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Branched-Chain Amino Acid Transport in *Streptococcus agalactiae*

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The transport of the branched-chain amino acids in *Streptococcus agalactiae* was characterized. Glucose-grown cells were able to utilize only glucose as an energy source for transport of L-leucine, whereas lactose-grown cells could utilize both glucose and lactose. It was determined from metabolic inhibitor studies that energy from glycolysis and substrate level phosphorylation was required for active transport. Energy was found to be coupled to transport by the action of adenosine triphosphatase and the generation of a proton motive force. The branched-chain amino acids were found to share a common transport system that may consist of multiple components.

Streptococcus agalactiae has long been recognized as a primary agent of bovine mastitis. Although *S. agalactiae* strains are highly sensitive to the penicillins in vitro (10, 19), the incidence of new infections of the bovine udder has remained a problem. More recently, this organism has also been identified as the cause of serious neonatal infections in humans (10). Antibiotic prophylaxis of pregnant women has been reported to be of questionable value, and treatment of infected infants with large doses of antibiotics has often been ineffective (10). Another approach that may prove to be useful is the study of nutritional immunity, which has been described by Kochan (14) as the study of nutritional requirements of parasites in host tissues to uncover new ways for developing effective immunity to microbial parasites. The most documented application of this concept has been the infection-promoting activity of iron (13, 15, 16).

The concept of nutritional immunity is being pursued in this laboratory by studying the factors in milk that can affect the growth of *S. agalactiae* and influence the susceptibility of the udder to infection (4, 5, 22). The diet of dairy cows has been shown to influence the composition of milk and the incidence of clinical mastitis (4, 30). Since *S. agalactiae* grows in the milk inside the mammary gland, the composition of milk may have a role in determining whether the organism can establish a successful infection. Brown (4) has found that the addition of small amounts (4 to 6 μg/ml) of L-isoleucine and other branched-chain amino acids to milk partially inhibits growth and acid production of *S. agalactiae*. Milk is a complex system containing very small amounts of free amino acids (17, 30), some peptides, and various proteins which are available to the organism as nitrogen sources (17). Since the organism does not have a biosynthetic capacity for the branched-chain amino acids (22, 34), this study was initiated to determine if the growth inhibition caused by L-isoleucine could be due to the inhibition of a branched-chain amino acid transport system. The transport of these amino acids has not been described in the streptococci.

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MATERIALS AND METHODS

Cultures and maintenance. *S. agalactiae* 660 was obtained from R. W. Brown and has been used in previous studies from this laboratory (4, 5, 22, 23). The culture was maintained in brain heart infusion broth at 4°C.

Growth conditions. Cultures for all experiments were grown in Mickelson (22, 23) chemically defined medium. A 4-ml tube of this medium was inoculated with the stock culture and grown at 37°C to the late logarithmic phase of growth. A portion of this culture was inoculated into the required amount of fresh medium and incubated overnight (17 h) at 37°C. The cells were in the stationary phase of growth. They were harvested by centrifugation in a Sorvall RC-2B refrigerated centrifuge (0 to 2°C) at 10,000 × *g* for 5 min. The cell pellet was washed twice in 100 mM potassium phosphate buffer (pH 7.0) containing 10 mM $MgSO_4$.

Cell dry weights were determined by trapping cells on tared 0.45-μm membrane filters (type HA; Millipore Corp., Bedford, Mass.). The filters were thoroughly washed in sterile distilled water and dried at 85°C to a constant weight.

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Transport assays. The washed cells were suspended in 100 mM tris(hydroxymethyl)aminomethane (Tris)-maleate buffer (pH 7.0) to a density of 2 mg/ml (dry weight). For measuring transport, a portion of these cells was diluted in 100 mM Tris-maleate buffer (pH 7.0) to a density of 1 mg/ml (dry weight) and equilibrated to 30°C. An appropriate energy source (10 mM), usually glucose, was added, and the suspension was incubated at 30°C for 5 min. Chloramphenicol was not routinely included in the reaction mixture, since, under the conditions of this experiment, the incorporation of amino acids into protein was negligible. The reaction was initiated by the addition of ^{14}C-amino acid (0.1 mM); 200-μl samples were removed at the indicated intervals and rapidly filtered through 0.45-μm membrane filters. The filters were immediately washed with 5 ml of 100 mM Tris-maleate buffer (pH 7.0) at room temperature. The filters were dried and placed in scintillation vials containing 10 ml of scintillation fluid. The scintillation fluid was composed of 0.5% 2,5-diphenyloxazole (PPO) and 0.03% 1,4-bis-[2]-(4-methyl-5-phenyloxazolyl)benzene (dimethyl-POPOP) in toluene. The radioactive samples were counted in a Beckman LS-8000 liquid scintillation counter.

