

CALORIC CATASTROPHE

LAWRENCE MINKOFF *and* RAYMOND DAMADIAN

From the Biophysical Laboratory, Department of Medicine and Biophysics, State University of New York at Brooklyn, Brooklyn, New York 11203

ABSTRACT Six solutes known to be actively transported by bacteria were studied with the cell in a "minimum energy" state to determine if sufficient energy were available from cellular stores of ATP to supply the energy necessary to run postulated membrane-situated "pumps." Steady-state cellular concentrations of potassium, calcium, magnesium, leucine, glycine, and α -methyl glucoside were determined together with tracer fluxes, oxygen consumption, ATP turnover, and the P:O ratio. From these measurements, it was calculated that the energy supply, 4.20 cal/340 min-g dry wt, fell far short of the energy necessary (28.28 cal/340 min-g dry wt), by classical membrane theory, to operate "pumps."

INTRODUCTION

Since the 1940s when Dean (1) and Krogh (2) first proposed the concept of a membrane-situated pump to account for the transfer of solutes across the biological interface, the model has been extensively exploited. Progressively it has grown more and more elaborate. A specific pump has now been proposed for almost every solute that has been observed to enter the cell (Fig. 1) (3-10), until the laws of energy conservation dictated that the capacity of the cell to deliver the energy necessary to operate all of the "pumps" be experimentally verified.

This question was considered by G. N. Ling in 1952. From measurements made in frog muscle, he determined it to be inadequate (11). The development in recent years of techniques for studying transport phenomena utilizing bacteria provided the opportunity to reexamine this question for a single cell with the advantage that a large variety of solutes could be individually studied and subjected to kinetic analysis. In particular, bacteria could be grown in a "minimum energy" state so that the caloric requirements of transport could be isolated from other energy-consuming processes in the cell, such as cell division and growth, diverse metabolic pathways, protein synthesis, DNA replication, etc.

The growth of bacteria on media containing a marginal supply of glucose results in abrupt cessation of growth when the substrate supply is exhausted (Fig. 2). The arrested culture is then in a "minimum energy" state. The kinetics of solute transport

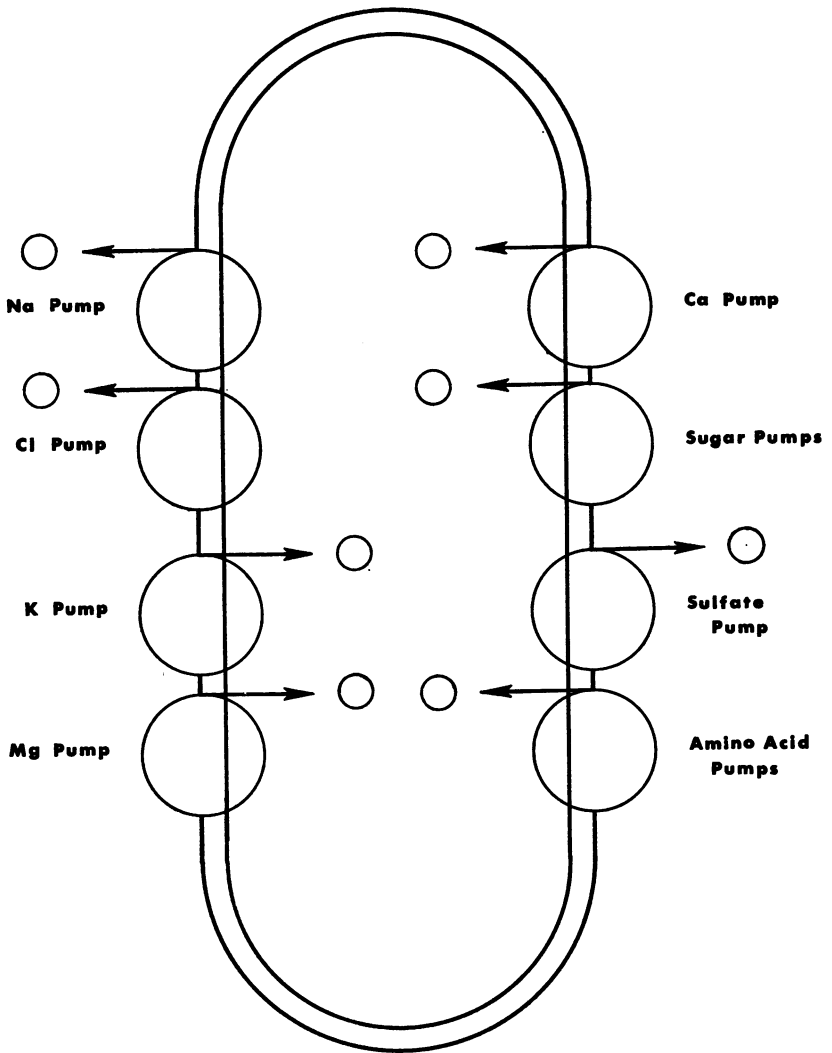


FIGURE 1 The membrane-situated pumps that have been postulated. Chloride (3), calcium (4), magnesium (5), sugar (6), phosphate (7), amino acids (8), sulfate (9), sodium, and potassium (10).

and the concentration "gradients" could then be studied when the supply of metabolic energy was at a minimum. This experimental model avoids the difficulty of assigning an accurate figure for the drain of numerous nontransport reactions on the cell's energy resources. Thus, there is permitted an assessment of the quantitative agreement between the energy necessary to operate "pumps" and the metabolic energy available to them.

