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Ubiquinone-mediated coupling of NADH dehydrogenase to active transport in membrane vesicles from *Escherichia coli**

(energy-coupling site/specificity of energy-coupling/orientation of vesicle membrane/membrane potentials)

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ABSTRACT **Addition of ubiquinone-1 to *E. coli* ML 308-225 membrane vesicles dramatically increases coupling between NADH oxidation and active transport such that initial rates and steady-state levels of lactose and amino-acid accumulation are comparable to those observed during D-lactate oxidation. Similar but less dramatic effects are observed with the quinone and succinate or L-lactate. In the presence of NADH and ubiquinone-1, the vesicles also generate a membrane potential (interior negative) that is similar in magnitude to that observed in the presence of D-lactate. Stimulation of NADH-dependent transport by ubiquinone-1 cannot be accounted for by increased rates of oxidation of NADH, and the effect of the quinone on NADH-dependent lactose transport is not observed in vesicles depleted of NADH dehydrogenase activity. Thus, it is apparent that ubiquinone-1 shunts electrons from NADH dehydrogenase [NADH:(acceptor)oxidoreductase; EC 1.6.99.3] to the portion of the respiratory chain containing the energy-coupling site. The findings demonstrate unequivocally that inefficient coupling of NADH oxidation to active transport cannot be due to the presence of inverted vesicles. In addition, they provide further support for specific localization of the energy-coupling site.**

Membrane vesicles isolated from various bacterial cells catalyze active transport by a respiration-dependent mechanism that does not involve the generation or utilization of ATP or other high-energy phosphate compounds (1–4). In vesicles prepared from *Escherichia coli* and *Salmonella typhimurium*, most transport systems are coupled primarily to the oxidation of D-lactate or reduced phenazine methosulfate (or pyocyanine) via a membrane-bound respiratory chain with oxygen or, under appropriate conditions (5–7), fumarate or nitrate as terminal electron acceptors.

One of the most striking and controversial aspects of the vesicle system is the specificity of the physiological electron donors that generate the membrane potential (interior negative) and drive active transport (1–4, 8–15)†. In *E. coli* vesicles, of a large number of potential energy sources tested, very few replace D-lactate to any extent whatsoever, and none is as effective, although many are oxidized at least as rapidly and reduce the same membrane-bound cytochromes, qualitatively and quantitatively (1–4, 8–10, 12, 14, 15)†. For this and other reasons, it was suggested that the energy-coupling site for active transport is located in a relatively specific segment of the respiratory chain between D-lactate dehydrogenase and cytochrome b_1, the first cytochrome in the common portion of the *E. coli* respiratory chain. It has been argued, however, that significant numbers of membrane vesicles become inverted during preparation, and that these inverted vesicles oxidize NADH and other electron donors but do not catalyze active transport (16–21). There is an accumulating body of evidence that this is not the case, and that all of the vesicles have the same orientation as the membrane in the intact cell. Some of this evidence is as follows: (*i*) Initial rates of transport in the vesicles are similar to those observed in whole cells in many instances (14, 22). Moreover, the steady-state level of accumulation of transport substrates is comparable frequently to that observed in the intact cell. (*ii*) Freeze fracture studies of membrane vesicles (1–4, 23–25) demonstrate that the "texture" of the convex surface of the vesicles is distinctly different from that of the concave surface, and that the vesicles are homogeneous in this regard. In addition, the texture observed on the respective surfaces is the same as that observed in the intact cell. (*iii*) As mentioned above, all electron donors oxidized by the vesicles reduce the same cytochromes, qualitatively and quantitatively. If a significant percentage of the vesicles is inverted, and only the inverted vesicles oxidize NADH, it is difficult to understand how NADH is able to reduce all of the cytochromes in the preparations. (*iv*) Recent experiments utilizing radioautography and electron microscopy demonstrate directly that essentially all of the vesicles catalyze active transport (26). (*v*) Studies with antibodies against D-lactate dehydrogenase and Ca^{++},Mg^{++}-stimulated ATPase demonstrate that both of these membrane-bound enzymes are present exclusively on the inner surface of the vesicle membrane (27, 28). (*vi*) Fluorescence of 1-anilino-8-naphthalenesulfonate (ANS) is quenched upon addition of D-lactate to *E. coli* ML 308-225 membrane vesicles (29), an observation similar to that observed in energized mitochrondria and ethylenediaminetetraacetic acid-treated intact *E. coli*. In chloroplasts and submitochondrial particles, in which the polarity of the membrane is opposite to that of intact mitochondria, ANS fluorescence is enhanced upon energization. It follows that any inverted membrane vesicles in the preparations would exhibit enhancement of ANS fluorescence in the presence of D-lactate. If more than 50% of the vesicles are inverted (20, 21), no net change or an increase in ANS fluorescence should have been observed. (*vii*) Inverted vesicles catalyze calcium accumulation but do not catalyze D-lactate-dependent proline transport (30). In contrast, vesicles prepared by osmotic lysis (23) do not exhibit calcium transport.