For determination of the kinetic parameters, the initial rate of uptake at 0.5 min for various substrate concentrations was measured. The apparent K_m and V_{max} values were determined from the biphasic Lineweaver-Burk plots by the formula of Neal (25).

Chemicals. The branched-chain amino acids, carbonyl cyanide-*m*-chlorophenyl hydrazone (CCCP), and oligomycin were obtained from Calbiochem, La Jolla, Calif. Other amino acids, valinomycin, adenylyl imidodiphosphate, and 2-heptyl-4-hydroxyquinoline-*N*-oxide (HOQNO) were obtained from Sigma Chemical Co., St. Louis, Mo. *N,N'*-Dicyclohexylcarbodiimide (DCCD) was obtained from Aldrich Chemical Co., Milwaukee, Wis. Sodium amytal was obtained from Eli Lilly & Co., Indianapolis, Ind. Dinitrophenol, CCCP, DCCD, HOQNO, valinomycin, and oligomycin were dissolved in ethanol and added to the cell suspension so that the final ethanol concentration did not exceed 0.1%. L-[*U*-^{14}C]leucine (10 mCi/mmol), L-[*U*-^{14}C]isoleucine (10 mCi/mmol), and L-[*U*-^{14}C]valine (10 mCi/mmol) were purchased from Amersham Corp., Arlington Heights, Ill.

RESULTS

Energy requirements for L-leucine uptake. When *S. agalactiae* cells were grown in Mickelson chemically defined medium with glucose as the carbon source, they were able to utilize only glucose, of the catabolites examined, as an energy source for transport (Fig. 1). Lactose and arginine did not serve as energy sources, although they gave slightly greater accumulation than did no energy source. There was no evidence of significant nonmetabolic uptake such as has been observed in *Streptococcus faecalis* (2, 3). When the glucose in the growth medium was replaced with lactose, both glucose and lactose served as energy sources for transport (Fig. 2). Arginine has been found to serve as an energy source for the transport of amino acids in *Streptococcus lactis* when the cells are grown on a carbohydrate other than glucose (32); this was not the case in *S. agalactiae* (Fig. 2).

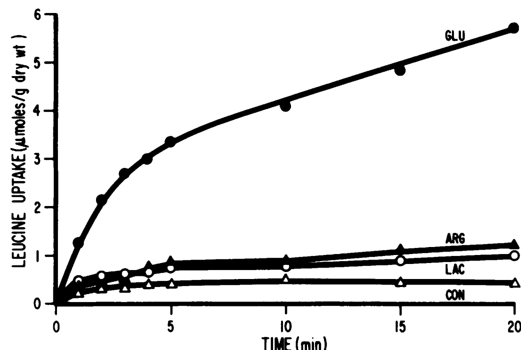


FIG. 1. *Energy requirement for L-leucine uptake by glucose-grown cells of S. agalactiae. Cells were incubated for 5 min at 30°C with an energy source (10 mM) before the addition of L-[U-^{14}C]leucine (0.1 mM). Symbols: △, control (no exogenous energy source); ●, glucose; ○, lactose; ▲, arginine.*

Effects of metabolic inhibitors on L-leucine uptake. The uptake of L-leucine in the presence of various metabolic inhibitors was measured with glucose-grown cells to determine how energy is coupled to transport. The proton-conducting uncoupler CCCP and the inhibitor of membrane-bound adenosine triphosphatase (ATPase) DCCD completely stopped active transport (Fig. 3a). Dinitrophenol, another uncoupler, was not as effective as CCCP. Arsenate, which interferes with adenosine triphosphate (ATP) generation from glycolysis, was only partially inhibitory. Two sulfhydryl group reagents, iodoacetate and *p*-chloromercuribenzoate, completely inhibited uptake of L-leucine (Fig. 3b). Another sulfhydryl group reagent, *N*-ethylmaleimide, was only slightly inhibitory. The glycolytic inhibitor fluoride was also very inhibitory. Azide, which acts primarily as an inhibitor of cytochrome oxidase, caused about 50% inhibition of uptake. Sodium amytal was a potent inhibitor of L-leucine uptake (Fig. 3c). It has been found to inhibit reduced nicotinamide adenine dinucleotide (NADH) oxidation in *S. agalactiae* (21). HOQNO had no effect on L-leucine uptake; it is an inhibitor of NADH oxidation in some bacteria, but not in *S. agalactiae* (21). Valinomycin, a potassium ionophore (28), inhibited L-leucine uptake. Oligomycin, which inhibits membrane-bound ATPase in mitochondria, but not bacterial ATPase (6), did not inhibit uptake. Adenylylimidodiphosphate also did not inhibit uptake; it is an analog of ATP