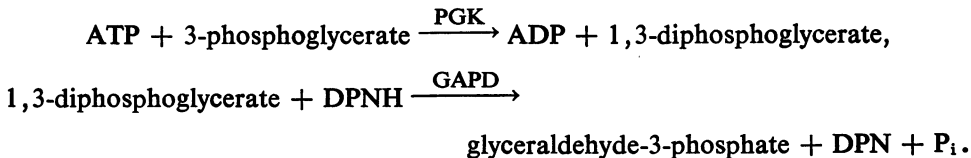
MATERIALS AND METHODS

Bacteria

A phage-resistant, nonpathogenic wild strain of *Escherichia coli* isolated from a clinical source by Dr. Barnett Sulzer of this institution was used exclusively throughout this study. Bacteria were grown freshly for each experiment in medium KA (12, 13) by methods previously described (12, 13), and were cultivated at 37°C throughout these experiments unless otherwise indicated. Bacterial dry wt was determined by turbidimetry using a gravimetrically standardized procedure described elsewhere (12, 13). The cells were harvested while in the logarithmic phase of growth and transferred to medium NaA (12) lacking citrate and supplemented with a marginal supply of glucose (0.5 mg/ml).

Measurement of Triphosphate

Samples of cells growing in glucose-limited media were taken periodically, cooled immediately by immersion of the sample tube in an ice slurry, and centrifuged. The resulting pellet was then resuspended in perchloric acid to a final concentration of 0.3 M. The cells were lysed by repetitive freeze-thawing, centrifuged at 12,000 *g* and the resulting supernatant assayed for triphosphate by phosphoglyceric phosphokinase (PGK) according to the reactions:

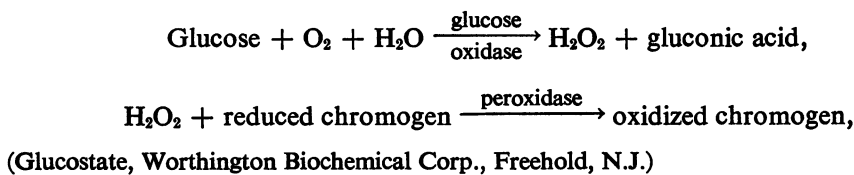


Difference in DPNH was followed at 340 nm on a Gilford spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio; Sigma, Ultraviolet, Determination of ATP).

The procedure is not specific for ATP. Other nucleoside triphosphates such as GTP, ITP, and UTP are also substrates for the reaction.

Measurement of Glucose

Samples of cells and media were taken and the cells removed by Millipore filtration. The media were then assayed for glucose by the coupled enzyme colorimetric procedure:



Potassium Flux

Potassium flux was determined by methods described in previous publications (12, 13).

Other Flux Determinations

Other solutes studied were the divalent ions Ca^{++} and Mg^{++} , a sugar, α -methyl-D-glucoside, and two amino acids, glycine and L-leucine. The nonmetabolizable analogue of glucose, α -

methyl-D-glucoside, was chosen since it utilizes the same transport system as glucose (14). Glycine and L-leucine were chosen because Roberts et al. (15) have shown that exogenous glycine and L-leucine appear totally in protein and are not transaminated or deaminated to form other substrates. The utilization of these amino acids as sources of energy was further guarded against by confining these experiments to "endogenous respiration" (discussed later in this paper) where it is specifically known not to occur (16).

Nonradioactive carriers of each of the solutes (Ca, Mg, α -methyl-D-glucoside, glycine, L-leucine) were added to "minimum energy" cells and incubated at 37°C until solute equilibrium was attained across the cell boundary. Radioactive tracers¹ were then added to the media, and incorporation was followed by periodic sampling and determination of intracellular radioactivity. The size of the internal pool was determined from the extracellular specific activity (counts per minute per milliliter medium/millimoles of solute added by weight to 1 ml of medium) and the final intracellular activity at tracer equilibrium. The unidirectional flux of these solutes was estimated from the initial rate of tracer accumulation when tracer movement out of the cell at time close to zero was infinitesimal.

