amino acids in liver cells[2,3,7]. The situation seems to be different in vivo and ethanol may alter the supply of amino acids e.g. through an activation of the pituitary-adrenal system[6]. The rapid activation of protein synthesis in avian liver after estrogen administration is probably a sensitive system to detect the possible effects of ethanol on protein synthesis. In the absence of any effects of ethanol treatment on plasma levels of vitellogenin and on the size distribution of liver polyribosomes under these conditions we conclude that despite of its large effects on oxidative metabolism and substrate usage acute ethanol administration does not influence avian liver protein synthesis in vivo.

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2 M.A. Rothschield, M. Oratz, J. Mongelli and S.S. Schreiber, J. clin. Invest. *50,* 1812 (1971).
3 M. Oratz, M.A. Rothschield and S.S. Schreiber, Ann. N.Y. Acad. Sci. *252,* 51 (1975).
4 A. Perin, G. Scalabrino, A. Sessa and A. Arnaboldi, Biochim. biophys. Acta *366,* 101 (1974).
5 J. Mørland and A. Bessesen, Biochim. biophys. Acta *474,* 312 (1977).
6 G.-S.D. Nadkarni, Biochem. Pharmac. *23,* 389 (1974).
7 K.N. Jeejeebhoy, M.J. Phillips, A. Bruce-Robertson, J. Ho and U. Sodtke, Biochem. J. *126,* 1111 (1972).
8 P.H. Mäenpää and M.R. Bernfield, Biochemistry *8,* 4926 (1969).
9 P.H. Mäenpää, Biochem biophys. Res. Commun. *72,* 437 (1976).
10 E. Bernt and I. Gutmann, in: Methods of Enzymatic Analysis, p. 1499. Ed. H.U. Bergmeyer. Verlag Chemie, Weinheim 1974.

Energetics of myo-inositol transport in *Pseudomonas putida*[1]

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Summary. The effects of specific inhibitors on the high-affinity transport system of cyclitols and on the respiration of *Pseudomonas putida* shows that the transport activity is dependent on high energy phosphate bond.

Two systems for active transport of cyclitols have been reported in *Pseudomonas putida*[2]. These systems differ from each other in their affinity for the cyclitol and in sensitivity to osmotic shock. In this report the nature of the energy sources linked to the high affinity, binding protein-dependent, transport system is described. *P. putida* mutants defective in the electron transport chain or in Mg^{++}-Ca^{++} ATPase were not available. Therefore correlation of the transport activities of the cells with their ATP content and respiration rates in the presence of various inhibitors acting at different sites of metabolism was used to investigate the energy source for transport.

Materials and methods. P. putida strain isolation and identification have been described, as has the transport assay[2]. The organism was grown in mineral medium[2] supplemented with either 1% myo-inositol (induced cells) or 1% D-glucose (non-induced cells). Growth temperature was 20 °C. The cells were washed 3 times with mineral medium and resuspended in the assay medium (unless otherwise indicated, mineral medium). 2-/^{3}H/myo-inositol (10 µCi/µmole) concentration was 25 µM, which corresponds to the saturating concentration of the high-affinity transport system. The inhibitors were added to the cells 10 min prior to the transport assay. Anaerobic assays were performed in sealed penicillin vials under O_2-free nitrogen flushing. Flushing was initiated 15 min before the uptake assays. N_2-flushed substrate solution was added and samples were removed through the caps with Hamilton syringes. Conductivity was measured in a RDM_3 conductimeter (Radiometer, Copenhagen). Respiration measurements were performed with the aid of a Clark electrode (Yellow Spring Inc., Ohio, USA), the cell suspension was flushed for 30 sec with air and oxygen consumption was recorded before and after the addition of 0.25 mM myo-inositol. ATP determination and pH measurement during transport under anaerobic conditions were performed as previously described[3].