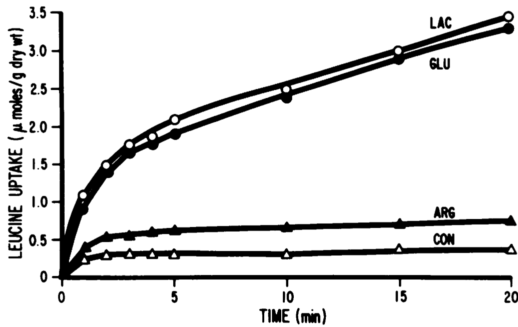
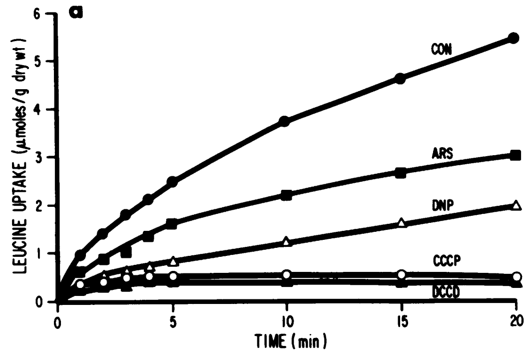


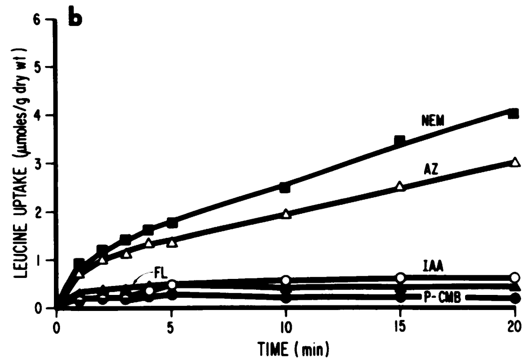
FIG. 2. *Energy requirement for L-leucine uptake by lactose-grown cells of S. agalactiae. Cells were incubated for 5 min at 30°C with an energy source (10 mM) before the addition of L-[U-^{14}C]leucine (0.1 mM). Symbols: △, control (no exogenous energy source); ●, glucose; ○, lactose; ▲, arginine.*

that competitively inhibits mitochondrial ATPase (27).

Effect of unlabeled amino acids on branched-chain amino acid uptake. The uptake of L-leucine was measured in the presence of 1 mM (10-fold excess) and 10 mM (100-fold excess) unlabeled amino acids. The branched-chain amino acids, L-leucine, L-isoleucine, and L-valine, were very inhibitory to labeled L-leucine uptake even at only a 10-fold excess (Table 1). D-Leucine was inhibitory at a 100-fold excess, and L-norleucine was slightly more inhibitory. L-Alanine, L-phenylalanine, and L-methionine were somewhat inhibitory when present in a 100-fold excess. L-Cysteine was also somewhat inhibitory. Twelve other unlabeled amino acids did not inhibit L-leucine uptake. Branched-chain amino acids strongly inhibited the uptake of L-isoleucine (Table 2) and L-valine (Table 3) by *S. agalactiae* cells. In contrast to the situation with leucine and isoleucine, D-valine was not inhibitory to L-valine uptake.

