Proton-linked L-fucose transport in *Escherichia coli*

Stuart A. BRADLEY, Colin R. TINSLEY, Jennifer A. R. MUIRY and Peter J. F. HENDERSON

Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, U.K.

1. Addition of L-fucose to energy-depleted anaerobic suspensions of *Escherichia coli* elicited an uncoupler-sensitive alkaline pH change diagnostic of L-fucose/H⁺ symport activity. 2. L-Galactose or D-arabinose were also substrates, but not inducers, for the L-fucose/H⁺ symporter. 3. L-Fucose transport into subcellular vesicles was dependent upon respiration, displayed a pH optimum of about 5.5, and was inhibited by protonophores and ionophores. 4. These results showed that L-fucose transport into *E. coli* was energized by the transmembrane electrochemical gradient of protons. 5. Neither steady state kinetic measurements nor assays of L-fucose binding to periplasmic proteins revealed the existence of a second L-fucose transport system.

INTRODUCTION

Escherichia coli grows on L-fucose (6-deoxy-L-galactose, Fig. 1) as sole carbon source. The cytoplasmic enzymes, L-fucose isomerase, L-fuculose kinase and L-fuculose-1-phosphate aldolase, have been purified and characterized (Green & Cohen, 1956; Heath & Ghalambor, 1962; Ghalambor & Heath, 1962). The L-fucose transport and metabolic enzymes are regulated coordinately, and are induced during growth on L-fucose (Hacking & Lin, 1976, 1977). We present evidence that the initial transport of L-fucose is coupled to the transmembrane electrochemical gradient of protons, $\Delta\mu_{H^+}$. This gradient was postulated by Mitchell (1961, 1963, 1973) to explain the energization of ATP synthesis and transport processes by the oxidoreductive reactions of respiration or photosynthesis (reviewed by Nicholls, 1982). The existence of $\Delta\mu_{H^+}$ across the bacterial cytoplasmic membrane has been substantially confirmed, the polarity being outside acid and positive (Harold, 1970; Ingledew & Poole, 1984; Kagawa, 1978). In *E. coli* $\Delta\mu_{H^+}$ was shown to energize transport of lactose (West, 1970; West & Mitchell, 1972; Flagg & Wilson, 1977; Kaback, 1977), D-galactose (Henderson, 1974; Henderson *et al.*, 1977; Horne & Henderson, 1983), L-arabinose (Henderson, 1974; Daruwalla *et al.*, 1981) and D-xylose (Lam *et al.*, 1980; Davis *et al.*, 1984). Each of these sugars is transported by a separate, biochemically

and genetically distinguishable, transport system, with the common property of coupling inward sugar transport to uptake of protons, sugar/H⁺ symport [or the experimentally indistinguishable antiport of hydroxyl ions (Mitchell, 1973)].

MATERIALS AND METHODS

L-Fucose, L-galactose, D-arabinose, D-fucose, D-galactose, L-glucose, L-rhamnose, L-arabinose and L-mannose were obtained from Sigma Chemical Co. [1-³H]-L-Fucose or [1-¹⁴C]-L-fucose were from Amersham International and were diluted with unlabelled L-fucose to give stock 2 mM solutions of specific radioactivity 1–5 Ci/mol.

E. coli strain JM2418 [$\Delta(his\ gnd)\ \Delta lac$] was grown on basal salts medium (Henderson *et al.*, 1977) supplemented with 10 mM-L-fucose, 20 mM-glycerol, and 80 μ g of L-histidine/ml with shaking at 30 °C. Culture volumes were 1–3 ml in a 12 ml test tube, 200 ml in a 250 ml conical flask, 400 ml in a 500 ml conical flask, or 1250 ml in a 2 litre flask, for experiments investigating induction, energized transport into intact cells, sugar/H⁺ symport assay, or vesicle preparation, respectively. Right-side-out subcellular vesicles were made from spheroplasts (Witholt *et al.*, 1976; Witholt & Boekhout, 1978) by the method of Kaback (1972).

Unless indicated otherwise cultures of *E. coli* strain JM2418 were harvested, depleted of energy reserves, and washed as described by Henderson *et al.* (1977). For measurements of radioactive L-fucose transport into intact cells 150 mM-KCl/5 mM-Mes (pH 6.5) was the supporting medium, the assays being carried out with 10 mM-glycerol plus aeration as described by Henderson *et al.* (1977). Transport of radioactive L-fucose (80 μ M) into subcellular vesicles in 50 mM-potassium phosphate (pH 6.5)/10 mM-MgSO₄ was energized by 20 mM-ascorbate/0.1 mM-phenazine methosulphate plus oxygen and was measured as described by Horne & Henderson (1983). Possible inhibitors were added to the suspensions 3 min before the labelled sugar.