The experiments presented in this paper provide an unequivocal demonstration that oxidation of NADH by the vesicles cannot be the result of artifactual inversion of a portion of the vesicle population. In addition, the results support the suggestion that the energy-coupling site for active trans-

Abbreviations: ANS, 1-anilino-8-naphthalenesulfonic acid; $TPMP^+$, triphenylmethylphosphonium bromide; NADH, reduced nicotinamide adenine dinucleotide; NADPH, reduced nicotinamide adenine dinucleotide phosphate.

* This is paper XXXII in the series "Active Transport in Isolated Bacterial Membrane Vesicles". Paper XXXI is S. Schuldiner, R. Weil, and H. R. Kaback, submitted for publication.

† S. Schuldiner and H. R. Kaback, submitted for publication.

port is located in a relatively specific segment of the respiratory chain.

METHODS

Growth of Cells and Preparation of Membrane Vesicles. *E. coli* ML 308-225 ($i^-z^-y^+a^+$) was grown in minimal medium A containing 1.0% sodium succinate (hexahydrate) as sole carbon source, and membrane vesicles were prepared as described (23, 28).

Transport Assays were carried out as described (23, 31). [*1*-^{14}C]Lactose (22 mCi/mmol) and [^{3}H]triphenylmethylphosphonium bromide ($TPMP^+$) (114 mCi/mmol) were used at final concentrations of 0.4 mM. The specific activities and final concentrations of the uniformly labeled L-[^{14}C]aminoacids used were as follows: alanine (153 mCi/mmol), 25 μM; proline (232 mCi/mmol), 16 μM; serine (156 mCi/mmol), 25 μM; aspartic acid (208 mCi/mmol), 18 μM; tyrosine (404 mCi/mmol), 6.9 μM; leucine (280 mCi/mmol), 46 μM; and lysine (306 mCi/mmol), 12 μM. Lactose and amino-acid transport was assayed with Schleicher and Schuell Selectron filters (0.45 μm pore size) and $TPMP^+$ uptake was assayed with Millipore Cellotate filters (0.5 μm pore size)†.

Where indicated, an aliquot of an ethanolic solution of ubiquinone-1 was added to the reaction mixtures to yield the final concentration of ubiquinone-1 desired. The stock solutions of ubiquinone-1 were sufficiently concentrated so that the final concentration of ethanol in the reaction mixtures was 0.1%. Ethanol, at this concentration, had no effect on either transport or oxygen consumption.

Oxygen Utilization. Rates of oxygen uptake were determined with a Clark electrode (YSI model 53 oxygen monitor) as described (10).

Protein Determinations were carried out as described by Lowry *et al.* (32).

Chemicals. Ubiquinone-1 was generously provided by Hoffmann-LaRoche, Inc., Basel, Switzerland. Pyocyanine perchlorate was obtained from K & K Laboratories, Inc., Plainview, N.Y. All other materials were of reagent grade obtained from commercial sources.