Oxygen Consumption

Oxygen uptake was measured in a Gilson Respirometer (Gilson Medical Electronics, Inc., Middleton, Wis.) (17, 18). 10 ml of growing bacteria (0.1 OD 620 at start) in glucose-limited KA medium was placed in the respirometer, under 100% oxygen. Oxygen consumption and OD were followed simultaneously. Cessation of growth was established when OD became fixed. Termination of growth marked the onset of the "minimum energy" state.

ATP Turnover

E. coli was grown to glucose limitation in KA. Bacteria were then harvested and suspended to a final concentration of 1.5 mg dry wt/ml. The study of ³²P_i incorporation into ATP was initiated by an injection of ³²P_i as the Na₃ salt (2 μ Ci/ml final suspension, Cambridge Nuclear Corp.) into the bacterial suspension. Samples of the suspension were then taken at timed intervals and immediately cooled to 0°C in an ice slurry. Cells were then washed twice by centrifugation with cold 400 mM sucrose to remove extracellular ³²P_i. ATP was extracted from the bacterial pellet by suspending the cells in 1 ml of cold 0.6 M perchloric acid for 30 min. The extract was neutralized with concentrated KOH (5 N) and buffered by the addition of 2 ml of Tris-Cl (0.1 M, pH 7.6). This extract was divided into equal halves, one for hydrolysis by ATPase and one for measurement of unhydrolyzed ATP. ATP hydrolysis was carried out by the addition of myosin ATPase (1 mg) kindly supplied by Dr. Paul Dreizen of this institution, and prepared in this laboratory by methods described elsewhere (19). The extract together with enzyme was incubated at 37°C until all the ATP was hydrolyzed (1 h). 2.0 ml of perchloric acid was then added to both hydrolyzed and unhydrolyzed samples and the nucleotides in each mixture sorbed to 0.2 g of Norit A charcoal by the procedure of Crane and Lipmann (20). The Norit A was then washed twice in conical centrifuge tubes with neutral 10 mM potassium phosphate, taken up quantitatively in toluene scintillate containing 0.75 ml ethanol, 1.0 ml hyamine hydroxide, and 10 ml of scintillate (5 g 2,5-diphenyloxazole [PPO] and 100 mg 1,4-bis-[2-(5-phenyloxazolyl)]benzene [POPOP]/liter toluene), and counted in a Picker Licomat 220 scintillation counter (Picker Corp., Cleveland, Ohio) equipped with a radium

¹ [U-¹⁴C]Glycine, L-[U-¹⁴C]leucine, and α -[methyl-¹⁴C]-D-glucoside were obtained from Calatomic, Los Angeles, Calif. Calcium-45 chloride was obtained from Cambridge Nuclear Corp., Cambridge, Mass. Magnesium-28 chloride was obtained from Brookhaven National Laboratory, Upton, N. Y.

external standard to detect variation in quenching. The difference in the activity of the hydrolyzed and nonhydrolyzed samples after correcting for volume and efficiency of the method represented $^{32}\text{P}_i$ incorporated in the terminal phosphate.

Energy Calculations

Analysis of the energy required to maintain a given transport process consisted basically of two parts; calculation of the concentration ratio of the two compartments, cellular and external reservoir, and determination of the steady-state flux. The power consumption (P) of the transport reaction (calories per minute) is then simply the product of the electrochemical potential difference between the two compartments ($\Delta\bar{\mu}$ [calories per mole]) and the steady-state flux (Φ [moles per minute]).

P (calories per minute) = $\Delta\bar{\mu}$ (calories per mole) \times Φ (moles per minute) where $\Delta\bar{\mu}$ = the partial molar free energy difference in the two compartments,

$$\left(\frac{\partial G}{\partial n}\right)_{\text{in}} - \left(\frac{\partial G}{\partial n}\right)_{\text{out}} = RT \ln \frac{a_1}{a_2} + zF\Delta\psi, \quad (1)$$

where a_1 and a_2 represent the activities of the solutes in cellular and external compartment respectively, z is the ionic valence, and $\Delta\psi$ is the transmembrane potential difference. Hence,

$$P = \left(RT \ln \frac{a_1}{a_2} + zF\Delta\psi \right) \times \Phi. \quad (2)$$

Activities chosen for intracellular solutes were the activities of an equivalent aqueous solution at the same solute concentration. Intracellular ionic activities approximating the activities of aqueous solutions comprise a basic premise upon which "membrane theory" rests, which in effect is one of the premises being tested by these studies. The value utilized for the transmembrane potential was that estimated by Schultz et al. from the Cl distribution ratio in *E. coli*, 29 mV, interior negative (21).

RESULTS

Fig. 2 illustrates the growth characteristics of a glucose-limited culture. Cessation of growth occurs instantly when substrate is exhausted. The cell population of the culture increases exponentially until the glucose concentration of the suspension reaches "0," where cell multiplication stops abruptly.