Sugar-promoted pH changes (sugar/H⁺ symport) were measured with energy-depleted anaerobic suspensions of intact cells in 150 mM-KCl/2mM-glycylglycine (pH 6.1–6.7) as detailed by Henderson & Macpherson (1986).

Fig. 1. Structural relationships of sugars similar to L-fucose

The β -form of each sugar is shown.

RESULTS

L-Fucose/H⁺ symport in *E. coli* cells

E. coli strain JM2418 was grown on glycerol plus L-fucose, harvested, depleted and resuspended as described (Henderson & Macpherson, 1986). Addition of L-fucose to the anaerobic suspension elicited a rapid alkaline pH change followed by acidification (Figs. 2 and 3). The initial alkaline pH change was diagnostic of sugar/H⁺ symport activity (West, 1970; West & Mitchell, 1972), while the acidification was probably due to metabolism of L-fucose to acidic end-products (Hacking & Lin, 1976, 1977). The initial rate and the extent of the proton influx (Table 1) was comparable with that described for other sugar/H⁺ symport systems in wild-type *E. coli* strains (West & Mitchell, 1972; Henderson *et al.*, 1977; Lam *et al.*, 1980; Daruwalla *et al.*, 1981).

The appearance of the alkaline pH change was abolished by reagents that render the membrane permeable to protons (Mitchell, 1961, 1970): 4 μ M-tetrachloro-

Fig. 2. Uncoupler-sensitive alkaline pH change promoted by L-fucose

E. coli strain JM2418 was grown on 20 mM-glycerol + 10 mM-L-fucose and additions as described in the Materials and methods section. The organisms were harvested and depleted as described by Henderson & Macpherson (1986). In the two separate experiments shown 10 μ mol of L-fucose was added to an anaerobic suspension of cells (17.3 mg dry mass) in 3.7 ml of 150 mM-KCl/2 mM-glycylglycine, pH 6.24–6.34. The pH was measured as described by Henderson & Macpherson and in Table 1. An upward deflection represents an alkaline pH change. For the lower trace 15 nmol of tetrachlorosalicylanilide was added 35 min before the L-fucose. In a separate experiment (not shown) 4 μ mol of 2,4-dinitrophenol also prevented appearance of the L-fucose-promoted alkaline pH change.

Fig. 3. Alkaline pH changes promoted by L-galactose and D-arabinose in L-fucose-induced *E. coli*

Sugar-promoted pH changes were measured as described for Fig. 2 and Table 1. The top three traces used *E. coli* strain JM2418 (16.5 mg dry mass) originally grown on 20 mM-glycerol + 10 mM-L-fucose and the bottom three the same strain grown on 20 mM-glycerol without L-fucose. The pH at the point of sugar addition was in the range 6.39–6.45. The calibration mark is the mean value from all the experiments.

salicylanilide (Fig. 2) or 1 mM-2,4-dinitrophenol (results not shown). This indicated that the external pH change reflected a difference of pH across the membrane rather than a net change of pH inside and outside the cells.

The pH changes were absent from cells grown on glycerol alone (Table 1), confirming their association with L-fucose-inducible functions of transport or metabolism.

Substrate specificity of the L-fucose/H⁺ symport system

L-Galactose and D-arabinose, which are structural analogues of L-fucose (Fig. 1), each elicited an alkaline pH change in experiments with L-fucose-induced *E. coli* strain JM2418 (Fig. 3). In neither case was a subsequent acidification observed (Fig. 3), consistent with the inability of wild-type *E. coli* strains to grow on either analogue, although mutations in the *fuc* operon permit growth on D-arabinose (LeBlanc & Mortlock, 1971a,b).

Neither L-galactose nor D-arabinose promoted a pH change when L-fucose was omitted from the growth medium (Fig. 2, Table 1), so their activity was associated with L-fucose-inducible functions. D-Fucose, L-glucose, L-rhamnose (6-deoxy-L-mannose), L-mannose, L-arabinose and D-xylose did not promote significant pH changes (Table 1). This confirmed that L-fucose was not being transported by the other sugar/H⁺ symport systems for D-galactose, L-arabinose, or D-xylose (use of a *lac* mutant eliminated the lactose/H⁺ symport system), or on an L-rhamnose/H⁺ symport system (T. Gunn, J. A. R. Muir & T. McDonald, unpublished work).

