ and gluconate concentrations $50\text{ }\mu\text{M}$. n.d., Not detected.

Organism	Carbon source	Rate of transport ($\mu\text{mol/min per g dry wt.}$)	
		Glucose	Gluconate
Wild-type PAO1	Glucose	9.9	22.7
		10.7	18.9
	Gluconate	n.d.	24.6
			27.2
Mutant PAOL6	Glucose	13.2	n.d.
		11.7	
	Gluconate	n.d.	19.8
			21.7
Mutant PAOFDL6	Glucose	11.5	n.d.
		12.2	

extending earlier observations (Midgley & Dawes, 1973) made with batch cultures and confirmed here in Table 3.

Effect of increasing glucose and gluconate concentrations on the steady-state specific activities of citrate-grown organisms

The foregoing experiments had revealed that increasing the glucose concentration in the chemostat above 15 mM repressed the glucose-transport system and that gluconate-grown organisms in batch culture have repressed activity of the glucose-transport system (Table 3). The possibility that

Fig. 8. Effect of various concentrations of citrate, glucose and gluconate in the inflowing medium on the transport systems for glucose, gluconate and 2-oxogluconate in NH_4^+ -limited *Ps. aeruginosa* PAO1

Transport of glucose, $\circ$; gluconate, $\bullet$; 2-oxogluconate, $\square$.

gluconate, produced by the extracellular action of glucose dehydrogenase, was an important factor in the chemostat experiments was therefore examined by increasing the concentrations of glucose and gluconate in an NH_4^+ -limited medium containing 75 mM -citrate (Figs. 8 and 9).

The induction of the transport systems for glucose, gluconate and 2-oxogluconate (Fig. 8) by glucose alone was similar to that observed in Fig. 4, with a marked repression of glucose transport occurring when the glucose concentration exceeded 15 mM .

However, decreasing the glucose from 20 to 15 mM restored the activity of the glucose-transport system to that previously observed at 15 mM. With the glucose concentration then held constant at 15 mM, the introduction of increasing concentrations of gluconate markedly repressed the glucose-transport system while inducing further the activities of the transport systems for gluconate and 2-oxogluconate. The addition of gluconate to the medium also caused further induction of the glucose-catabolizing enzymes (Fig. 9), especially gluconate dehydrogenase. The enzymes of 2-oxogluconate catabolism were also induced to low values (specific activity 0.2–0.5 $\mu\text{mol/h}$ per mg of protein) when the gluconate concentration attained 5 mM.

Again, high concentrations of gluconate and 2-oxogluconate were present in the culture when only glucose was present in the inflowing citrate medium (Table 4). The addition of gluconate to the medium resulted in an inhibition of citrate utilization, increasing with increasing gluconate concentrations.

Experiments with mutants

Mutants deficient in the extracellular pathway of glucose metabolism have been used to investigate three problems, namely (1) the sequential nature of the induction of gluconate transport by glucose, (2) to confirm the above evidence, which indicates that gluconate is a major inhibitory factor for the induction of the glucose-transport system, and (3) to determine whether glucose or gluconate induces the Entner–Doudoroff system in *Ps. aeruginosa*; for example, in *Pseudomonas fluorescens* (Eisenberg *et al.*, 1972) and in *Escherichia coli* (Eisenberg & Drobrogosz, 1967) gluconate is considered to be the true inducer.

Tables 3 and 5 reveal that both mutants, which are defective in glucose dehydrogenase and therefore unable to produce gluconate from glucose, have no detectable gluconate- or 2-oxogluconate-transport activity when grown on glucose in the presence or absence of citrate. However, when gluconate is added to strain PAOL6 (deficient only in glucose

Fig. 9. Effect of various concentrations of citrate, glucose and gluconate in the inflowing medium on the glucose-catabolizing enzymes of NH_4^+ -limited *Ps. aeruginosa* PAO1

Glucose dehydrogenase, Δ ; gluconate dehydrogenase, $\blacktriangle$; hexokinase, $\circ$; glucose 6-phosphate dehydrogenase, $\bullet$; Entner–Doudoroff enzymes, ∇ ; gluconate kinase, $\square$.

Table 4. Effect of various concentrations of citrate, glucose and gluconate in the inflowing medium on the steady-state utilization of these substrates and their residual concentrations and that of 2-oxogluconate with chemostat-grown *Ps. aeruginosa* PAO1

The values are the means of duplicate determinations, which did not differ by more than 5%. The dilution rate was 0.41 h^{-1} .

