LETTERS TO THE EDITOR

Comment on the "Caloric Catastrophe"

Dear Sir:

Minkoff and Damadian (1973) reported that they "... find membrane models of solute transport dependent on ATP for energy to be thermodynamically untenable." This conclusion was based on calculations from data obtained from *Escherichia coli* in a "minimum energy state." Their calculations indicated that over a 340 min period 28.28 cal/g dry wt were required for the simultaneous active transport of several solutes while only 4.2 cal/g dry wt were available from the turnover hydrolysis of ATP. Ergo, there is a "caloric catastrophe." We believe that Minkoff and Damadian's conclusion may be wrong.

Minkoff and Damadian report that *E. coli* in the minimum energy state consume O_2 at the rate of 9.6 μmol/g-min so that over 340 min 3.3×10^{-3} mol/g dry wt of O_2 is consumed. If one assumes that some carbon source is being oxidized, then about 10^5 cal will be released for each mole of carbon oxidized to CO_2. There will be about 3.3×10^2 cal released over the experimental period which is 10-fold more energy than is consumed by active transport. Therefore, active transport by membrane processes *is* tenable thermodynamically ignoring metabolic details.

The metabolic detail which must be considered is the coupling of ATP synthesis to carbon source oxidation. Minkoff and Damadian calculate the P:O ratio to be 0.085 while the expected value is 3 (Hempfling, 1970 *a*). This low value suggests that electron transport has been uncoupled from ATP synthesis. It seems unlikely that the glucose starvation of the minimum energy state could cause uncoupling. In fact, the data of Hempfling (1970 *b*) indicate that glucose starvation stimulates oxidative phosphorylation. The central issue is, then, whether or not the P:O ratio was correctly determined. We believe it was not. They measured the rate at which $^{32}P_i$ placed in the suspending medium was incorporated into the γ-phosphate of ATP. This measurement tells how rapidly $^{32}P_i$ entered the nucleotide pool but not necessarily how rapidly the pool turns over, which is of course the critical point. Turnover would be measured only if the hydrolysis of ATP leads to the removal of P_i from the pool. In addition, it must be shown that the rate-limiting step in $^{32}P_i$ uptake is in the oxidative phosphorylation process and not in the permeation of the cell surface structures.

It is probable that the P:O ratio for *E. coli* under the conditions described by Minkoff and Damadian is closer to 3 than to 0.085 (Hempfling, 1970 *b*). If the value is 3, then over the 340 min time period 147 cal/g dry wt would be available from ATP hydrolysis. Minkoff and Damadian's value for the minimum amount of energy required for active transport processes during the same period is 28.28 cal/g dry wt. Thus, only about 19% of the available energy would be required for active transport and we would conclude that there is no caloric catastrophe.

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Hempfling, W. P. 1970 *a*. *Biochem. Biophys. Res. Commun.* **41**:9.
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Caloric Recalculation

Dear Sir:

In a recent paper Minkoff and Damadian (1) claim that insufficient energy is available from the use of ATP in *Escherichia coli* cells in "minimum energy state" to power six solute pumps which they investigated. This claim is based on a consideration of the measure P/O ratio in these cells; together with the ATP content and a value for the free energy of ATP hydrolysis, this allows the rate of energy production for ATP-dependent pumping to be calculated. The measured P/O of 0.085 in the minimum energy state is lower than that found by van der Beek and Stouthammer (2) in "resting" bacteria, including *E. coli*, i.e., 0.3–1.0. The low value of P/O obtained by Minkoff and Damadian could be due to an underestimate of the rate of ATP turnover. They measured this by supplying $^{32}P_i$ in the medium, and measuring the incorporation of label into the γ-phosphate of ATP. The implicit assumption here is that the specific activity of the orthophosphate used in oxidative phosphorylation is the same as that of the exogenous phosphate. This is unlikely to be true; the rate of labeling of ATP is likely to be limited under these conditions by the rate of ^{32}P uptake into the intracellular orthophosphate pool. The data of Weiden et al. (3) for phosphate uptake and inorganic phosphate pool size in growing *E. coli* suggest that the half-time of labeling of this pool is 1 min, while the half-time of ATP labeling in *E. coli* measured by Minkoff and Damadian is 1.5 min. The agreement between these two values is close and suggests that Minkoff and Damadian have underestimated the ATP turnover due to the slow orthophosphate uptake rate.

Thus it is possible that the P/O ratio could be as high as 1.0 under these circumstances; this would allow adequate ATP for the solute transport processes under investigation. A further point releated to the energy supply for solute transport is the evidence that oxidative phosphorylation could power solute fluxes in prokaryotes directly via a proton gradient (4), i.e., ATP would be an alternative to solute transport. A P/O of 1 would allow adequate energy supply to solute pumps via H^+ gradients.

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1. Minkoff, L., and R. Damadian 1973. *Biophys. J.* **13**:167.
2. van der Beek, E. G., and A. H. Stouthammer 1973. *Arch. Mikrobiol.* **89**:327.