The average initial rates of H⁺ uptake (mean $\pm$ S.D. in nmol/min per mg dry mass) were 1.05 ± 0.38 (14) for L-

Table 1. Substrate specificity of sugar/H⁺ symport

E. coli strain JM2418 was grown on 20 mM-glycerol and 80 µg of histidine/ml with or without 10 mM-L-fucose. The cultures were harvested, depleted of energy reserves and resuspended in 150 mM-KCl/2 mM-glycylglycine, pH 6.5, as described by Henderson *et al.* (1977). Samples corresponding to about 17 mg dry mass were resuspended under anaerobic conditions in 3.7 ml suspensions, final pH 5.7–6.7. Each of the indicated sugars (10 µmol) was added to one suspension and the subsequent pH change measured from the point of sugar addition to the point where the alkaline change ceased. The values were converted to nmol of H⁺ using deflections produced by addition of 3 nmol of NaOH (not shown in Fig. 2), correcting for any drift rate existing before addition of sugar. They are means of the indicated number of measurements, usually from more than one culture. Abbreviations: n.t., not tested.

Sugar	Proton uptake (nmol of H ⁺ /mg dry mass)	
	L-Fucose-induced	Uninduced
L-Fucose	*1.2 (14)	*0.1 (8)
L-Galactose	6.4 (5)	0.0 (3)
D-Arabinose	2.1 (5)	0.0 (3)
L-Rhamnose	0.0 (2)	0.0 (3)
L-Arabinose	0.0 (3)	0.0 (4)
L-Glucose	0.0 (2)	0.0 (1)
D-Fucose	0.0 (3)	n.t.
D-Xylose	0.0 (2)	n.t.

* Rapid proton extrusion followed the proton uptake in the induced, but not the uninduced, cells.

Table 2. Specificity for inducer of L-fucose transport

E. coli strain JM2418 (0.1 ml in 2.5 % nutrient broth) was inoculated into 1.5 ml or 3 ml of basal salts with 20 mM-glycerol, 80 µg of histidine/ml and one of the indicated sugars (10 mM). After overnight growth to A₆₈₀ 0.7–1.7 each culture was harvested by sedimentation and resuspended in the same volume of 150 mM-KCl/5 mM-Mes, pH 6.5. This washing procedure was repeated a further three times, and a final suspension (A₆₈₀ 1.0–1.9) was used for measurements of the initial rate of transport of 50 µM-L-fucose (³H- or ¹⁴C-labelled) energized by 10 mM-glycerol (see the Materials and methods section). Each value is the mean of at least four measurements on more than one culture expressed as a percentage of the mean value for the same cultures grown with L-fucose (6–18 nmol/min per mg dry mass).

Sugar added	L-Fucose transport activity (%)
L-Fucose	100
L-Galactose	2
L-Arabinose	3
L-Rhamnose	53
L-Glucose	3
D-Fucose	1
D-Xylose	7
L-Xylose	3
L-Lyxose	9
L-Mannose	5
None	4

fucose, 0.71 ± 0.24 (5) for L-galactose and 0.33 ± 0.20 (5) for D-arabinose, reflecting the relative substrate specificities at the concentration used.

Inducer specificity of L-fucose transport

Transport of L-fucose into intact cells of *E. coli* is conveniently measured using the radioisotope-labelled sugar. When strain JM2418 was grown in the presence of different sugars, only cells grown with L-fucose or L-rhamnose displayed subsequent accumulation of L-fucose (Table 2); L-galactose, D-arabinose, D-fucose, L-glucose or L-mannose were not effective inducers (Table 2). This was consistent with a previous observation that D-arabinose was not an inducer of the L-fucose operon in wild-type *E. coli* strains (LeBlanc & Mortlock, 1971a,b; Skjold & Ezekiel, 1982). In *E. coli* and *Klebsiella pneumoniae* L-fuculose 1-phosphate was the true inducer rather than L-fucose (St. Martin & Mortlock, 1976; Bartkus & Mortlock, 1986). Therefore, the reason that L-galactose and D-arabinose were not gratuitous inducers may be that they were not metabolized to the corresponding phosphorylated analogue of L-fucose.