Kinetics of branched-chain amino acid transport. The initial rates of uptake of L-leucine, L-isoleucine, and L-valine were measured for the concentration range of 10 to 500 μM. A double-reciprocal plot of the data for L-leucine uptake is shown in Fig. 4. Similar plots were observed for L-isoleucine and L-valine uptakes. The biphasic nature of the plot is suggestive of a multiple-component transport system such as has been reported for *Escherichia coli* (1, 29) and *Salmonella typhimurium* (12). These plots cannot be described by a single set of kinetic constants. The kinetic constants shown in Table 4 were determined by assuming the presence of two transport systems for each amino acid. Some evidence for a leucine-specific component, which accounted for less than 10% of the total L-leucine uptake, was obtained by measuring the initial rate of L-leucine uptake in the presence of up to a 500-fold excess of L-isoleucine. It was also noted that L-isoleucine displayed typical competitive inhibition Michaelis-Menten kinetics for L-leucine uptake (data not shown).





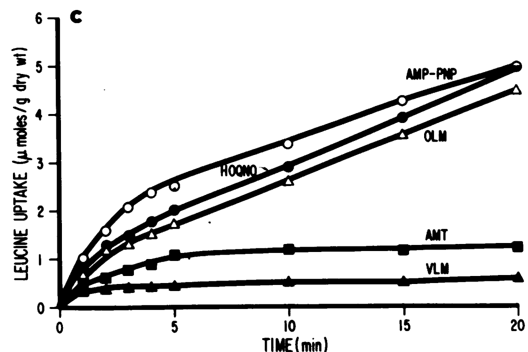


FIG. 3. *Effect of metabolic inhibitors on L-leucine uptake by cells of S. agalactiae. Cells were incubated for 5 min at 30°C with 10 mM glucose plus the appropriate inhibitor before the addition of L-[U-^{14}C]leucine (0.1 mM). (a) Symbols: ●, control (ethanol only); ○, CCCP (50 μM); ▲, DCCD (0.3 mM); △, dinitrophenol (4 mM); ■, arsenate (10 mM). (b) Symbols: ●, p-chloromercuribenzoate (1 mM); ○, iodoacetate (10 mM); ▲, fluoride (10 mM); △, azide (10 mM); N-ethylmaleimide (1 mM). (c) Symbols: ●, HOQNO (10 μm); ○, adenylyl imidodiphosphate (0.17 mM); ▲, valinomycin (10 μM); △, oligomycin (0.1 mg/ml); ■, amytal (10 mM).*

TABLE 1. *Inhibition of L-[^{14}C]leucine uptake by addition of unlabeled amino acids*[a]

Amino acid	Concn (mM)	Uptake (%)[b]	Amino acid	Concn (mM)	Uptake (%)[b]
L-Leucine	1	15	L-Cystine	1	131
	10	5		10	97
L-Isoleucine	1	26	L-Glutamic acid	1	109
	10	8		10	93
L-Valine	1	17	Glycine	1	90
	10	8		10	75
D-Leucine	1	88	L-Histidine	1	98
	10	38		10	78
L-Norleucine	1	54	L-Lysine	1	103
	10	35		10	91
L-Alanine	1	115	L-Proline	1	126
	10	55		10	107
L-Cysteine	1	66	L-Serine	1	91
	10	33		10	75
L-Phenylalanine	1	92	L-Threonine	1	112
	10	45		10	102
L-Methionine	1	88	L-Tryptophan	1	106
	10	33		10	105
L-Arginine	1	145	L-Tyrosine	1	87
	10	111		10	79
L-Aspartic acid	1	120			
	10	104			

[a] Control uptake for 1 min was 1.15 μmol/g (dry weight).
[b] Uptake is shown as a percentage of the control.

DISCUSSION

The results reported in this study demonstrate that energy is required for active transport of L-leucine in *S. agalactiae* cells. Glucose-grown cells can use only glucose as an energy source for transport, presumably because of the effects of catabolite repression (26) on the enzymes necessary to use other energy sources. Lactose-grown cells were able to transport L-leucine by using either glucose or lactose for energy. Arginine was not an efficient energy source for transport. *S. lactis* can convert arginine to ornithine with the production of 1 mol of ATP per mol of arginine utilized (31); this energy can then be used for amino acid transport (32). *S. agalactiae* apparently does not have this capability.

The inhibitors that were most effective in preventing active transport of L-leucine were those that interfered with efficient ATP synthesis by glycolysis or with the coupling of that energy to transport. In agreement with the findings of this study, it has recently been reported that *p*-chloromercuribenzoate and iodoacetate specifically inhibited glycolysis in *S. lactis* by acting on glyceraldehyde-3-phosphate dehydrogenase, whereas 1 mM *N*-ethylmaleimide had no significant inhibitory effect (33). Fluoride was another potent inhibitor of L-leucine transport; it stops glycolysis by inhibiting enolase (11). These sulfhydryl group reagents and fluoride have also been found to inhibit sugar transport in *S. agalactiae* (23). Arsenate was only partially inhibitory to L-leucine uptake, perhaps because of limited uptake (7).