Results and discussion. Myo-inositol uptake was measured at various pH values. The experiments were performed in 0.1 M Good's buffer[4] adjusted with Tris-base to avoid the presence of alkali ions. Transport activity has a maximum at pH 6 in Good's Tris media (figure 1). However in H_3PO_4-Tris 0.1 M buffers the activities were always lower and the maximum displaced to pH 7.5 (figure 1); ionic strength does not account for the difference observed, since conductivity was not significantly different. Transport activity was also measured at pH 7.5 in different media: it is maximal in either mineral medium or in 0.1 M Tris-HCl, but it is decreased 60% in 0.1 M Tris-H_3PO_4 and 80% in

The effects of inhibitors on myo-inositol transport, ATP content and respiration. Cells were incubated with the inhibitor for 10 min at room temperature. Aliquots were withdrawn for transport assay in the presence of 25 µM myo-inositol; ATP and respiration rate determinations are described in materials and methods. The results are expressed as the percent of the values obtained with untreated cells.

Additions	Transport activity Uptake 15 sec	Time 3 min	ATP content	Respiration rate Endogenous (no substrate)	After substrate addition
None	100%	100%	100%	100%	100%
DNP 1 mM	17%	6%	6%	105%	75%
NaN_3 10 mM	8%	24%	43%	110%	60%
Arsenate 10 mM	76%	87%	80%	100%	100%
Arsenate 10 mM (in absence of phosphate)	34%	44%	50%	97%	100%
NaF 1 mM	95%	100%	100%	100%	100%
KCN 10 mM	8%	9%	0%	0%	0%
Anaerobiosis	75%	42%	55%	-	-

0.2 M Tris-HCl buffer (of ionic strength identical to that of mineral medium). Ionic strength is therefore not a critical factor. It seems more likely that high Tris concentration impares the membrane function.

Since cyclitol transport in *Klebsiella aerogenes* proceeds through a proton symport[3] the presence of such a mechanism was sought in *P. putida.* The cells were suspended in unbuffered 0.1 M NaCl or choline chloride. Anaerobiosis was maintained for 15 min prior to and during the test. Transport activity and pH variation in the medium were simultaneously recorded. Non-induced glucose-grown cells, myo-inositol-grown cells poisoned with 1 mM N-ethylmaleimide showed the same absence of pH response to the addition of substrate; however only the untreated inositol grown cells were able to transport the myo-inositol. These results rule out the involvement of a proton symport for inositol transport in *P. putida.*

The effect of Na^+ of K^+ ions on the transport was tested in 0.1 M Tris-HCl buffer pH 7.5 supplemented with 0.1 M

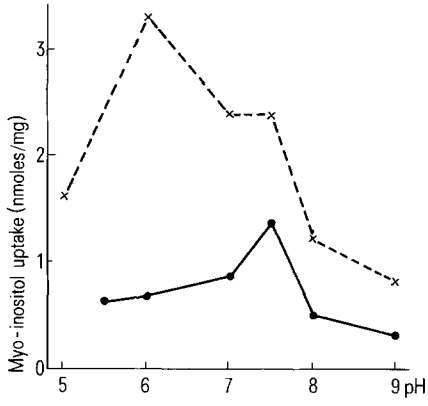

Fig. 1. The effect of pH and buffer composition on myo-inositol transport. Cells were resuspended either in a mixture of 0.1 M H_3PO_4 plus 0.1 mM Tris (●——●) or in 0.1 M Good's buffer (× – – – ×) adjusted with Tris (pH 5 and pH 6 morpholinoethatesulfonic acid; pH 7, morpholinoethane sulfonic acid; pH 7.5, N-Tris-(hydroxymethyl)-methyl-2-aminopropanesulfonic acid; pH 8, N-Tris-(hydroxymethyl)-methyl-glycine; pH 9, N-Tris-(hydroxymethyl)-methyl-3-amino-propanesulfonic acid). The uptake assay was initiated by the addition of 25 µM 2-/^{3}H/myo-inositol 0.25 ml aliquots were filtered after 0.5 min.