Citrate:glucose: gluconate ratio in in- flowing medium (mM)	Rate of utilization (mmol/h per g dry wt.)			Residual concentration (mM)			
	Citrate	Glucose	Gluconate*	Citrate	Glucose	Gluconate	2-Oxogluconate
75:0:0	7.3	0	0	27.1	0	0	0
75:5:0	7.1	0.7	0	29.0	0.6	1.1	0.6
75:10:0	6.5	1.2	0	33.0	2.4	1.0	1.0
75:15:0	6.4	1.1	0	33.9	7.1	1.9	1.9
75:20:0	5.9	1.9	0	35.0	7.4	1.5	2.4
75:15:0	6.2	1.2	0	35.2	6.4	1.7	2.5
75:15:2.5	5.9	1.4	0.5	35.8	5.0	1.4	2.3
75:15:5	6.1	1.3	0.9	38.2	6.4	1.0	2.7
75:15:7.5	5.1	2.0	1.3	41.7	2.8	1.4	3.1
75:15:10	4.2	1.9	1.6	48.7	2.8	1.6	4.6

* Calculated on the assumption that the conversion of glucose into gluconate was unaffected by added gluconate.

dehydrogenase), both the gluconate- and 2-oxo-gluconate-transport systems are induced. These findings suggest that gluconate (or some closely related compound) is the inducer of gluconate transport.

Although they do not furnish definitive evidence for 2-oxogluconate transport, our previous observation (Roberts *et al.*, 1973) that the transport systems for gluconate and 2-oxogluconate are independently regulated suggests that gluconate must be converted into 2-oxogluconate for induction to occur.

The results in Table 5 also show that the transport of glucose by mutants unable to produce gluconate from glucose is unaffected by high concentrations of glucose in the medium; glucose transport increases steadily as the glucose concentration is increased in the presence of 75 mM-citrate in the NH_4^+ -limited medium. Higher activities of glucose transport were manifest in both organisms when grown on 45 mM-glucose alone. However, the addition of 10 mM-gluconate to the growth medium of strain PAOL6 severely repressed the glucose-transport system, confirming the inhibitory effect of this compound.

Table 6 records the specific activities of the glucose-catabolizing enzymes in the same experiment. Again, increasing concentrations of glucose induced increased activities of the enzymes. Noteworthy are the activities of glucose 6-phosphate dehydrogenase in these mutants, about three times higher than that of the wild type when grown on glucose alone. Further, the Entner-Doudoroff enzymes are induced, indicating that gluconate is not the inducer under these conditions, in contrast with *Ps. fluorescens* (Eisenberg *et al.*, 1972). The addition of gluconate caused the induction of the 2-oxogluconate-catabolizing enzymes in strain PAOL6, but not in

strain PAOFDL6; however, the latter organism is unable to grow on 2-oxogluconate.

The residual concentrations of glucose, gluconate, 2-oxogluconate and citrate in the media of these experiments are recorded in Table 7. Little gluconate or 2-oxogluconate was detected with either mutant. However, the addition of gluconate to the medium caused the production of 2-oxogluconate with strain PAOL6, which possesses gluconate dehydrogenase activity, but not with strain PAOFDL6, which does not have this enzyme.

Table 5. *Effect of various concentrations of citrate, glucose and gluconate in the inflowing medium on the activity of the transport systems for glucose, gluconate and 2-oxogluconate in Ps. aeruginosa mutants PAOL6 and PAOFDL6*

The conditions for assay were as for Table 3. The dilution rate was 0.42 h^{-1} . n.d., Not detected.

Organism	Citrate:glucose:gluconate ratio in inflowing medium (mM)	Rate of transport ($\mu\text{mol/min per g dry wt.}$)		
		Glucon- 2-Oxo-		
		Glucose	ate	gluconate
PAOFDL6	75:0:0	0.1	n.d.	n.d.
	75:10:0	3.3	n.d.	n.d.
	75:20:0	5.5	n.d.	n.d.
	0:45:0	15.2	n.d.	n.d.
	0:45:10	13.6	n.d.	n.d.
PAOL6	75:10:0	4.9	n.d.	n.d.
	75:30:0	6.3	n.d.	n.d.
	75:45:0	6.6	n.d.	n.d.
	0:45:0	8.9	n.d.	n.d.
	0:45:10	3.2	9.7	26.9

Table 6. *Effect of various concentrations of citrate, glucose and gluconate in the inflowing medium on the steady-state activities of the glucose-catabolizing enzymes of Ps. aeruginosa mutants PAOL6 and PAOFDL6*

The dilution rate was 0.42 h^{-1} . n.d., Not detected.

