RESEARCH COMMUNICATIONS

REPLY TO LETTERS ON "CALORIC CATASTROPHE"

INADEQUACY OF THE ENERGY AVAILABLE FROM ATP FOR MEMBRANE TRANSPORT

LAWRENCE MINKOFF *and* RAYMOND DAMADIAN

From the Biophysical Laboratory, State University of New York Downstate Medical Center, Brooklyn, New York 11203

Recommended by Carlton F. Hazlewood and Freeman W. Cope

We respond to the letters of White and Ibsen (1) and of Raven (2) that questioned results in our paper "Caloric Catastrophe" (3). We concluded in this paper that transport reactions dependent on ATPase's for energy do not comply with the Laws of Energy Conservation. It is best to begin by reiterating what we have done in our experiments and what we claim, so that no ambiguities remain to fuel debate.

ATP and membrane ATPase's are a cornerstone of membrane "pump" theory. Cleavage of the *terminal phosphate* of ATP by ATPase's such as the Na-K ATPase's of ion transport is the source of energy in membrane theory for "pumps". There is no doubt that the discrete molecular models that have been proposed for "pumping" sodium and potassium are all built around Na-K ATPases (4–6). Numerous Na-K ATPases that have been reported to power sodium and potassium pumps have been reviewed (7).

We therefore addressed ourselves, in "Caloric Catastrophe" to the question of whether membrane pumps that derive their energy from hydrolysis of the terminal phosphate of ATP comply with the Laws of Energy Conservation.

We measured the rate at which the terminal bond of ATP was being split and we simultaneously calculated the energy required by thermodynamics for pumps to operate. They did not agree. During a 340 min period, only 4.2 cal/g dry wt were liberated by ATP while 28.28 cal/g dry weight were needed for transport. Since cell

ATP concentrations were steady state throughout the course of our experiments, the rate of hydrolysis of ATP was measured by the rate of incorporation of extracellular $^{32}P_i$ (Figs. 3 and 6 in reference 3) into the terminal phosphate of ATP.

The P:O ratio in our study, referred to by Doctors White and Ibsen and by Dr. Raven, is therefore quite extraneous. It is not the "central issue" as suggested by White and Ibsen. Rather, it is somewhat beside the point. We measured directly the rate of ATP hydrolysis and consequently the energy available from the terminal bond of this molecule to power "pumps".

CELL $^{32}P_i$ PENETRATION

Both letters raise the possibility that the rate of ATP hydrolysis was underestimated because $^{32}P_i$ incorporation into ATP was rate-limited by penetration of $^{32}P_i$ into the cell rather than by ATP turnover rate itself. This is a legitimate question since the controls for this possibility were not included with the paper. From the controls performed with the study, however (Table I), $^{32}P_i$ penetration was virtually instantaneous (10 experiments) in *glucose-limited Escherichia coli*, and this alternative is excluded. The approximation noted by Raven of the labeling half-time of *E. coli* (8) and our ATP labeling half-time is coincidental.

Cellular penetration by $^{32}P_i$ was complete by 0.50 min, and in other experiments where we succeeded in obtaining points at times as low as 0.16 min, we also observed that $^{32}P_i$ accumulation was finished by the time the first point was obtained. The more usual $^{32}P_i$ uptake in the presence of glucose is shown in the right-hand part of the table for comparison.

The speed with which $^{32}P_i$ entered glucose-limited *E. coli*, in fact, prevented the use of $^{32}P_i$ in our study as one of the transported solutes since intake rate was so rapid as to preclude an accurate determination of $^{32}P_i$ influx rate.

TABLE I

CELL $^{32}P_i$ PENETRATION

Glucose-limited *E. coli*		*E. coli* in the presence of glucose	
Time	cpm	Time	cpm
min		*min*	
0.50	6,558	0.57	6,635
2.02	6,339	2.12	7,570
3.65	6,391	3.73	9,792
5.14	6,207	5.31	11,272
6.80	6,072	7.01	13,729
8.46	5,797	8.62	15,067

THE P:O RATIO

While the actual P:O ratio we devised has no bearing on the question of the adequacy of energy for membrane transport—our result would have been the same had we never computed a P:O ratio—we are nonetheless interested in the academic aspects of our P:O value relative to others in the literature.