RESULTS

Effect of ubiquinone-1 on lactose and amino-acid transport

As shown by the data presented in Fig. 1 (open symbols), lactose transport by *E. coli* ML 308-225 membrane vesicles is simulated maximally by D-lactate (panel B). Although succinate (panel C) and L-lactate (panel D) replace D-lactate to some extent, they stimulate the initial rate of transport less than half as well. NADH (panel A) is a poor electron donor for active transport, eliciting almost no effect on either the initial rate or steady-state level of lactose accumulation. Similar effects of these electron donors on active transport (1–4, 10, 14, 15). ANS fluorescence (29), and 2′-(*N*-dansyl)aminoethyl 1-thio-β-D-galactopyranoside fluorescence (33) have been reported. Strikingly, when ubiquinone-1 is added to the vesicles (Fig. 1, closed symbols), NADH-, succinate-, and L-lactate-dependent transport are stimulated, and in each case, transport activity approaches that observed in the presence of D-lactate. Ubiquinone-1 has little or no effect on lactose transport in the presence of D-lactate. The stimulatory effect of ubiquinone-1 is most marked with NADH as electron donor, and there is a 20- to 30-fold increase in the rate and extent of lactose transport. With succinate and L-lactate,

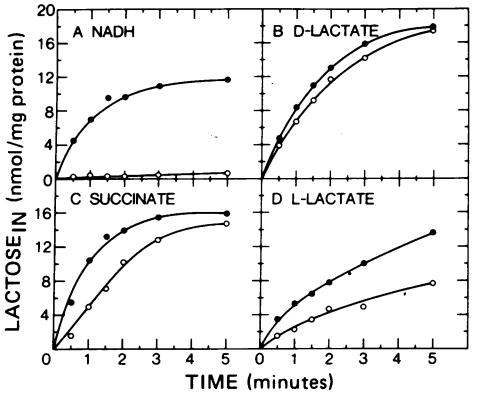

FIG. 1. Effect of ubiquinone-1 on lactose transport by *E. coli* ML 308-225 membrane vesicles in the presence of various electron donors. Aliquots (25 μl) of membrane vesicles containing 5.6 mg of protein per ml were diluted to a final volume of 50 μl containing (in final concentrations) 50 mM potassium phosphate (pH 6.6), 10 mM magnesium sulfate, and 80 μM ubiquinone-1 and 0.1% ethanol, where indicated. Ethanol (0.1% final concentration) was added to the control samples. After 30 sec of incubation at 25°, the indicated dehydrogenase substrate was added, and immediately thereafter, [*1*-^{14}C]lactose (22 mCi/mmol) at a final concentration of 0.4 mM. Final concentrations of the dehydrogenase substrates were 5 mM, 20 mM, 20 mM, and 20 mM for NADH (A), D-lactate (B), succinate (C), and L-lactate (D), respectively. The samples were incubated at 25° for the times shown, and the reactions were terminated and assayed as described (23, 31). ○, Control; ●, plus ubiquinone-1.

stimulation observed on addition of the quinone is at least an order of magnitude less than that observed with NADH.

The dramatic effect of ubiquinone-1 on NADH-dependent active transport is also observed with the amino-acid transport systems (Fig. 2). In the absence of ubiquinone-1, NADH has little effect on the transport of proline, serine, alanine, aspartate, tyrosine, leucine, or lysine. In the presence of the quinone, however, NADH-dependent transport of each amino acid is comparable to that observed in the presence of D-lactate.

The concentration dependence for ubiquinone-1 with respect to the initial rate of proline transport in the presence of NADH is shown in Fig. 3. From 0 to about 40 μM ubiquinone-1, proline transport activity increases markedly, achieving a maximum which remains constant up to about 100 μM. Although not shown, concentrations higher than 100 μM result in progressive inhibition. Accordingly, throughout the experiments reported here ubiquinone-1 was used at a final concentration of 80 μM (corresponding to about 28 nmol of ubiquinone-1 per mg of membrane protein).