Organism	Citrate:glucose:gluconate ratio in inflowing medium (mM)	Specific activity ($\mu\text{mol of substrate converted/h per mg of protein}$)						
		Glucose dehydrogenase	Gluconate dehydrogenase	Glucose 6-phosphate dehydrogenase	Hexokinase	Gluconate kinase	2-Oxo-gluconate enzymes	Entner-Doudoroff enzymes
PAOFDL6	75:0:0	n.d.	n.d.	2.9	3.7	0.5	n.d.	0.1
	75:10:0	n.d.	n.d.	11.5	9.5	0.3	n.d.	9.1
	75:20:0	n.d.	n.d.	17.9	10.0	0.2	n.d.	10.6
	0:45:0	n.d.	n.d.	92.8	12.2	1.3	n.d.	18.2
	0:45:10	n.d.	n.d.	93.6	13.0	1.4	n.d.	15.2
PAOL6	75:10:0	n.d.	18.6	11.6	9.5	0.4	n.d.	7.5
	75:30:0	n.d.	24.6	16.3	10.0	0.5	n.d.	9.8
	75:45:0	n.d.	25.5	17.0	12.2	0.7	n.d.	10.2
	0:45:0	n.d.	63.1	81.1	13.0	0.7	n.d.	13.4
	0:45:10	n.d.	94.2	90.0	12.8	0.7	1.3	17.3

Table 7. Effect of various concentrations of citrate, glucose and gluconate in the inflowing medium on the steady-state utilization of these substrates and on their residual concentrations, and that of 2-oxogluconate with chemostat cultures of *Ps. aeruginosa* mutants PAOL6 and PAOFDL6

The values are means of duplicate determinations, which did not differ by more than 5%. The dilution rate was 0.42 h⁻¹.

Organism	Citrate:glucose: gluconate ratio in inflowing medium (mM)	Rate of utilization (mmol/h per g dry wt.)		Residual concentration (mM)			
		Citrate	Glucose	Citrate	Glucose	Gluconate	2-Oxogluconate
PAOFDL6	75:0:0	9.4	0	30.1	0	0	0
	75:10:0	8.6	1.5	35.3	3.1	0.5	0
	75:20:0	8.3	2.4	36.0	8.9	1.1	0
	0:45:0	0	6.6	0	19.0	1.0	0
	0:45:10	0	5.9	0	18.8	10.8	0
PAOL6	75:10:0	9.8	1.7	29.5	2.3	0.2	0
	75:30:0	10.4	1.9	27.0	21.2	0.4	0.2
	75:45:0	10.3	1.9	28.3	36.0	0.4	0.3
	0:45:0	0	5.5	0	15.6	0.2	0.4
	0:45:10	0	4.5	0	9.5	9.8	1.5

Discussion

The necessity for a threshold concentration of 6–8 mM-glucose to induce the enzymes of glucose catabolism is probably explicable in terms of catabolite repression by citrate on the appropriate transport systems, effectively preventing glucose from inducing its own transport system until a significant concentration gradient exists across the cell membrane. Induction of the transport system appears to allow subsequent induction of both the intra- and extra-cellular enzymes; the consequent extracellular generation of gluconate by the action of glucose dehydrogenase is probably the major factor initiating the induction of the gluconate-transport system. In contrast with the behaviour of the enzymes immediately concerned with glucose and gluconate catabolism, the enzymes of 2-oxogluconate catabolism cannot be induced by increasing glucose concentrations in the presence of citrate, confirming the independent regulation observed with glycerol-grown organisms (Roberts *et al.*, 1973).

We cannot, at present, say whether the increased activity of the glucose transport system is a direct result of an inducer–repressor type of interaction at the genetic level or due to the relief of catabolite repression, caused by metabolism of glucose, thus acting initially at the cytoplasmic level. Induction of the glucose-transport system and the enzymes of glucose metabolism readily occurs in the absence of citrate, suggesting that citrate metabolism in some way controls the rate of expression of the genes specifying these components. Consequently the site at which glucose, or derived metabolites, overcomes this effect must await clarification of the mechanism of catabolite repression in this organism. Thus, although we have used the term induction to describe the increase in activity of glucose transport and

related systems, we recognise that this is a complex process probably involving both cytoplasmic and genetic elements.

The unexpected observation that although glucose transport is induced by glucose concentrations in excess of 6 mM it is subsequently repressed when the concentration exceeds 15 mM can be attributed to the accumulation of gluconate in the medium. Thus mutants defective in glucose dehydrogenase, and therefore unable to produce gluconate, do not exhibit this effect. Gluconate not only represses the glucose transport system but also inhibits its activity, as previously shown by Midgley & Dawes (1973).

The contribution of the extracellular pathway to the overall metabolism of glucose is revealed by the residual concentrations of gluconate and 2-oxogluconate in the chemostat media (e.g. Tables 1, 2, 4 and 7), although these values are necessarily an underestimate of the activity of the pathway, owing to the utilization of gluconate and possibly of 2-oxogluconate. The importance of the relative concentrations of gluconate, glucose and citrate for the expression of the glucose-catabolic enzymes and the transport systems accords with the regulation of these enzymes and transport systems by induction with glucose or its metabolites and repression by gluconate and citrate; similar competition between glucose and citrate was observed with *Ps. aeruginosa* 2F32 (Ng & Dawes, 1973).