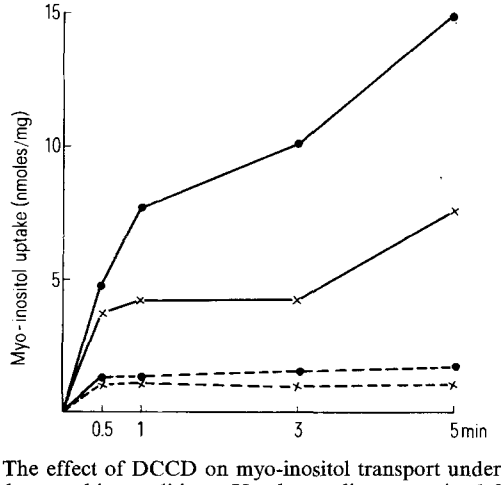

Fig. 2. The effect of DCCD on myo-inositol transport under aerobic and anaerobic conditions. Uptake medium contained 25 µM 2-/^{3}H/myo-inositol. Control cells in the presence of 10 mM EDTA: ●——● aerobic conditions; ×——× anaerobic conditions. Cells treated with 0.1 mM DCCD: ●·····● aerobic conditions; ×·····× anaerobic conditions.

Na^+ or K^+ chloride. As a control 0.1 M choline chloride was used. On the basis of 30-sec uptake, Na^+ and K^+ ions decrease transport 60% as compared to cells incubated in the absence or in the presence of choline chloride. In contrast respiration rates remained unaffected upon addition of Na^+, K^+ or choline. Therefore the inhibition is due to the presence of alkali ions and not to chloride or an osmotic effect.

The transport system is totally inactivated by a treatment with N-ethylmaleimide (1 mM) for 10 min. When the treatment is performed in the presence of 50 mM myo-inositol only 10% of the initial activity is retained, which indicates that the substrate does not protect against the alkylation by the maleimide in contrast to the observation made with the *K. aerogenes* system[5]. p-Hydroxymercuribenzoate abolished transport activity at 1 mM concentration, while at 0.1 mM it reduces activity 50%. No inhibition is observed with 10 µM.

Since all *Pseudomonas* are obligatory respiratory organisms it was interesting to test the transport activity under anaerobic conditions. Figure 2 and the table indicate that under anaerobic conditions both the initial rate of myo-inositol entry and total accumulation are decreased when compared to the values obtained in aerobic conditions. After 15-min of N_2 flushing the ATP content was found to be 55% of that of the aerobically maintained cells (1.9 nmoles ATP/mg dry weight of cells). To test the role of ATPase the cells were treated with 0.1 mM DCCD[6] and 10 mM EDTA for 15 min, centrifuged and washed with mineral medium. Transport assays were performed under either aerobic or anaerobic conditions. As shown in figure 2, DCCD drastically lowers transport in both experiments to the same level as compared with cells treated with EDTA only.

The source of energy linked to the transport process has been investigated with the use of various inhibitors. The results are listed in the table. The uncoupling action of DNP[6] drastically reduces the ATP level and abolishes transport. The substrate-induced respiration is also decreased, but to a lesser extent. In the presence of phosphate, arsenate moderately decreases both transport and ATP content without affecting the respiration rate. In the absence of phosphate, the ATP level and transport process are more deeply reduced, but again respiration is not affected. Azide does not affect endogenous respiration, whereas the ATP content is lowered 57%; transport is then strongly reduced. Fluoride (1 mM) seems to have no effect on *P. putida* metabolisms; the cells are fully de-energized by cyanide (10 mM).

From the effects of inhibitors and from the close correlation between the ATP content and transport, it can be deduced that cyclitol transport in *P. putida,* like transport in most of the systems dependent upon periplastic binding protein[7], is driven by high energy phosphate bonds. Furthermore the combined effect of anaerobiosis and DCCD indicate that the transport requires the participation of a functional ATPase and that oxidase reactions are not coupled directly to transport. Cotransport with alkali ions or protons can also be excluded.

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2 G. Reber, M. Belet and J. Deshusses, J. Bact. *131,* 872 (1977).
3 G. Reber, M. Mermod and J. Deshusses, Eur. J. Biochem. 72, *93* (1977).
4 N.E. Good, G.D. Winget, N. Winter, T.N. Coronolly, S. Izawa and R.M.M. Sing, Biochemistry *5,* 467 (1966).
5 J. Deshusses and G. Reber, Experientia *30,* 996 (1974).
6 Abbreviations. DCCD, dicyclohexylcarbodiimide; DNP, 2,4-dinitrophenol.
7 E. Berger and L.A. Heppel, J. biol. Chem. *249,* 7767 (1976).