Measurement of the total triphosphate content (ATP, CTP, UTP, GTP, etc.) of the bacteria, the total energy reservoir for active transport, also fell precipitously at the onset of the "minimum energy" state (Fig. 3), declining from 8 to 2.7 μmol . The 2.7 μmol remnant of triphosphate remained unchanged at 2.7 $\mu\text{mol/g}$ dry cells for the duration of the "minimum energy" state. Solute transport, on the other hand, persisted throughout, as demonstrated by the concentration "gradients" and fluxes tabulated in Table I. The persistence of transport in the minimum energy state indicated transport could be maintained in the *absence of net ATP synthesis*.

Potassium, the main intracellular cation of *E. coli*, was the first solute studied with respect to the kinetics of its movements. The onset of the "minimum energy" state was initially accompanied by a 23% decrease in cell potassium content (Fig. 4), from

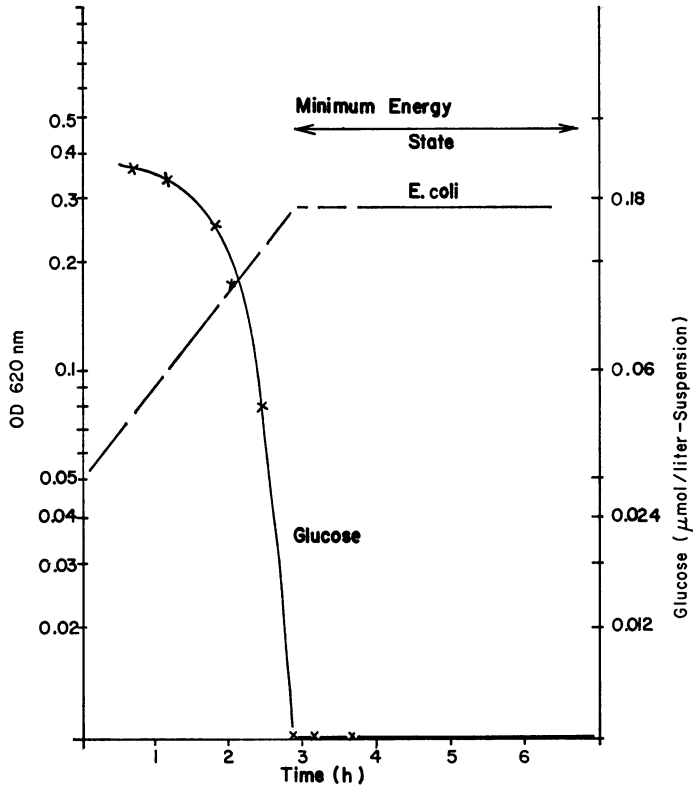


FIGURE 2 Cessation of growth upon exhaustion of medium glucose. Bacterial population density (broken line) monitored by OD measurements of the culture at 620 nm. Glucose disappearance (X) designates the onset of the minimum energy state.

600 to 459 $\mu\text{mol/g}$. It remained at 459 $\mu\text{mol/g}$ for 6 h despite the absence of substrate. Throughout this period, the measured steady-state exchange rate of potassium across the cell boundary was 0.150 $\text{pmol}/\text{cm}^2\text{-s}$ (Table I), where the concentration "gradient" was 700:1. Calcium flux was 0.181 $\text{pmol}/\text{cm}^2\text{-s}$ at a concentration "gradient" of 75:1 and the magnesium flux was 2.13 $\text{pmol}/\text{cm}^2\text{-s}$ at a concentration "gradient" of 25:1.

Of the biologically important organic ions, the steady-state flux value for L-leucine was 0.166 $\text{pmol}/\text{cm}^2\text{-s}$ with a concentration "gradient" of 300:1 and 0.202 $\text{pmol}/\text{cm}^2\text{-s}$ for glycine at a concentration "gradient" of 1000:1. The only nonionic solute studied, α -methyl-D-glucoside, had a flux rate of 0.330 $\text{pmol}/\text{cm}^2\text{-s}$ when the transcellular concentration gradient was 25:1.

Endogenous Respiration

A minimal amount of respiration, the endogenous respiration of incubating bacteria (16), persisted during the minimum energy state. Combination of the oxygen con-

sumption rate with measurements of the rate of $^{32}\text{P}_i$ incorporation into the phosphate of ATP permitted determination of the P:O ratio for the minimum energy state. Fig. 5 demonstrates the change in oxygen consumption with the onset of substrate limitation. The rate of oxygen consumption decreased from 130 to 9.6 $\mu\text{mol/g}\cdot\text{min}$ (Fig. 5).