Gluconate exerts a similar inductive effect to glucose, except that glucose transport is not induced, thus confirming previous observations with batch cultures (Midgley & Dawes, 1973; Hyleman & Phibbs, 1974). However, no marked threshold gluconate concentration was apparently required for gluconate transport and induction increased with increasing

gluconate concentration (Fig. 6). Additionally gluconate at concentrations greater than 15 mM induces the enzymes of 2-oxogluconate metabolism in the presence of citrate; again a significant amount of the inflowing gluconate is converted into 2-oxogluconate by the action of gluconate dehydrogenase. The extracellular oxidation of glucose to gluconate and 2-oxogluconate clearly plays an important role in the growth of *Ps. aeruginosa* on glucose in batch culture and in NH_4^+ -limited continuous culture. Indeed, we have previously estimated that the flow of carbon in the extracellular pathway is some 10-fold that transported into the cell via the glucose-transport system when the organism is presented with high glucose concentrations (Midgley & Dawes, 1973; Roberts *et al.*, 1973).

The question of what benefit accrues to the organism by possessing an extracellular pathway is pertinent. From our results, it is evident that the extracellular conversion of glucose into gluconate and 2-oxogluconate, and their subsequent uptake, enables *Ps. aeruginosa* to circumvent any limitation imposed by the rate of operation of a single transport system and the subsequent phosphorylation step. However, we suggest that the extracellular pathway might benefit *Ps. aeruginosa* in its natural habitat, because it enables the organism to convert glucose rapidly into compounds that are less available to other microbes, but which may effectively compete for glucose. In support of this sequestration hypothesis we have shown that when the nutrient limitation in a chemostat is changed from NH_4^+ to glucose *Ps. aeruginosa* responds by switching its metabolism of glucose substantially from the extracellular to the intracellular pathway (Whiting *et al.*, 1976). Thus under conditions of glucose limitation, glucose dehydrogenase, gluconate dehydrogenase, gluconate kinase and the enzymes of 2-oxogluconate metabolism are all significantly repressed, whereas the activities of hexokinase and glucose 6-phosphate dehydrogenase are increased; further, glucose transport is increased some fivefold whereas the activities of gluconate and 2-oxogluconate

transport are decreased by seven- and five-fold respectively. These observations indicate that, when the supply of glucose is restricted, *Ps. aeruginosa* adjusts its metabolism to take the available substrate into the cell as rapidly as possible.

We are grateful to the Science Research Council for the award of a research studentship to P. H. W.

References

- Claridge, C. A. & Werkman, C. H. (1953) *Arch. Biochem. Biophys.* **47**, 99–106
- Claridge, C. A. & Werkman, C. H. (1954) *J. Bacteriol.* **68**, 77–79
- Dawes, E. A., Midgley, M. & Whiting, P. H. (1975) *Proc. Int. Symp. 6th and Study Group on the Continuous Culture of Micro-organisms*, Ellis Horwood, Chichester, in the press
- Eisenberg, R. C. & Drobrogosz, W. J. (1967) *J. Bacteriol.* **93**, 941–949
- Eisenberg, R. C., Butters, S. J., Quay, S. C. & Friedman, S. B. (1972) *J. Bacteriol.* **120**, 147–153
- Hamilton, W. A. & Dawes, E. A. (1960) *Biochem. J.* **76**, 70P
- Hamlin, B. T., Ng, F. M.-W. & Dawes, E. A. (1967) in *Microbial Physiology and Continuous Culture* (Powell, E. O., Evans, C. G. T., Strange, R. E. & Tempest, D. W., eds.) pp. 211–231, H.M.S.O., London
- Hyleman, P. B. & Phibbs, P. V., Jr. (1974) *Biochem. Biophys. Res. Commun.* **49**, 1041–1048
- Lessie, T. G. & Neidhardt, F. C. (1967) *J. Bacteriol.* **93**, 1397–1399
- Midgley, M. & Dawes, E. A. (1973) *Biochem. J.* **132**, 141–154
- Ng, F. M.-W. & Dawes, E. A. (1973) *Biochem. J.* **132**, 129–140
- Roberts, B. K., Midgley, M. & Dawes, E. A. (1973) *J. Gen. Microbiol.* **78**, 319–329
- Stokes, F. N. & Campbell, J. J. R. (1951) *Arch. Biochem. Biophys.* **30**, 121–125
- Tiwari, N. P. & Campbell, J. J. R. (1969) *Biochim. Biophys. Acta* **192**, 395–401
- Wang, C. H., Stern, I. J. & Gilmour, C. M. (1959) *Arch. Biochem. Biophys.* **81**, 489–492
- Whiting, P. H., Midgley, M. & Dawes, E. A. (1976) *J. Gen. Microbiol.* **92**, 304–310