Effect of ubiquinone-1 on oxidase activities of membrane vesicles

As shown previously (1–4, 10, 14, 15), the ability of *E. coli* ML 308-225 membrane vesicles to oxidize a particular electron donor bears no quantitative relationship to the ability of the electron donor to drive active transport. This observation is confirmed in Table 1. Although D-lactate is the best physiological electron donor for transport (Fig. 1), NADH and succinate are oxidized more rapidly, and L-lactate is oxidized approximately 60% as well as D-lactate. When the ability of the vesicles to oxidize these electron donors is measured in the presence of ubiquinone-1, there is a mild increase in the rate of oxidation of each electron donor, although the relative rates remain essentially unchanged. It is

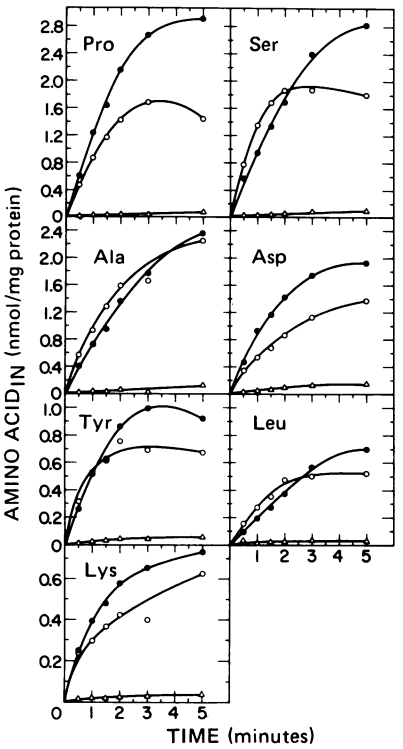

FIG. 2. Effect of ubiquinone-1 on amino-acid transport by *E. coli* ML 308-225 membrane vesicles in the presence of NADH. Assays were carried out as described in the legend of Fig. 1, using the amino acids indicated at specific activities and final concentrations given in *Methods*. △, NADH; ○, NADH plus ubiquinone-1; ●, D-lactate.

apparent from these data that the increased ability of NADH in particular, but also succinate or L-lactate, to drive transport in the presence of ubiquinone-1 cannot be attributed simply to increased rates of oxidation of these electron donors. Rather, it is clear that the quinone increases the efficiency of the system with respect to these electron donors.

Effect of ubiquinone-1 on the ability of membrane vesicles to generate an electrical potential (interior negative) in the presence of NADH

Recent experiments (4, 34–36)† demonstrate that oxidation of D-lactate by isolated membrane vesicles results in the generation of a membrane potential (interior negative), and that this potential is intimately involved in the mechanism of active transport. Moreover, electron donors such as NADH, which do not drive active transport, also do not generate a membrane potential†. As shown previously (4)† and in Fig. 4, in the presence of D-lactate, ML 308-225 vesicles accumulate the lipophilic cation TPMP$^+$ rapidly, achieving a steady state within 1–2 min. In contrast, the vesicles do not accumulate TPMP$^+$ in the presence of NADH or NADPH. On addition of ubiquinone-1, however, NADH stimulates TPMP$^+$ uptake as well as D-lactate, indicating that under these conditions, NADH oxidation results in the generation of a membrane potential (interior negative). It is also noteworthy that in the presence of ubiquinone-1, NADPH also stimulates TPMP$^+$ uptake, but only about 20% as well as NADH. Although not shown, similar results were obtained with lactose and amino-acid transport. The significance of this observation will be discussed below.