The unexpected induction of the L-fucose/H⁺ transport system by L-rhamnose (Table 2) raised the possibility that L-fucose/H⁺ symport occurred on an L-rhamnose transport system (and *vice versa*). However, in experiments using L-fucose-induced cells, radiolabelled L-rhamnose was not accumulated significantly (results not shown), neither did L-rhamnose inhibit L-fucose transport (see Table 4), nor did it elicit an alkaline pH change (Table 1). The L-fucose and L-rhamnose transport systems are therefore different, and the induction of L-fucose transport by L-rhamnose probably reflects an ability of L-rhamnulose 1-phosphate to substitute for L-fuculose 1-phosphate.

Steady state kinetics of L-fucose transport

The initial rates of L-fucose transport were measured over a wide range of substrate concentrations, 2–400 µM, at pH 6.5. Provided that the external concentration was not depleted by more than 15 %, the relationship between *v* and *s* was a rectangular hyperbola in three independent experiments, yielding apparent *K_m* values in the range 20–40 µM and *V_{max}* of 9–30 nmol/min per mg dry mass. The linearity of the reciprocal plots, one of which is shown in Fig. 4, was an important indication that only one L-fucose transport system was present.

L-Fucose transport into subcellular vesicles of *E. coli*

Right-side-out subcellular vesicles made by the method of Kaback (1972) have been invaluable for studying the energization of substrate transport into micro-organisms (see, e.g., Kaback, 1972, 1974, 1976, 1977; Ramos *et al.*, 1976; Ramos & Kaback, 1977).

Radioisotope-labelled L-fucose was accumulated by such vesicles made from cells grown on L-fucose (Fig. 5, Table 3). The transport was critically dependent upon the presence of a respiratory substrate, ascorbate + phenazine methosulphate (Fig. 5) and was prevented by a respiratory inhibitor, cyanide (Table 3); respiration was necessary to generate Δμ_{H⁺} (Ramos *et al.*, 1976; Ramos & Kaback, 1977). Even when respiratory substrate was present, transport of L-fucose was inhibited by protonophores (dinitrophenol and tetrachlorosalicylanilide) or ionophores [valinomycin or nigericin

Fig. 4. Monophasic steady-state kinetics of L-fucose transport

E. coli strain JM2418 was grown on 20 mM-glycerol + 10 mM-L-fucose, harvested and resuspended in 150 mM-KCl/5 mM-Mes, pH 6.5, + 1 mM-mercaptoethanol for 1 h at 30 °C. The resedimented cells were resuspended in the same medium but without mercaptoethanol, sedimented again and finally resuspended to a density of 0.68 mg dry mass/ml. Aerated samples (0.2 ml) were incubated at 25 °C for 3 min with 25 mM-glycerol before addition of 50 μ l of [1-¹⁴C]-L-fucose solution to give the indicated concentration range of 2–400 μ M. After 15 s 0.2 ml was filtered rapidly and washed twice with approx. 2 ml of the above medium. The radioactivity trapped on the filter was measured and converted to nmol of sugar as described elsewhere (Henderson *et al.*, 1977). At L-fucose concentrations below 8 μ M measurements were made in triplicate, and in duplicate above this concentration. The deduced K_m was 20.9 ± 0.9 μ M and the V_{max} was 34.7 ± 0.5 nmol/min per mg dry mass.

(Henderson, 1970; Harold *et al.*, 1974)], compounds that discharge the protonmotive force (Fig. 5 and Table 3).

Maximum activity of the L-fucose transport system occurred at pH 5.5, a value similar to the pH optimum for proton-linked lactose or galactose transport into subcellular vesicles (Ramos & Kaback, 1977; Ramos *et al.*, 1976; Horne & Henderson, 1983). This corresponded closely to the pH dependence of the protonmotive force (Ramos *et al.*, 1976). At pH 6.5 the average apparent K_m for L-fucose was in the range of 40–55 μ M with an average apparent V_{max} of about 4 nmol/min per mg of protein (data from three batches of vesicles).