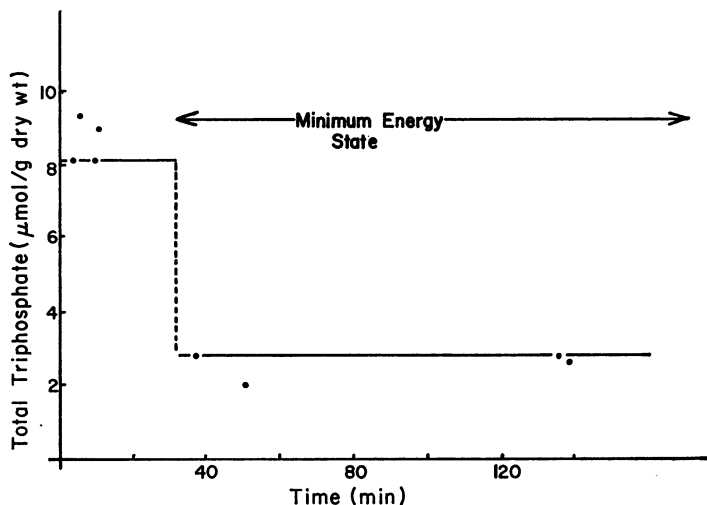


FIGURE 3 Cellular triphosphate in the minimum energy state. Bacterial density of the assayed suspension was 0.3 mg dried bacteria/ml.

TABLE I
EXPERIMENTALLY DETERMINED CALORIC REQUIREMENTS DURING 340
MIN INCUBATION IN THE MINIMUM ENERGY STATE

	Flux*	Internal concentration	External concentration	"Concentration gradient"	Energy required in 340 min	Energy available from turnover hydrolysis of ATP (340 min)
	<i>pmol/cm²-s</i>	<i>mM</i>	<i>mM</i>		<i>cal/g dry wt</i>	<i>cal/g dry wt</i>
Potassium	0.150	175	0.25	700	3.03	
L-Leucine	0.166	3	0.01	300	3.40	
Glycine	0.202	10	0.01	1000	5.00	
α -Methyl-D-glucoside	0.330	2.37	0.10	25	3.71	
Calcium	0.181	768	0.01	75	1.68	
Magnesium	2.13	10	0.40	25	11.46	
Total energy required					28.28	4.2
Percent of necessary energy available from ATP hydrolysis					14.8	

Caloric requirements for solute transport in *E. coli* in the minimum energy state. Data are for 340 min of incubation in the minimum energy state.

* 1 mg dry wt = 2.829×10^8 cm² membrane area.

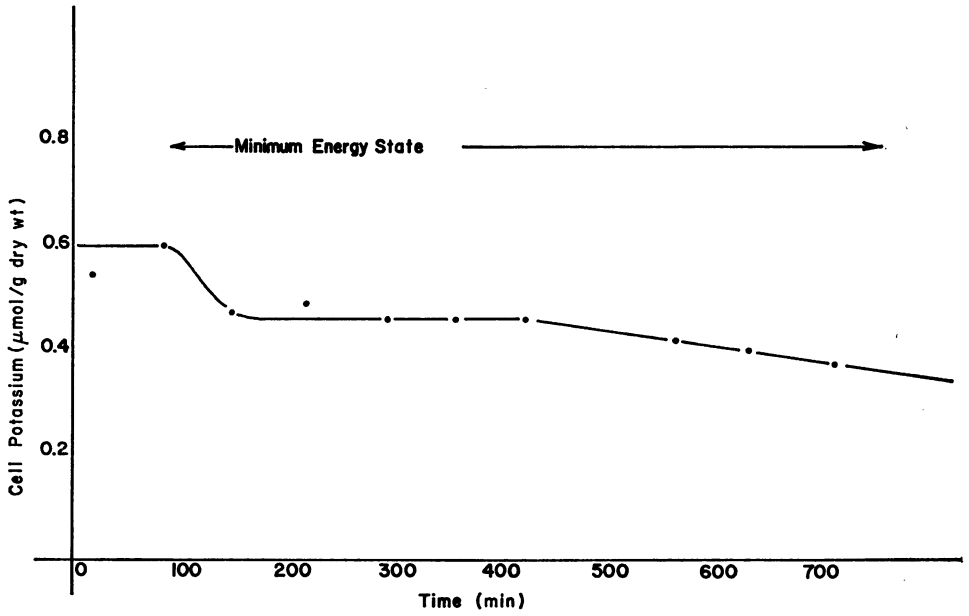


FIGURE 4 Cell potassium content in the minimum energy state. Bacteria were grown in glucose-limited sodium-A medium (0.5 mg glucose/ml). Samples were taken periodically, collected on Millipore filters (0.45 μ M), and washed with 400 mM sucrose. Details of the procedure for determining bacterial potassium are described in reference 12.

