Interaction between Uncouplers of Oxidative Phosphorylation and *Staphylococcus aureus* Infected Liver Mitochondria

Mice intraperitoneally infected with a virulent strain of *Staphylococcus aureus* Smith (1×10^9 cells) have been observed to die within 260 min ($\pm$ 20 min) following the challenge[1]. The mechanism of death is as yet unknown. During the past 10 years sufficient data have been accumulated in our laboratory to indicate that the pathogen profoundly influences the normal metabolism of the host[2]. Recently, the possibility of uncoupling of oxidative phosphorylation by the staphylococcal 'toxin' (whole staphylococci and all their toxins as produced in the host are encompassed in this expression 'toxin') has been speculated[3]. This report is concerned with the P/O ratio of staphylococcal infected liver mitochondria as well as the effects of uncouplers of oxidative phosphorylation in vitro. Changes in the population to 'energized' and 'non-energized' liver mitochondria[4] following *S. aureus* infection in vivo have also been recorded from electron micrographs.

White mice (30 g) were fasted overnight and then inoculated with *S. aureus* as previously described[5]. Studies were made 3 h after the challenge or 80 min before the average death time of 260 min. Liver homogenates were freshly made in 0.25 *M* sucrose using a high speed tissue grinder. Mitochondria were used for the oxidative phosphorylation studies in LEHNINGER's medium[7] except that fluoride was replaced with malonate in equimolar amounts[8]. Respiratory studies were done by the standard manometric techniques[9]. Respiration was followed for 60 min at 30 °C. The uncoupling agents used were 2,4-dinitrophenol ($3 \times 10^{-5} M$), oligomycin (2 µg/3 ml) and antimycin A (1 µg/3 ml).

Electron micrographs of the mitochondrial population of normal and infected liver homogenates in 0.5 *M* sucrose were obtained by the methods described elsewhere[10]. 'Energized' and 'non-energized' mitochondria were identified[4] and their relative distribution (%) in the entire population was calculated and averaged from 5 electron micrographs.

The infected liver mitochondria have a high rate of oxygen uptake (Table). The uncoupling actions of dinitrophenol, oligomycin and antimycin A are clearly indicated by the significant fall of P:O ratio in the normal liver mitochondria. Interactions of the chemical uncouplers in vitro with the uncoupling action of *S. aureus* in vivo indicate the similarity of the latter with that of dinitrophenol. The 'toxemic' mitochondria from infected mouse livers show less response to the stimulative action of dinitrophenol in regard to oxygen uptake compared to normal liver mitochondria. Phosphate esterification of infected mitochondria is not suppressed by dinitrophenol to the extent found in the uninfected mitochondria. The P:O ratios indicate that the uncoupling action of *S. aureus* 'toxin' in vivo is more effective than that of

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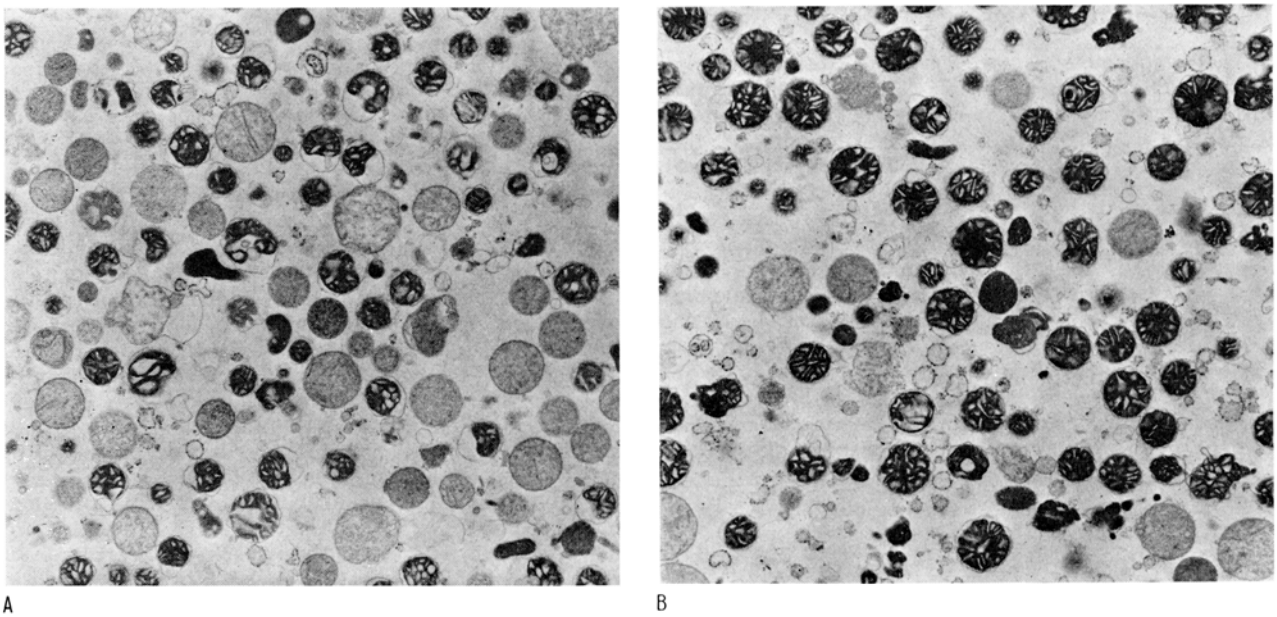
Uncoupling of oxidative phosphorylation by staphylococcal toxin action in vivo (n = 5)