CCCP strongly inhibited active transport of L-leucine; this compound acts to collapse the proton motive force by making the cell membrane freely permeable to protons (2, 8). It also partially inhibits both substrate and oxidative phosphorylation in *S. agalactiae* (21). DCCD, which inhibits membrane-bound ATPase (9), also strongly inhibited active transport of L-leucine. Valinomycin was a potent inhibitor of L-leucine transport in *S. agalactiae*; it has been shown to act by making cell membranes freely

TABLE 2. *Inhibition of* L-*[^{14}C]isoleucine uptake by addition of unlabeled branched-chain amino acids*[a]

Amino acid	Concn (mM)	Uptake (%)[b]
L-Isoleucine	1	13
	10	5
L-Leucine	1	21
	10	9
L-Valine	1	26
	10	10
D-Isoleucine	1	83
	10	37

[a] Control uptake for 1 min was 1.08 μmol/g (dry weight).
[b] Uptake is shown as a percentage of the control.

TABLE 3. *Inhibition of* L-*[^{14}C]valine uptake by addition of unlabeled branched-chain amino acids*[a]

Amino acid	Concn (mM)	Uptake (%)[b]
L-Valine	1	25
	10	7
L-Leucine	1	19
	10	9
L-Isoleucine	1	27
	10	12
D-Valine	1	120
	10	107

[a] Control uptake for 1 min was 1.23 μmol/g (dry weight).
[b] Uptake is presented as a percentage of the control.

permeable to potassium ions (28). The effect of valinomycin on amino acid transport in *S. faecalis* has been found to be dependent on the concentration of potassium in the cytoplasm and in the medium (2); transport was inhibited at high external potassium concentrations. Valinomycin has been used with a potassium-free medium to artificially create a membrane potential and induce transport (2, 18). In *S. lactis*, this valinomycin-imposed membrane potential was dissipated after 5 min, and the intracellular ATP level fell to the steady-state level (18). Thus, with the low-potassium 100 mM Tris-maleate buffer used in the current study, any membrane potential generated would probably have been dissipated during the 5-min incubation period with valinomycin. The intracellular potassium ion concentration would have approached the extracellular concentration (by intracellular loss), with the corresponding collapse of the electrical component of the proton motive force (2).

The respiratory inhibitor azide is a classical inhibitor of cytochrome oxidase and was found to be only partially inhibitory to L-leucine uptake. *S. agalactiae* has been found to possess an aerobic metabolism mediated by NADH oxidase, but no evidence for the presence of cytochromes was obtained (20). Recently, it has also been found that incubation of cells with cyanide did not affect the transport of glucose (23). Some evidence for the involvement of respiratory energy in L-leucine transport was obtained by the finding that amytal, but not HOQNO, inhibited transport. Mickelson (21) has previously shown that amytal, but not HOQNO, inhibits NADH oxidation in *S. agalactiae*.

The results of the inhibitor studies suggest that *S. agalactiae* transports L-leucine with energy derived from glycolysis and substrate level phosphorylation; additional energy from oxidative phosphorylation via the NADH oxidase may also be used. Results also suggest that the energy in the form of ATP is coupled to transport via the action of ATPase, which couples the hydrolysis of ATP to the extrusion of protons from the cell (18). This action of proton extrusion creates a proton motive force that drives the energy-dependent transport of L-leucine in accordance with the chemiosmotic concept of Mitchell (2, 24).

The results of the specificities of branched-

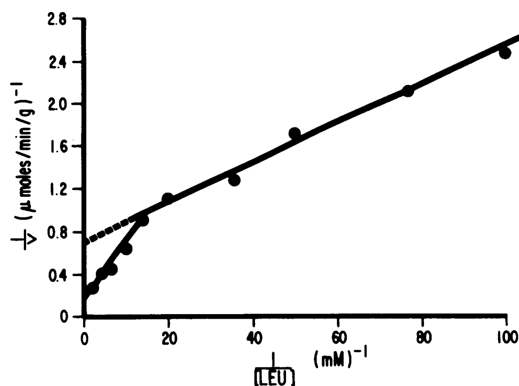


FIG. 4. *Double-reciprocal plot of the initial rate of* L-*leucine uptake by cells of S. agalactiae.*