In bacteria one cannot assume any P:O ratio in advance as Ibsen and White have. The P:O ratio varies widely depending on the experimental conditions as Hempfling (9, 10) and van der Beek and Stouthammer (11) (the authors referred to by Doctors Ibsen, White, and Raven) have demonstrated. In Hempfling's study, the P:O ratio for example, varied from 0.10 to 3.8 depending on the substrate used. The P:O ratio we have computed, therefore, for our energy-starved cell cannot be compared with any P:O ratio but a P:O ratio derived from an energy-starved cell.

Other conspicuous differences between our study and the ones referred to by White and Ibsen and Raven preclude comparison. The P:O ratios in both the studies referred to were computed from the *net* formation of nucleotide (phosphate-esters in Hempfling's case). By these criteria, our P:O ratio would not have been 0.085. It would have been 0.0. *Net* formation of ATP did not occur during the "minimum-energy" state characteristic of our experiments (Fig. 3 in reference 3).

Since no *net* formation of ATP occurred in the "minimum-energy" state, we measured the amount of ATP formed during turnover to determine if the steady-state formation of ATP could supply the necessary energy. It could not. We took pains to measure turnover formation of ATP, rather than settle for the P:O ratio of 0.0, in order to demonstrate that even if every molecule of the cell's ATP were assigned to transport functions and diverted away from other cell needs for energy, there was not enough energy to power pumps.

In addition, Hempfling's P:O ratio was determined from the net amount of phosphate ester formed which can be as high as 112 μmol/g dried bacteria (Table IV *b* in reference 12). Our P:O ratio was determined by direct measurement of ATP formed, which for *E. coli* in the "minimum-energy" state was 2.7 μmol/g dried bacteria.

Why the oxidation of energy-starved *E. coli* does not end up in ATP—there is neither *net* synthesis of ATP nor sufficient turnover to account for it—as Ibsen and White have inquired remains open to question. It is possible that the relatively small remnant of oxidation ($\sim$12% of growing *E. coli*) that persists in the "minimum-energy" state is not coupled to phosphorylation or does not proceed via the cytochrome pathway.

We conclude, therefore, as we began. Membrane models of solute transport dependent on the hydrolysis of ATP for energy are thermodynamically untenable. Transport reactions dependent on ATPase's for the release of energy from the terminal phosphate bond of ATP do not comply with the Laws of Energy Conservation.

Received for publication 25 September 1973.

REFERENCES

1. WHITE, S. H., and K. H. IBSEN. 1973. *Biophys. J.* **13**:1001.
2. RAVEN, J. A. 1973. *Biophys. J.* **13**:1002.
3. MINKOFF, L., and R. DAMADIAN. 1973. *Biophys. J.* **13**:167.
4. KEYNES, R. D. 1961. *In* Membrane Transport and Metabolism. A. Kleinzeller and A. Kotyk, editors. Academic Press, Inc., New York. 131.
5. SOLOMON, A. K. 1962. *Biophys. J.* **2**:79.
6. SKOU, J. C. 1965. *Physiol. Rev.* **45**:596.
7. ALBERS, R. W. 1967. *Ann. Rev. Biochem.* **36**:727.
8. WEIDEN, P. L., W. EPSTEIN, and S. G. SCHULTZ. 1967. *J. Gen. Physiol.* **50**:1641.
9. HEMPFLING, W. P. 1970. *Biochem. Biophys. Res. Commun.* **41**:9.
10. HEMPFLING, W. P. 1970. *Biochem. Biophys. Acta.* **205**:169.
11. VAN DER BEEK, E. G., and A. H. STOUTHAMMER. 1973. *Arch. Mikrobiol.* **89**:327.
12. DAMADIAN, R. 1971. *Biophys. J.* **11**:739.