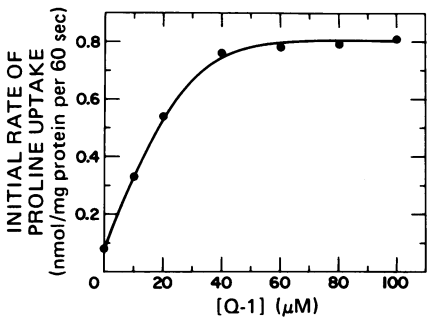

FIG. 3. Effect of increasing concentrations of ubiquinone-1 (Q-1) on NADH-dependent proline uptake by *E. coli* ML 308-225 membrane vesicles. Assays were carried out in the presence of 5 mM NADH and ubiquinone-1 at the designated concentrations as described in the legend of Fig. 1. At each ubiquinone-1 concentration, proline uptake was determined at 0.25, 0.5, 0.75, and 1 min, and initial rates were determined from these data.

Nature of ubiquinone-1 stimulation of active transport

Since it is well known that certain artificial electron carriers, such as phenazine methosulfate (31, 37) or pyocyanine (31), are able to drive active transport in the presence of appropriate reducing agents, the effects of ubiquinone-1 presented thus far might be trivial. That is, it is possible that ubiquinone-1 is reduced nonenzymatically by NADH in the bulk phase, and that the reduced quinone then donates electrons to the respiratory chain in a manner similar to that of the aforementioned electron carriers. The following experiments demonstrate that this is not the case.

The artificial electron carrier pyocyanine is able to drive active transport in the vesicles system with either NADH or NADPH as reductant. This is expected since both reduced nicotinamide coenzymes have essentially the same redox potential (38). On the other hand, stimulation of TPMP$^+$ uptake (Fig. 4) or lactose and amino-acid transport (not shown) by NADPH in the presence of ubiquinone-1 is only about 20% of that observed in the presence of NADH and the quinone, an activity which correlates reasonably well with the ability of the vesicles to oxidize NADPH relative to NADH (Table 1). Thus, stimulation of transport by NADH in the presence of ubiquinone-1 cannot be due to nonenzymatic re-

Table 1. Effect of ubiquinone-1 on oxidase activities of *E. coli* ML 308-225 membrane vesicles

Substrate*	Oxygen consumption (ng atoms O_2/min per mg protein) No additions	+80 μM ubiquinone-1	% Increase
NADH	188	238	27
D-Lactate	122	143	17
Succinate	244	254	4.1
L-Lactate	81.4	103	27
NADPH	19.0	24.5	29

Oxygen consumption was measured with a Clark-type oxygen electrode as described (10), using a membrane protein concentration of 1.1 mg/ml. The reaction mixtures also contained 0.1% ethanol in all cases.

* Substrates were used at the following final concentrations: sodium NADH, 5 mM; lithium D-lactate, 20 mM; sodium succinate, 20 mM; lithium L-lactate, 20 mM; and sodium NADPH, 5 mM.

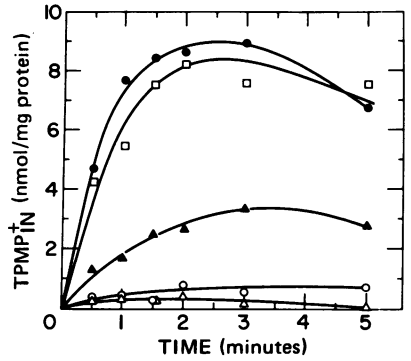

FIG. 4. Effect of ubiquinone-1 on NADH-dependent $TPMP^+$ uptake by *E. coli* ML 308-225 membrane vesicles. The assays were carried out as described in the legend of Fig. 1, except that [^{3}H]$TPMP^+$ (114 mCi/mmol) was used at a final concentration of 0.4 mM in place of [1-^{14}C]lactose and Millipore Cellotate filters were used to assay the samples†. Where indicated, D-lactate, NADH, NADPH, and ubiquinone-1 were used at final concentrations of 20 mM, 5 mM, 5 mM, and 80 μM, respectively. □, D-Lactate; ●, NADH plus ubiquinone-1; ▲, NADPH plus ubiquinone-1; ○, NADH; △, NADPH.