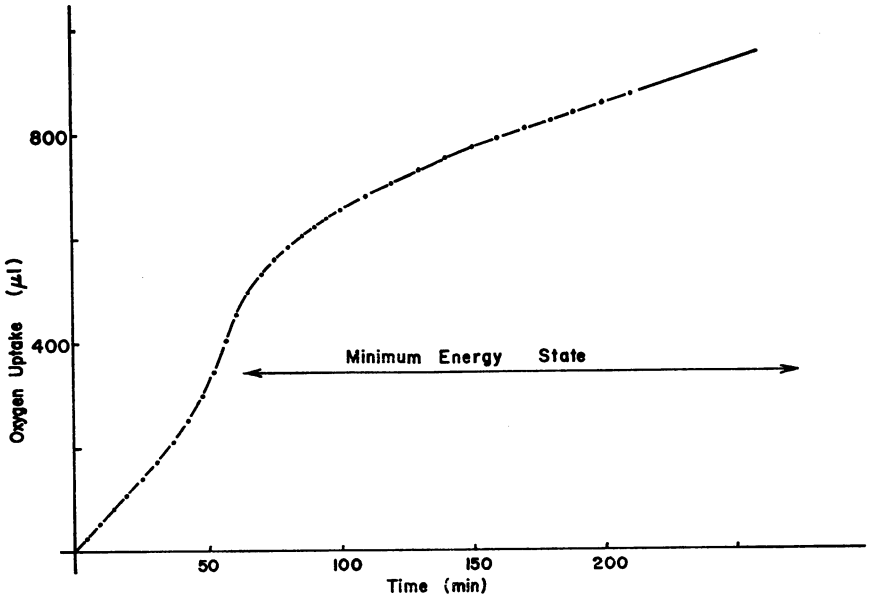


FIGURE 5 Oxygen consumption during the minimum energy state. Oxygen consumption was measured on a Gilford Respirometer under 100% oxygen, and in the presence of KOH (0.1 N). The oxygen leakage rate for our equipment at 100% oxygen was 20 μ l/h and constituted the background subtraction from the measured oxygen loss that provided the final bacterial oxygen consumption.

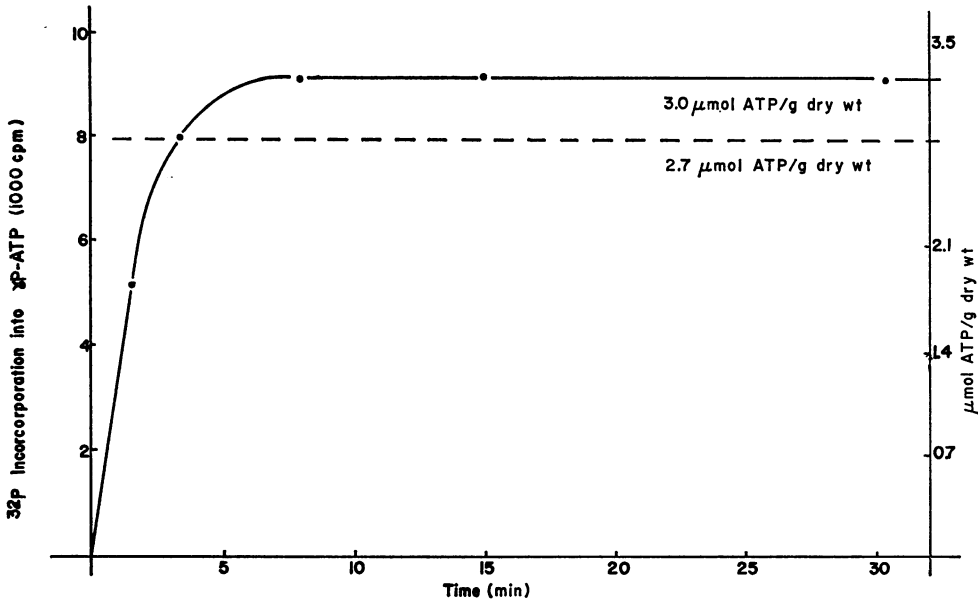


FIGURE 6 $^{32}\text{P}_i$ incorporation into ATP during the minimum energy state. Incorporation is shown as the accumulation of radioactivity in the ATP molecule and the corresponding concentration of ATP that was labeled at the γ -phosphate. The broken line represents the triphosphate concentration determined chemically by the PGK assay.

The rate of $^{32}\text{P}_i$ incorporation the γ -phosphate of ATP (Fig. 6, solid line) indicates that all of the cell ATP, as determined by the PGK assay for ATP (broken line, 2.7 $\mu\text{mol/g}$ dried cells), has been accounted for by incorporation of $^{32}\text{P}_i$ into the terminal phosphate. The P:O ratio determined from the oxygen consumption studies and $^{32}\text{P}_i$ incorporation into ATP was 0.085. The energy requirements for pump-mediated transport of the solutes studied totaled 28.28 cal/340 min-g dry wt, a deficit of 24.08 cal. Utilizing a free energy for ATP hydrolysis of 7.6 kcal/mol (22, 23), the estimated rate of energy delivery from this P:O ratio by the traditional rules of bioenergetics was 4.2 cal/340 min-g dry wt. Furthermore, since there is no net ATP synthesis during substrate limitation (Fig. 3), the energy for transport in the "minimum energy" state is derived exclusively from the *turnover hydrolysis* of ATP.