Attempts to detect L-fucose-promoted pH changes in vesicles were not successful, possibly because this requires higher levels of transport activity achieved by amplifying gene expression (see, e.g., Daruwalla *et al.*, 1981; Patel *et al.*, 1982; Horne & Henderson, 1983). Nevertheless,

Fig. 5. Respiration-dependence and uncoupler-sensitivity of L-fucose transport into subcellular vesicles of *E. coli*

Subcellular vesicles were prepared from glycerol + L-fucose-grown cells of strain JM2418 and transport of 80 μ M radioisotope-labelled L-fucose was measured as described by Horne & Henderson (1983). ○, Ascorbate/phenazine methosulphate as respiratory substrate; ●, ascorbate/phenazine methosulphate + 1 mM-2,4-dinitrophenol; △, no additions. Each point is the mean of at least four measurements on at least two batches of vesicles.

these results all showed that L-fucose transport was linked to $\Delta\mu_{H^+}$.

Inhibition of L-fucose transport by sugars

In order to substantiate the substrate specificity of the L-fucose transport system, the ability of unlabelled sugars to inhibit transport of radioactive L-fucose was measured using both intact cells and subcellular vesicles (Table 4). These results confirmed that L-galactose and D-arabinose were interacting with an L-fucose transport system; no significant interaction was observed with L-rhamnose, L-glucose, L-mannose, D-fucose or D-xylose. L-Galactose and D-arabinose were competitive inhibitors of L-fucose transport (J. A. R. Muir, unpublished work).

DISCUSSION

The observation of an L-fucose-promoted alkaline pH change in intact cells, the dependence of L-fucose transport into vesicles on respiration, and its sensitivity to uncoupling agents or ionophores confirmed the existence of an L-fucose/ H^+ symport system in *E. coli*. The possibility of L-fucose uptake by one of the other sugar/ H^+ symporters (for lactose, D-galactose, L-arabinose, D-xylose or L-rhamnose) was eliminated by its occurrence in a *lac* deletion mutant, and by its different substrate and inducer specificities compared with galactose/ H^+ symport (Henderson *et al.*, 1977; Henderson & Giddens, 1977), xylose/ H^+ symport (Lam *et al.*, 1980),

Table 3. Effects of inhibitors and ionophores on energized L-fucose transport into subcellular vesicles

Transport of radioactive L-fucose (40 μ M) into right-side-out vesicles made from *E. coli* strain JM2418 grown on L-fucose plus glycerol was measured as described by Horne & Henderson (1983) using 20 mM-ascorbate/0.1 mM-phenazine methosulphate (PMS) and oxygen for energization. The indicated additions were made 3 min before the sugar. Each value is the mean of at least four measurements on more than one batch of vesicles expressed as a percentage of the initial rate obtained with L-fucose alone (3–9 nmol/min per mg of protein). The controls contained an equivalent concentration of ethanol when this was a solvent for the compound added.

Addition	L-Fucose transport activity (%)
None	100
2,4-Dinitrophenol (1 mM)	7
Tetrachlorosalicylanilide (20 μ M)	11
KCN (4 mM)	22
Valinomycin (4 μ M)	17
Nigericin (4 μ g/ml)	31
Unenergized control (ascorbate/PMS omitted)	6

Table 4. Inhibition of L-fucose transport by substrate analogues

Transport of radioisotope-labelled L-fucose was measured using washed suspensions of L-fucose-induced *E. coli* strain JM2418 (Table 2) or right-side-out vesicles (Table 3). The indicated unlabelled sugar, 5.0 mM for the intact cells and 0.8 mM for the vesicles, was added to the incubation 3 min before the labelled L-fucose. Each value is the mean of measurements of initial rate expressed as a percentage of the rate obtained with L-fucose alone in the same culture or batch of vesicles. At least four measurements on more than one preparation were made. Abbreviation: n.t., not tested.

Sugar added	Initial rate of L-fucose transport (%)	
	Intact cells	Vesicles
None	100	100
L-Fucose	2	18
L-Galactose	9	44
D-Arabinose	29	105
L-Rhamnose	90	147
L-Glucose	94	162
D-Fucose	112	155
D-Xylose	102	167
L-Mannose	102	133
D-Galactose	n.t.	110
D-Altrose	n.t.	110

arabinose/H⁺ symport (Daruwalla *et al.*, 1981) or L-rhamnose/H⁺ symport (T. Gunn, unpublished work). Hence there are at least six separate sugar/H⁺ symport systems in *E. coli*. A similar L-fucose/H⁺ symport was present in *Salmonella typhimurium* LT2, and in *Klebsiella*

pneumoniae, but not in strains of *Erwinia carotovora* or *Erwinia atroseptica* (T. McDonald, unpublished work).