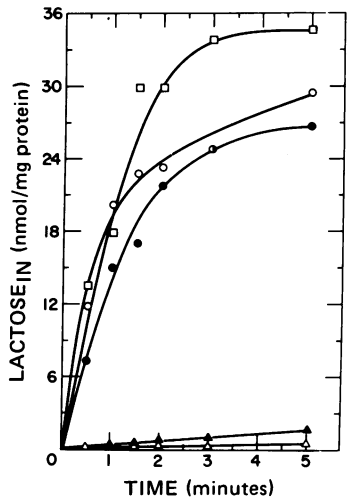

FIG. 5. Lactose uptake by *E. coli* ML 308-225 membrane vesicles depleted of NADH dehydrogenase activity. Membrane vesicles were extracted with 1.0 M guanidine·HCl, washed, and subsequently treated with 70 μM dicyclohexylcarbodiimide for 30 min as described previously (36). Vesicles treated in this fashion oxidized NADH at 10–15% of the rate of appropriately treated control preparations. Lactose uptake was measured using a final concentration of 0.4 mM [1-^{14}C]lactose (22 mCi/mmol). Where indicated, the following additions were made: □, ascorbate and phenazine methosulfate at 20 mM and 0.1 mM, respectively; ○, NADH and pyocyanine perchlorate at 5 mM and 0.1 mM, respectively; ●, NADPH and pyocyanine perchlorate at 5 mM and 0.1 mM, respectively; ▲, NADH and ubiquinone-1 at 5 mM and 80 μM, respectively; and △, NADPH and ubiquinone-1 at 5 mM and 80 μM, respectively.

duction of the quinone by reduced nicotinamide coenzyme in the bulk phase. It is also noteworthy in this regard that nonenzymatic reduction of ubiquinone-1 by NADH or NADPH is not observed by direct spectrophotometric measurements (data not shown).

Extraction of isolated membrane vesicles with chaotropic agents inactivates transport in a manner that can be completely reversed by treatment with various carbodiimides (36). These effects result from specific alterations in the permeability of the vesicle membrane to protons. In addition to effects on proton permeability, extraction of vesicles with chaotropic agents solubilizes 50–60% of the membrane-bound D-lactate dehydrogenase. It is especially noteworthy with respect to these experiments that extraction of the vesicles with these reagents also diminishes the ability of the vesicles to oxidize NADH by 85–90%. As reported previously (36) and confirmed in Fig. 5, when vesicles are extracted with 1.0 M guanidine·HCl and subsequently treated with dicyclohexylcarbodiimide, high rates of lactose transport are observed with ascorbate-phenazine methosulfate as the electron donor system. It is also apparent that NADH and NADPH drive transport effectively with pyocyanine as electron carrier. In marked contrast, however, neither NADH nor NADPH is able to drive lactose transport to a significant extent with ubiquinone-1. These results demonstrate clearly that ubiquinone-1 is unable to mediate the flow of electrons from NADH or NADPH to the membrane-bound respiratory chain in the absence of NADH dehydrogenase [NADH-(acceptor):oxidoreductase; EC 1.6.99.3] activity.

DISCUSSION

The results presented in this paper demonstrate that addition of ubiquinone-1 to *E. coli* ML 308-225 membrane vesicles in the presence of NADH results in the generation of a membrane potential (interior negative) and initial rates and steady-state levels of lactose and amino-acid transport that are comparable to those observed with D-lactate. Since the effects of ubiquinone-1 are dependent upon NADH dehydrogenase activity and markedly diminished with NADPH, a poor substrate for NADH dehydrogenase, it seems apparent that ubiquinone-1 is able to shunt electrons from NADH dehydrogenase to an energy-coupling site that is not located in the portion of the respiratory chain between NADH dehydrogenase and the cytochromes. As such, the observations support a previous hypothesis (1, 4, 10) that the energy-coupling site (i.e., the site at which the membrane potential is generated†) is located in a relatively specific segment of the respiratory chain between D-lactate dehydrogenase and cytochrome b_1.