DISCUSSION

Possible explanations were considered for the observed discrepancy between total caloric consumption of the membrane "pumps" and the available energy supply, namely:

(a) Sources of energy other than nucleoside triphosphate supply the cell's transport needs.

(b) The energy-limited state renders the cell membrane impermeable to potassium efflux thereby sustaining a normal intracellular potassium concentration and a high concentration gradient after the onset of the "minimum energy" state.

(c) A large fraction of the measured membrane flux is "exchange diffusion." This is a mechanism originally considered by Ussing (24, 25) to explain disparities in metabolic energy and pump requirements reported by other investigators (26-30).

(d) Since values of the activity coefficient for a single ion inside a cell are unknown, the values chosen were arbitrary. Calculations utilizing activity coefficients for equivalent aqueous solutions overestimate the energy requirements.

(e) There is insufficient metabolic energy to operate the postulated membrane "pumps." Experimental measurements of the caloric requirements of solute transport are not compatible with "pumps."

Sources of Metabolic Energy Other than ATP

In classical membrane theory, virtually all of the pump models that have been proposed for ion transport consider ATP the energy currency. In particular, discrete molecular models that have been proposed for membrane-mediated sodium and potassium transport are all built around Na-K-dependent ATPases to supply the energy needs of the pump by the cleavage of ATP (31-33). A recent review describes numerous Na-K ATPases that have been reported to provide for sodium and potassium transport in biological tissues (34).

Impermeability of the Cell Membrane to Potassium Efflux

In *E. coli*, possibility of membrane impermeability to potassium flux was excluded by the measured steady-state potassium flux. Potassium permeated the membrane at the rate of 0.150 pmol/cm²-s. In fact, the calculated energy consumption from the product of the flux and the electrochemical potential specifies the rate at which energy was consumed at this "permeability."

Exchange Diffusion

Exchange diffusion² is the point of this paper. If all or most of the isotopic exchange takes place at "fixed charge" sites within the cell (see Table IV of reference 35; also see references 36, 37), exchange diffusion constitutes the mechanism by which the living cell exchanges tracer for stable isotope. Quantitative correlation between the available metabolic energy and the energy necessary to maintain transmembrane concentration ratios would consequently be poor.

² Specifically, Ussing postulated the concept of exchange diffusion in 1947 (24). Briefly, the mechanism consisted of a mobile macromolecule within the cell membrane, a "carrier" molecule, that shuttled back and forth across the thickness of the cell membrane binding a radioactive isotope at one interface and exchanging it for a nonradioactive isotope at the other (25). This facilitated isotopic equilibration is envisioned as a non-energy-consuming process. Experimentally, it cannot be distinguished from the isotopic self-exchange that occurs at fixed charge sites in ion exchangers, and is equivalent to it.

Values Selected for the Activity Coefficients of Intracellular Solutes

The values selected for the activity coefficients of single ions inside cells were those conventionally assumed in traditional "pump" theory. The membrane theory requires that intracellular solutions, and hence intracellular activity coefficients, approximate aqueous solutions. The activity coefficients used in the calculation are therefore the maximum values provided by the "pump" theory. Any overestimates of energy due to overestimates of the activity coefficients contradict the "pump" hypothesis itself. "Pumps" exist solely to maintain apparent "concentration gradients" brought about by the principle of membrane theory that intracellular solutes are in "free solution." Selection of smaller values for intracellular ion activities than those utilized would require significant intracellular ion pair association or significant departure of the internal solution from simple aqueous behavior violating the fundamental basis of "pump" theory.

Insufficient Metabolic Energy

Consequently, by exclusion, it can be said that the rate of energy delivery, calculated from traditional bioenergetics and the P:O ratio, is insufficient to operate membrane-situated pumps. The degree of disparity for the experimentally determined solutes was severe: a deficit of 24.08 cal/g dried cells over a period of 340 min (Table I). Only 14.8% of the necessary energy was available from the turnover hydrolysis of ATP.

In addition, our studies have only accounted for the transport requirements of six solutes. The amino acids arginine, histidine, tyrosine, phenylalamine, methionine, threonine, valine, glutamine, asparagine; the organic acids acetate, pyruvate, succinate, α -ketoglutarate, malate, lactate, fumarate, formate, bicarbonate; and the inorganic ions, sulfate, manganese and phosphate, all of which accumulate in *E. coli* (Table IV of reference 35), are likely to compound this discrepancy when their caloric requirements are known. On the basis of the measurements and calculations in this paper, we find membrane models of solute transport dependent on ATP for energy to be thermodynamically untenable. We therefore conclude that the present pump models as constituted pose a serious dilemma, a caloric catastrophe, so to speak.