For each of the sugars D-galactose, L-arabinose and D-xylose there exists, in *E. coli*, a second transport system containing a binding protein (see, e.g. Boos, 1969; Brown & Hogg, 1972; Schleif, 1969, Ahlem *et al.*, 1982). These are not energized by $\Delta\mu_{H^+}$, but by a phosphorylated product of glycolysis (see, e.g., Berger, 1973; Berger & Heppel, 1974). We examined the possible existence of an L-fucose binding protein in L-fucose-induced *E. coli* strain JM2418 by extracting periplasmic proteins, concentrating them, and performing a binding protein assay with 1 μ M radioactive L-fucose using equilibrium dialysis as described by Clark & Hogg (1981). No binding of L-fucose was detected. An identical procedure performed on D-xylose-induced cells clearly detected the xylose binding protein described by Ahlem *et al.* (1982). Arsenate (20 mM) inhibited the transport of L-fucose (50–200 μ M) into intact cells by 30–50%; generally, binding protein transport systems are inhibited 60–90% by such arsenate concentrations and proton-linked systems are inhibited by 0–50% (see, e.g. Berger, 1973; Berger & Heppel, 1974; Daruwalla *et al.*, 1981; Davis *et al.*, 1984). The steady state kinetic plots were monophasic (Fig. 4). It therefore seemed unlikely that there was a second L-fucose transport system.

L-Galactose and D-arabinose will be useful alternative substrates for studying the L-fucose/H⁺ symport. The absence of acid extrusion (Fig. 3) showed that they were not metabolized completely, but they could still be substrates for the initial enzymes of L-fucose metabolism. Their failure to induce L-fucose transport was disappointing in view of the potential value of gratuitous inducers, but could be explained if they were not metabolized to the phosphorylated analogue of fucose 1-phosphate, the inducer (Bartkus & Mortlock, 1986). We obtained mutants impaired in L-fucose transport, but they also appeared to be defective in general expression of the L-fucose operon (see also Chakrabarti *et al.*, 1984; Chen *et al.*, 1983); this might be a consequence of there being only one transport system. Regulation of the L-fucose operon is an incompletely understood process, alteration of which enables *E. coli* to utilize D-arabinose (LeBlanc & Mortlock, 1971a,b; Skjold & Ezekiel, 1982) or propanediol (Hacking & Lin, 1976, 1977; Hacking *et al.*, 1978; Boronat & Aguilar, 1981).

However, the prime purpose of this work was an initial characterization of a different sugar/H⁺ symport system. Comparison of its similarities to, and differences from, other such systems in *E. coli* will aid elucidation of their molecular mechanism.

We thank the SERC for financial support through grant GR/D/22201. Mr. T. McDonald provided expert technical assistance.

REFERENCES

- Ahlem, C., Huisman, W., Neslund, G. & Dahms, A. S. (1982) J. Biol. Chem. **257**, 2926–2931
- Bartkus, J. M. & Mortlock, R. P. (1986) J. Bacteriol. **165**, 710–714
- Berger, E. A. (1973) Proc. Natl. Acad. Sci. U.S.A. **70**, 1514–1518