Since active transport is a vectorial process, it is generally believed that only vesicles that retain the same orientation as the membrane in the intact cell can catalyze active transport. If this assumption is valid, the results presented here demonstrate unequivocally that oxidation of NADH by the vesicle preparations cannot be due to the presence of inverted vesicles, since NADH oxidation via NADH dehydrogenase is able to drive transport and generate a membrane potential as effectively as D-lactate. The findings, especially when considered in the light of other observations summarized at the beginning of the paper, provide strong support for the contention that virtually none of the vesicles in these preparations is inverted.

In addition to their implications with regard to active transport, the results presented here are interesting with respect to the physical state of the quinones present endogenously in the vesicle membrane. The vesicles used in these experiments contain approximately 6.0 nmol of ubiquinone-8 per mg of membrane protein‡. Since quinones are electron carriers that are presumed to be freely diffusible within the

‡ The quinone content of the vesicles was determined as described (39, 40). In addition to ubiquinone-8, the vesicles also contain approximately 2.1 nmol of menaquinone-8 per mg of membrane protein.

membrane, it is not immediately obvious why the vesicles should require exogenous ubiquinone-1 in order for NADH oxidation to perform work. Furthermore, although data are not presented in this paper, it has been shown that menaquinone-1 does not stimulate active transport in the presence of NADH in *E. coli* vesicles, but does so effectively in vesicles prepared from *Staphylococcus aureus*, an organism which contains menaquinone but no ubiquinone (41). In contrast, ubiquinone-1 has no effect on NADH-dependent active transport in *S. aureus* vesicles. These preliminary observations suggest that the endogenous quinones may not be freely diffusible within the membrane, and that they may function in a more specific manner than previously thought to be the case.