Received for publication 20 May 1972.

REFERENCES

1. DEAN, R. 1941. *Biol. Symp.* 3:331.
2. KROGH, A. 1946. *Proc. R. Soc. Lond. B Biol. Sci.* 133:140.
3. GREEN, K. 1965. *Am. J. Physiol.* 209:1311.
4. CAMPBELL, W., and D. GREENBERG. 1940. *Proc. Natl. Acad. Sci. U.S.A.* 26:177.
5. SILVER, S. 1969. *Proc. Natl. Acad. Sci. U.S.A.* 62:764.
6. LEVINE, M., D. L. OXENDER, and W. D. STEIN. 1965. *Biochim. Biophys. Acta.* 109:151.

7. EGGLETON, M. G. 1933. *J. Physiol. (Lond.)*. **79**:31.
8. KABACK, H. R., and E. R. STADTMAN. 1966. *Proc. Natl. Acad. Sci. U.S.A.* **55**:920.
9. PARDEE, A. B., L. S. PRESTIDGE, M. B. WHIPPLE, and J. DREYFUSS. 1966. *J. Biol. Chem.* **241**:3962.
10. SCHULTZ, S. G., and A. K. SOLOMON. 1961. *J. Gen. Physiol.* **45**:355.
11. LING, G. N. 1965. *Fed. Proc.* **24**(Suppl 15): 5103.
12. DAMADIAN, R. 1968. *J. Bacteriol.* **95**:113.
13. DAMADIAN, R. 1966. School of Aerospace Medicine Technical Report. SAM-TR-66-19.
14. KEPES, A., and G. N. COHEN. 1962. In *The Bacteria*. I. C. Gansalus and R. Y. Stanier, editors. Academic Press, Inc., New York. **4**:179-222.
15. ROBERTS, R. B., P. H. ABELSON, D. B. COWIE, E. T. BOLTON, and R. J. BRITTEN. 1955. *Carnegie Inst. Washington Publ.* **607**.
16. DAWES, E. A., and D. W. RIBBONS. 1962. *Annu. Rev. Microbiol.* **16**:241.
17. GILSON, W. E. 1963. *Science (Wash. D. C.)*. **141**:531.
18. UMBREIT, W. W., R. H. BURRIS, and J. F. STAUFFER. 1964. *Manometric Techniques*. Burgess Publishing Company, Minneapolis.
19. DREIZEN, P. 1966. *J. Biol. Chem.* **241**:443.
20. CRANE, R. K., and F. LIPMANN. 1953. *J. Biol. Chem.* **201**:235.
21. SCHULTZ, S. G., N. L. WILSON, and W. EPSTEIN. 1962. *J. Gen. Physiol.* **46**:159.
22. ROBBINS, E. A., and P. D. BOYER. 1957. *J. Biol. Chem.* **224**:121.
23. MAHLER, H. R., and E. H. CORDES. 1966. *Biological Chemistry*. Harper & Row, Publishers, New York. **201**.
24. USSING, H. H. 1947. *Nature (Lond.)*. **160**:262.
25. USSING, H. H. 1948. *Cold Spring Harbor Symp. Quant. Biol.* **13**:193.
26. TOSTESON, D. 1955. In *Electrolytes in Biological System*. A. M. Shanes, editor. American Physiological Society, Washington, D.C. **123**.
27. DAVIES, R. E. 1954. *Symp. Soc. Exp. Biol.* **8**:453.
28. KEYNES, R. D., and R. H. ADRIAS. 1956. *Faraday Soc. Discuss.* **21**:265.
29. SWAN, R. C., and R. D. KEYNES. 1956. Abstracts of International Physiological Congress, Brussels. **869**.
30. CROGHAN, P. C. 1958. *J. Exp. Biol.* **35**:219.
31. KEYNES, R. D. 1961. In *Membrane Transport and Metabolism*. A. Kleinzeller and A. Kotyk, editors. Academic Press, Inc., New York. **131**.
32. SOLOMON, A. K. 1962. *Biophys. J.* **2**:79.
33. SKOU, J. C. 1965. *Physiol. Rev.* **45**:596.
34. ALBERS, R. W. 1967. *Annu. Rev. Biochem.* **36**:727.
35. DAMADIAN, R. 1971. *Biophys. J.* **11**:739.
36. DAMADIAN, R., M. GOLDSMITH, and K. S. ZANER. 1971. *Biophys. J.* **11**:761.
37. DAMADIAN, R. 1971. *Biophys. J.* **11**:773.