TABLE 4. *Kinetic constants for the branched-chain amino acid transport systems in S. agalactiae*

Substrate	K_m (mM)		V_{max} (μmol/min per g)	
	1	2	1	2
L-Leucine	0.006	0.31	1.00	0.49
L-Isoleucine	0.006	0.53	1.16	0.47
L-Valine	0.007	0.29	1.16	0.92

chain amino acid transport have revealed that these amino acids share a common transport system. The system apparently has some affinity for the D-isomers of leucine and isoleucine, but not of valine. Transport activity was somewhat inhibited by L-alanine, L-phenylalanine, L-methionine, and L-cysteine; these findings are very similar to those reported for *S. typhimurium* (12) and *E. coli* (1). The inhibition of various transport systems by L-cysteine has been reported in several organisms (1), including *Streptococcus faecium* (3); part of its action may be as a sulfhydryl group reagent acting on transport carriers. The kinetic studies revealed that the branched-chain amino acid transport system in *S. agalactiae* may consist of multiple components. This situation is well characterized in the gram-negative bacteria, such as *E. coli* (1, 29) and *S. typhimurium* (12), but has not been studied in the gram-positive bacteria. The elucidation and verification of these systems will await the isolation of specific transport mutants.

The results of this study have revealed that the observed growth inhibition of *S. agalactiae* by L-isoleucine (4) was probably due to an overloading of the common branched-chain amino acid transport system, with a subsequent inhibition of L-leucine and L-valine uptake. Complete growth inhibition was not observed because of the presence of peptides and various proteins in the milk system (17).