Condition	Oxygen uptake µatoms/mg N	% control	Phosphate uptake µ*M*	% control	P:O Ratio Ratio	% control	Energy State of Mitochondria «Energized» (%)	«Non-energized» (%)
Normal								
Control (normal)	3.4 ($\pm$ 0.2)	100	8.8 ($\pm$ 2.0)	100	2.5 ($\pm$ 0.3)	100	—	—
+ DNP	4.4 ($\pm$ 0.4)	130[b]	4.6 ($\pm$ 1.0)	52[b]	1.0 ($\pm$ 0.1)	40[b]	—	—
+ Oligomycin	2.2 ($\pm$ 0.3)	62[b]	1.8 ($\pm$ 0.1)	19[b]	0.2 ($\pm$ 0.02)	8[b]	—	—
+ Antimycin A	0.0	0[b]		0[b]	0	0[b]	—	—
Infected								
Control (infected)	4.2 ($\pm$ 0.4)	100	2.2 ($\pm$ 0.2)	100	0.5 ($\pm$ 0.1)	100	—	—
+ DNP	4.6 ($\pm$ 0.5)	110	1.6 ($\pm$ 0.1)	72[b]	0.3 ($\pm$ 0.07)	60[b]	—	—
+ Oligomycin	1.9 ($\pm$ 0.07)	45[b]	0.4 ($\pm$ 0.2)	14[b]	0.1 ($\pm$ 0.05)	20[b]	—	—
+ Antimycin A	0	0[b]	0	0[b]	0	0[b]	—	—
Mitochondria distribution in vivo								
Normal liver	—	—	—	—	—	—	35 ($\pm$ 4)	65 ($\pm$ 4)
Infected liver	—	—	—	—	—	—	22 ($\pm$ 5)	78 ($\pm$ 5)
Infection effect								
Infected control as % normal control		124[a]		25[b]		20[b]	62[b]	120[b]

[a] Difference is significant from the normal or infected control ($p < 0.05$).
[b] Difference is significant from the normal or infected control ($p < 0.01$).



A B

Electron micrographs of mouse liver mitochondria: (A) Normal and (B) after 3h of *S. aureus* challenge.

dinitrophenol at the optimum concentration used in vitro. Oligomycin, on the other hand, suppresses both oxygen uptake as well as the phosphate esterification rates of infected mitochondria more effectively than that of the normal mitochondria. Complete uncoupling is noted in mitochondria treated with antimycin A in both normal and infected mitochondria.

Electron micrographs show a decrease of the 'energized' mitochondrial population and an increase of 'non-energized' mitochondrial population due to the staphylococcal 'toxin' action in vivo (Table, Figure).

Staphylococcal 'toxin' causes degree or proportion uncoupling in vivo. The uncoupling action has a similarity with that of dinitrophenol acting in vitro. It is thus speculated that the staphylococcal toxin probably allows oxidation to proceed without phosphorylation. The interaction studies in vitro between dinitrophenol and infected mitochondria further suggests that the 'uncoupled' mitochondria from infected animals respond less effectively to the chemical uncoupler in vitro than normal mitochondria. The inhibition of phosphorylating electron transport caused by oligomycin[11,12] is aggravated under infected conditions.

In one of our recent studies[13] we have observed an increase of long-chain fatty acids of the lipid composition of liver following staphylococcal infection. This may contribute to the uncoupling of oxidative phosphorylation in infected mitochondria[11,12]. Further, the relative distribution of saturated long-chain fatty acid is found to be more than the unsaturated long-chain fatty acids in the infected liver[13]. The fall of unsaturated long-chain fatty acid, particularly linoleic acid, may induce a damaging effect on the mitochondria[14]. Thus the inhibitory effects of oligomycin on the electron transport system appear to have been accentuated under infected conditions. The increase of the 'non-energized' mitochondrial population in the infected liver substantiate the possibility of uncoupling action by the staphylococcal 'toxin' in vivo[4].

The mechanism of the uncoupling action of staphylococcal 'toxin' in the body of the host is yet to be resolved[3,15]. The present study, however, brings forth fresh evidences to support the site of uncoupling to be at the phosphorylating step of oxidation. Inhibition of electron transport in oxidation can be due to mitochondrial damage. The host shows signs of hypoglycemia when infected[16] while an increase of oxygen consumption by the whole animal has not been demonstrated[3]. On the other hand, ATP production is badly impaired due to uncoupling of oxidative phosphorylation caused by the staphyloccocal 'toxin'[15]. Thus the host is unable to succeed in this adaptation to maintain energy production.

Résumé. Des microphotographies électroniques de mitochondries du foie de souris infectées par des staphilocoques montrent une décroissance de la population énergisée, ce qui suggère un découplement. Les mitochondries du foie des animaux infectés avaient un taux élevé d'absorption d'oxygène et indiquent une action de découplement de la *S. aureus* plus effective que celle du dinitrophenol employée in vitro dans une concentration optimale. Le découplement semble avoir lieu au stage de phosphorylation de l'oxydation.

K. L. Mukherjee, I. M. Smith and R. Barker

Infectious Disease Laboratories, University Hospitals, and Department of Biochemistry, University of Iowa, Iowa City (Iowa 52240, USA), 18 May 1971.

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