

Letter to the Editor

Cyclic AMP Stimulation of Calcium Efflux from Isolated Mitochondria: A Negative Report*

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I have recently published results showing that cyclic AMP triggers the release of calcium from kidney, liver and heart mitochondria (Borle, 1974; 1975). My laboratory worked on this problem from November, 1972, to December, 1973. The effects of cyclic AMP could be demonstrated by five different methods: (1) measurements of the total calcium concentration in the medium bathing the isolated mitochondria after filtration through a 0.45 millipore filter; (2) measurements of the calcium activity of the suspending medium with a Beckman calcium ion electrode; (3) measurements of Ca^{45} uptake and release into the medium; (4) measurement of the Ca^{45} concentration in the mitochondria; and (5) shifts in pH of the suspending medium. These results have been duplicated in liver and adrenal mitochondria by Matlib and O'Brien (1974). Comparable results have been obtained with a different technique and with higher cyclic AMP concentrations by Howell *et al.* (1975).

In 1975, I was informed by Drs. Batra, Carafoli, Rasmussen, Scarpa and Saris that they were unable to reproduce these results. I agreed to investigate this problem anew, with the techniques used in 1972 and 1973. From July, 1975, to February, 1976, my laboratory performed 442 experiments and in only 6% of them did I obtain a significant release of calcium from liver or kidney mitochondria. In 53% of the experiments, the results were completely negative. In the remaining 40% of experiments, the results were so small or so inconsistent that they must be considered negative. I attempted many modifications of the published method: the age of the rats, their breed, their diet, the method of sacrifice (vertebral dislocation and decapitation) were varied. Different methods of mitochondrial preparation were also tried: sucrose *vs.* manni-

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tol, 250 vs. 300 mOsm, presence or absence of EDTA or EGTA, centrifugal speed varying from 8,000 to 20,000 $\times g$. The incubating medium was also modified: presence or absence of ATP or of respiratory substrates, various concentrations of magnesium and phosphate and different ratios, different concentrations of cyclic AMP (from 10^{-7} M to $5 \cdot 10^{-5}$ M), different batches and different sources of this nucleotide (Sigma, Boehringer). No consistent results and no consistent pattern was observed. I do not have, currently at least, a plausible explanation for the discrepancy between the present and the previous results.

Consequently, I can only agree with Scarpa *et al.* (1976) and subscribe to their very conservative conclusion that these effects are very difficult to reproduce. I propose that the results published by Matlib and O'Brien and by myself as well as their physiological implications be kept in abeyance until new and reproducible data are published. In personal communications to me, Drs. Matlib and O'Brien have agreed with this conclusion. Finally, to the impressive list of colleagues who have communicated to Scarpa *et al.* (1976) their inability to reproduce these results, the following names should be added (personal communications): Dr. Lehninger (Philadelphia), Dr. Baker (Philadelphia), Dr. Gunter (Rochester) and Dr. Fairhurst (Irvine).

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