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LITERATURE CITED

1. **Anraku, Y.** 1978. Transport of amino acids, p. 171-219. *In* B. P. Rosen (ed.), Bacterial transport. Marcel Dekker, Inc., New York.
2. **Asghar, S. S., E. Levin, and F. M. Harold.** 1973. Accumulation of neutral amino acids by *Streptococcus faecalis*. J. Biol. Chem. **248**:5225-5233.
3. **Brock, T. D., and G. Moo-Penn.** 1962. An amino acid transport system in *Streptococcus faecium*. Arch. Biochem. Biophys. **98**:183-190.
4. **Brown, R. W.** 1974. Compounds affecting *Streptococcus agalactiae* growth in milk. J. Dairy Sci. **57**:797-802.
5. **Brown, R. W., and M. N. Mickelson.** 1979. Lactoperoxidase, thiocyanate, and free cystine in bovine mammary secretions in early dry period and at the start of lactation and their effect on *Streptococcus agalactiae* growth. Am. J. Vet. Res. **40**:250-255.
6. **Haddock, B. A., and C. W. Jones.** 1977. Bacterial respiration. Bacteriol. Rev. **41**:47-99.
7. **Harold, F. M., and J. R. Baarda.** 1966. Interaction of arsenate with phosphate-transport systems in wild-type and mutant *Streptococcus faecalis*. J. Bacteriol. **91**: 2257-2262.
8. **Harold, F. M., and J. R. Baarda.** 1968. Inhibition of membrane transport in *Streptococcus faecalis* by uncouplers of oxidative phosphorylation and its relationship to proton conduction. J. Bacteriol. **96**:2025-2034.
9. **Harold, F. M., J. R. Baarda, C. Baron, and A. Abrams.** 1969. Inhibition of membrane-bound adenosine triphosphatase and of cation transport in *Streptococcus faecalis* by *N,N'*-dicyclohexylcarbodiimide. J. Biol. Chem. **244**:2261-2268.
10. **Jelinkova, J.** 1977. Group B streptococci in the human population. Curr. Top. Microbiol. Immunol. **76**:127-165.
11. **Kanapka, J. A., and I. R. Hamilton.** 1971. Fluoride inhibition of enolase activity *in vivo* and its relationship to the inhibition of glucose-6-P formation in *Streptococcus salivarius*. Arch. Biochem. Biophys. **146**:167-174.
12. **Kiritani, K., and K. Ohnishi.** 1977. Repression and inhibition of transport systems for branched-chain amino acids in *Salmonella typhimurium*. J. Bacteriol. **129**:589-598.
13. **Kluger, M. J., and B. A. Rothenburg.** 1979. Fever and reduced iron: their interaction as a host defense response to bacterial infection. Science **203**:374-376.
14. **Kochan, I.** 1973. The role of iron in bacterial infections, with special consideration of host-tubercle bacillus interaction. Curr. Top. Microbiol. Immunol. **60**:1-30.
15. **Kochan, I.** 1975. Nutritional regulation of antibacterial resistance, p. 273-288. *In* D. Schlessinger (ed.), Microbiology—1974. American Society for Microbiology, Washington, D.C.
16. **Kochan, I., J. Wasynczuk, and M. A. McCabe.** 1978. Effects of injected iron and siderophores on infections in normal and immune mice. Infect. Immun. **22**:560-567.
17. **Lawrence, R. C., and T. D. Thomas.** 1979. The fermentation of milk by lactic acid bacteria, p. 187-219. *In* A. T. Bull, D. C. Ellwood, and C. Ratledge (ed.), Microbial technology: current state, future prospects. Cambridge University Press, London.
18. **Maloney, P. C., and T. H. Wilson.** 1975. ATP synthesis driven by a proton-motive force in *Streptococcus lactis*. J. Membr. Biol. **25**:285-310.
19. **McDonald, J. S., T. J. McDonald, and D. R. Stark.** 1976. Antibiograms of streptococci isolated from bovine intramammary infections. Am. J. Vet. Res. **37**:1185-1188.
20. **Mickelson, M. N.** 1967. Aerobic metabolism of *Streptococcus agalactiae*. J. Bacteriol. **94**:184-191.
21. **Mickelson, M. N.** 1974. Effect of uncoupling agents and respiratory inhibitors on the growth of *Streptococcus agalactiae*. J. Bacteriol. **120**:733-740.
22. **Mickelson, M. N.** 1976. Effects of nutritional characteristics of *Streptococcus agalactiae* on inhibition of growth by lactoperoxidase-thiocyanate-hydrogen peroxide in chemically defined culture medium. Appl. Environ. Microbiol. **32**:238-244.
23. **Mickelson, M. N.** 1977. Glucose transport in *Streptococcus agalactiae* and its inhibition by lactoperoxidase-thiocyanate-hydrogen peroxide. J. Bacteriol. **132**:541-548.
24. **Mitchell, P.** 1976. Vectorial chemistry and the molecular mechanics of chemiosmotic coupling: power transmission by proticity. Biochem. Soc. Trans. **4**:399-430.
25. **Neal, J. L.** 1972. Analysis of Michaelis kinetics for two independent, saturable membrane transport functions. J. Theor. Biol. **35**:113-118.
26. **Pastan, I., and S. Adhya.** 1976. Cyclic adenosine 5'-monophosphate in *Escherichia coli*. Bacteriol. Rev. **40**: 527-551.
27. **Penefsky, H. S.** 1974. Differential effects of adenylyl imidodiphosphate on adenosine triphosphate synthesis and the partial reactions of oxidative phosphorylation. J. Biol. Chem. **249**:3579-3585.

28. **Pressman, B. C.** 1968. Ionophorous antibiotics as models for biological transport. Fed. Proc. **27**:1283–1288.
29. **Rahmanian, M., D. R. Claus, and D. L. Oxender.** 1973. Multiplicity of leucine transport systems in *Escherichia coli* K-12. J. Bacteriol. **116**:1258–1266.
30. **Shimbaiashy, K., U. Ide, and T. Yonemura.** 1975. Free amino acids and phosphorylethanolamine in milk whey of cow. Agric. Biol. Chem. **29**:13–19.
31. **Thomas, T. D., D. C. Ellwood, and V. M. C. Longyear.** 1979. Changes from homo- to heterolactic fermentation by *Streptococcus lactis* resulting from glucose limitation in anaerobic chemostat cultures. J. Bacteriol. **138**: 109–117.
32. **Thompson, J.** 1976. Characteristics and energy requirements of an α-aminoisobutyric acid transport system in *Streptococcus lactis*. J. Bacteriol. **127**:719–730.
33. **Thompson, J.** 1978. In vivo regulation of glycolysis and characterization of sugar:phosphotransferase systems in *Streptococcus lactis*. J. Bacteriol. **136**:465–476.
34. **Willett, N. P., and G. E. Morse.** 1966. Long-chain fatty acid inhibition of growth of *Streptococcus agalactiae* in a chemically defined medium. J. Bacteriol. **91**:2245–2250.