- Berger, E. A. & Heppel, L. (1974) *J. Biol. Chem.* **249**, 7747–7755
- Boos, W. (1969) *Eur. J. Biochem.* **10**, 66–73
- Boronat, A. & Aguilar, J. (1981) *J. Bacteriol.* **147**, 181–185
- Brown, C. E. & Hogg, R. W. (1972) *J. Bacteriol.* **111**, 606–613
- Chakrabarti, T., Chen, Y.-M. & Lin, E. C. C. (1984) *J. Bacteriol.* **157**, 984–986
- Chen, Y.-M., Lin, E. C. C., Ross, J. & Aguilar, J. (1983) *J. Gen. Microbiol.* **129**, 3355–3362
- Clark, A. F. & Hogg, R. W. (1981) *J. Bacteriol.* **147**, 920–924
- Daruwalla, K. R., Paxton, A. T. & Henderson, P. J. F. (1981) *Biochem. J.* **200**, 611–627
- Davis, E. O., Jones-Mortimer, M. C. & Henderson, P. J. F. (1984) *J. Biol. Chem.* **259**, 1520–1525
- Flagg, J. L. & Wilson, T. H. (1977) *J. Memb. Biol.* **31**, 233–255
- Ghahambor, M. A. & Heath, E. C. (1962) *J. Biol. Chem.* **237**, 2427–2433
- Green, M. & Cohen, S. S. (1956) *J. Biol. Chem.* **219**, 557–568
- Hacking, A. J. & Lin, E. C. C. (1976) *J. Bacteriol.* **126**, 1166–1172
- Hacking, A. J. & Lin, E. C. C. (1977) *J. Bacteriol.* **130**, 832–838
- Hacking, A. J., Aguilar, J. & Lin, E. C. C. (1978) *J. Bacteriol.* **136**, 522–530
- Harold, F. M. (1970) *Adv. Microb. Physiol.* **4**, 45–104
- Harold, F. M., Altendorf, K. & Hirata, H. (1974) *Ann. N.Y. Acad. Sci.* **235**, 149–160
- Heath, E. C. & Ghahambor, M. A. (1962) *J. Biol. Chem.* **237**, 2423–2426
- Henderson, P. J. F. (1970) *Annu. Rev. Microbiol.* **25**, 393–428
- Henderson, P. J. F. (1974) in *Comparative Biochemistry and Physiology of Transport* (Bolis, L., Bloch, K., Luria, S. E. & Lynen, F., eds.), pp. 409–424, North-Holland Publishing Co., Amsterdam
- Henderson, P. J. F. & Giddens, R. A. (1977) *Biochem. J.* **168**, 15–22
- Henderson, P. J. F., Giddens, R. A. & Jones-Mortimer, M. C. (1977) *Biochem. J.* **162**, 309–320
- Henderson, P. J. F. & Macpherson, A. J. S. (1986) *Methods Enzymol.* **125**, 387–429
- Horne, P. & Henderson, P. J. F. (1983) *Biochem. J.* **210**, 699–705
- Ingledew, W. J. & Poole, R. K. (1984) *Microbiol. Rev.* **48**, 222–271
- Kaback, H. R. (1971) *Methods Enzymol.* **22**, 99–122
- Kaback, H. R. (1972) *Biochim. Biophys. Acta* **265**, 367–416
- Kaback, H. R. (1974) *Science* **186**, 882–892
- Kaback, H. R. (1976) *J. Cell. Physiol.* **89**, 575–593
- Kaback, H. R. (1977) *FEBS Symp.* **42**, 598–625
- Kagawa, Y. (1978) *Biochim. Biophys. Acta* **505**, 45–93
- Lam, V. M. S., Daruwalla, K. R., Henderson, P. J. F. & Jones-Mortimer, M. C. (1980) *J. Bacteriol.* **143**, 396–402
- LeBlanc, D. J. & Mortlock, R. P. (1971a) *J. Bacteriol.* **106**, 82–89
- LeBlanc, D. J. & Mortlock, R. P. (1971b) *J. Bacteriol.* **106**, 90–96
- Mitchell, P. (1961) *Nature (London)* **191**, 144–148
- Mitchell, P. (1963) *Biochem. Soc. Symp.* **22**, 142–168
- Mitchell, P. (1970) *Symp. Soc. Gen. Microbiol.* **20**, 121–166
- Mitchell, P. (1973) *J. Bioenerg.* **4**, 63–91
- Nicholls, D. G. (1982) *Bioenergetics: An Introduction to the Chemiosmotic Theory*, Academic Press, New York
- Patel, L., Garcia, M. L. & Kaback, H. R. (1982) *Biochemistry* **21**, 5805–5810
- Ramos, S., Schuldiner, S. & Kaback, H. R. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1892–1896
- Ramos, S. & Kaback, H. R. (1977) *Biochemistry* **16**, 854–859
- Schleif, R. (1969) *J. Mol. Biol.* **46**, 185–196
- Skjold, A. S. & Ezekiel, D. H. (1982) *J. Bacteriol.* **152**, 521–523
- St. Martin, E. J. & Mortlock, R. P. (1976) *J. Bacteriol.* **127**, 91–97
- West, I. C. (1970) *Biochem. Biophys. Res. Commun.* **41**, 655–661
- West, I. C. & Mitchell, P. (1972) *J. Bioenerg.* **3**, 445–462
- Witholt, B., Boekhout, M., Brock, M., Kingma, J., Van Heerikhuizen, H. & De Leij, L. (1976) *Anal. Biochem.* **74**, 160–170
- Witholt, B. & Boekhout, M. (1978) *Biochim. Biophys. Acta* **508**, 296–305