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1. Kaback, H. R. (1972) *Biochim. Biophys. Acta* **265,** 367–416.
2. Kaback, H. R. & Hong, J.-s. (1973) in *CRC Critical Reviews in Microbiology,* eds. Laskin, Allen I. & Lechevalier, H. (CRC Press, Ohio), Vol. 2, pp. 333–376.
3. Kaback, H. R. (1973) in *Bacterial Membranes and Walls,* ed. Leive, L. (Marcel Dekker, New York), Vol. I, pp. 241–292.
4. Kaback, H. R. (1974) *Science* **186,** 882–892.
5. Konings, W. N. & Kaback, H. R. (1973) *Proc. Nat. Acad. Sci. USA* **70,** 3376–3381.
6. Boonstra, J., Huttunen, M. T., Konings, W. N. & Kaback, H. R. (1975) *J. Biol. Chem.*, in press.
7. Konings, W. N. & Boonstra, J. (1975) in *Current Topics in Membranes and Transport,* in press.
8. Kaback, H. R. & Milner, L. S. (1970) *Proc. Nat. Acad. Sci. USA* **66,** 1008–1015.
9. Barnes, E. M., Jr. & Kaback, H. R. (1970) *Proc. Nat. Acad. Sci. USA* **66,** 1190–1198.
10. Barnes, E. M., Jr. & Kaback, H. R. (1971) *J. Biol. Chem.* **246,** 5518–5522.
11. Short, S., White, D. & Kaback, H. R. (1972) *J. Biol. Chem.* **247,** 298–304.
12. Kerwar, G. K., Gordon, A. S. & Kaback, H. R. (1972) *J. Biol. Chem.* **247,** 291–297.
13. Konings, W. N. & Freese, E. (1972) *J. Biol. Chem.* **247,** 2408–2418.
14. Lombardi, F. J. & Kaback, H. R. (1972) *J. Biol. Chem.* **247,** 7844–7857.
15. Lombardi, F. J., Reeves, J. P. & Kaback, H. R. (1973) *J. Biol. Chem.* **248,** 3551–3565.
16. Harold, F. M. (1972) *Bacteriol. Rev.* **36,** 172–230.
17. Mitchell, P. (1973) *J. Bioenerg.* **4,** 63–91.
18. Weiner, J. H. (1974) *J. Membr. Biol.* **15,** 1–14.
19. Futai, M. (1974) *J. Membr. Biol.* **15,** 15–28.
20. Van Thienen, G. & Postma, P. W. (1973) *Biochim. Biophys. Acta* **323,** 429–440.
21. Hare, J. B., Olden, K. & Kennedy, E. P. (1974) *Proc. Nat. Acad. Sci. USA* **71,** 4843–4846.
22. Short, S. A., White, D. C. & Kaback, H. R. (1972) *J. Biol. Chem.* **247,** 4752–4758.
23. Kaback, H. R. (1971) in *Methods in Enzymology,* ed. Jakoby, W. B. (Academic Press, New York), Vol. XXII, pp. 99–120.
24. Altendorf, K. H. & Staehelen, L. A. (1974) *J. Bacteriol.* **117,** 888–899.
25. Konings, W. N., Bisschop, A., Voenhuis, M. & Vermeulen, C. A. (1973) *J. Bacteriol.* **116,** 1456–1465.
26. Short, S. A., Kaback, H. R., Kaczorowski, G., Fisher, J., Walsh, C. T. & Silverstein, S. (1974) *Proc. Nat. Acad. Sci. USA* **71,** 5032–5036.
27. Short, S. A., Hawkins, T., Kohn, L. D. & Kaback, H. R. (1975) *J. Biol. Chem.* **250,** 4285–4290.
28. Short, S. A., Kaback, H. R. & Kohn, L. D. (1975) *J. Biol. Chem.* **250,** 4291–4295.
29. Reeves, J. P., Lombardi, F. J. & Kaback, H. R. (1972) *J. Biol. Chem.* **247,** 6204–6211.
30. Rosen, B. P. & McClees, J. S. (1974) *Proc. Nat. Acad. Sci. USA* **71,** 5042–5046.
31. Kaback, H. R. (1974) in *Methods in Enzymology,* eds. Fleischer, S. & Packer, L. (Academic Press, New York), Vol. XXXI, pp. 698–709.
32. Lowry, O. H., Rosebrough, N. J., Farr, A. J. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
33. Reeves, J. P., Shechter, E., Weil, R. & Kaback, H. R. (1973) *Proc. Nat. Acad. Sci. USA* **70,** 2722–2726.
34. Hirata, H., Altendorf, K. & Harold, F. M. (1973) *Proc. Nat. Acad. Sci. USA* **70,** 1804–1808.
35. Altendorf, K., Hirata, H. & Harold, F. M. (1975) *J. Biol. Chem.* **249,** 4587–4593.
36. Patel, L., Schuldiner, S. & Kaback, H. R. (1975) *Proc. Nat. Acad. Sci. USA,* in press.
37. Konings, W. N., Barnes, E. M., Jr. & Kaback, H. R. (1971) *J. Biol. Chem.* **246,** 5857–5861.
38. Sober, H. A. & Harte, R. A. (1968) "Selected data for molecular biology," in *CRC Handbook of Biochemistry,* pp. 527–534.
39. Crane, F. L. & Barr, R. (1971) in *Methods in Enzymology,* eds. McCormick, D. B. & Wright, L. D. (Academic Press, New York), Vol. XVIII, pp. 137–165.
40 Dunphy, P. J. & Brodie, A. F. (1971) in *Methods in Enzymology,* eds. McCormick, D. B. & Wright, L. D. (Academic Press, New York), Vol. XVIII, pp. 407–461.
41. Hammond, R. K. & White, D. C. (1969) *J. Bacteriol.* **100,** 573–578.