The Effects of *Chrysaora* and *Physalia* Venoms on Mitochondrial Structure and Function (37451)

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Jellyfish venoms are toxic to nerves and muscles of lower animals (1–4). *Chironex fleckeri* (sea wasp) venoms have been shown to affect the transport of calcium ions between the internal spaces of the muscle fibers and the external media. It has been postulated that this effect is due to the ability of this venom to prevent calcium uptake by the sarcoplasmic reticulum (5). Two other jellyfish, *Physalia physalis* (Portuguese man-of-war) and *Chrysaora quinquecirrha* (sea nettle), have venoms which depress calcium uptake by the sarcoplasmic reticulum (6). *C. quinquecirrha* venom also exerted an effect on the isolated canine Purkinje fiber which mimicked the action resulting from decreased calcium ion concentration at the membrane level (7). These Ca^{2+} mediated phenomena prompted the present studies into the effects of *Physalia* (P) and *Chrysaora* (C) venoms on calcium uptake and related functions in isolated mitochondria.

Materials and Methods. Lethal and nonlethal (inactivated by heat) (6) C and P venoms of known protein content and lethal potency (mouse intravenous LD_{100}) were obtained according to previously described techniques (8, 9).

Mitochondria were isolated from rat liver by the method of Weinbach (10). Aliquots of the mitochondrial suspensions containing 2 mg protein were incubated for 15 min at 25° in the presence of jellyfish venoms or control solutions in previously described media (11). At the end of this period calcium binding was determined using the Millipore filter technique previously employed in the experiments on calcium uptake by sarcoplasmic reticulum (6, 12).

Experiments were performed with and without added ATP (4 m*M*), oligomycin (1 μg/mg mitochondrial protein), antimycin A (1 μg/mg mitochondrial protein), malonate (20 m*M*), and succinate (6.7 m*M*).

Electron microscopy was performed on the mitochondrial pellets which had been incubated for the usual 15 min period with jellyfish venom or control solutions containing either equal volume or equal protein concentration (bovine serum albumin). The pellets were fixed in 1.5% glutaraldehyde for 1 hr, washed with Millonig's buffer and postfixed in 1% OsO_4 for 1 hr. All of the above procedures were performed at 4°.

Dehydration through graduated concentrations of alcohol was completed before embedding in Epon 812 according to established techniques (13). The specimens were examined under a Siemans 101 electron microscope.

Oxidative phosphorylation was measured polarographically using an oxygen electrode at 25° as previously described (14). The vessel contained 1.8 ml of a reaction mix consisting of: 120 m*M* KCl; 20 m*M* Tris–HCl (pH 7.4); 5 m*M* potassium phosphate (pH 7.4); 5 m*M* $MgCl_2$ and 0.2 ml of mitochondrial suspension (4 mg protein). The final concentration of substrate was 10 m*M* and 0.25 μmoles of ADP were added to initiate oxidative phosphorylation.

Protein concentrations of mitochondrial preparations were determined by the previously described methods (15).

Results. Venoms from both jellyfish markedly depressed calcium binding in isolated mitochondria (Table I). The minimum dosage of venom necessary to alter mito-

TABLE I. Depression of Ca^{2+} Uptake by *Chrysaora* and *Physalia*.[a]

Dosage (mouse iv LD_{100}):	0.1	0.3	0.5	1	3	5
	% Depression of calcium uptake					
Chrysaora venom	—	—	—	—16	—68	—89
Heated (nonlethal) *Chrysaora* venom	—	—	—	—13	—37	—
Physalia venom	—3	—79	—85	—95	—	—
Heated (nonlethal) *Physalia* venom	—	—77	—	—95	—	—

[a] Values given are expressed as percentage of depression of calcium uptake. Mitochondria were incubated for 15 min at 25°.

chondrial calcium transport was 3 LD_{100} (2.25 mg protein) for C venom and 0.3 LD_{100} (1.1 mg protein) for P venom. Heating of threshold doses of C and P venoms, thereby destroying their lethal action, resulted in a loss (50%) of only the effect of C venom upon calcium binding. All subsequent experiments involving both venoms were performed utilizing 1.67 times the minimal active dosage. Prior incubation of the mitochondria with either venom for 15 min followed by replacement of the incubating solution with radioactive buffer demonstrated blockage of calcium binding.

The effect of the jellyfish venoms on the low and high affinity calcium uptake of mitochondria was examined in media containing 80 μmoles Ca^{2+} and 2 μmoles CA^{2+}/mg protein, respectively (16). More than 50% depression in both low and high affinity calcium binding was observed for both toxins.

The ability of these venoms to prevent calcium binding in mitochondria was not affected by antimycin A or oligomycin in either the presence or absence of ATP (Table II). Bovine serum albumin (3.5 mg/ml) was more effective in counteracting the action of P venoms on mitochondrial calcium uptake than it was against C venom (Table II).

The introduction of C or P toxin to mitchondrial suspensions previously incubated with radioactive calcium for 15 min at 25° resulted in a 45 or 64% decrease, respectively, in the amount of mitchondrial calcium binding during the subsequent 15 min.

A mitchondrial suspension was incubated at 4° with radioactive calcium and either venom or control solutions. Aliquots were removed from each mixture at 1, 2.5 and 5 min. At the end of 5 min, the suspension was centrifuged (12,000*g*, 5 min) and suspended in nonradioactive buffer without venom (see Fig. 1). Aliquots were then taken 2.5, 5 and 10 min. This experiment demonstrated that only 2.5 min were required for maximum calcium binding and that only the *Physalia* venom depressed the uptake of calcium by the mitochondria during this period. Mitochondria in contact with *Chrysaora* venom transported calcium normally. If the mitochondria were preincubated with either venom for 15 min at 4° before the addition of radioactive calcium and then following the above regimen, significant depression of calcium binding was induced by both venoms (Fig. 1).

TABLE II. Depression of Ca^{2+} Uptake by Chemical Agents in the Presence or Absence of ATP.[a]

	Control (%)	*Physalia* (%)	*Chrysaora* (%)
ATP present	0	—83	—73
ATP absent, antimycin present	—90	—99	—99
ATP present, antimycin present	0	—97	—68
ATP present, antimycin present, oligomycin present	—50	—99	—89
ATP present, bovine serum albumin present	0	—6	—54

[a] Mitochondria were incubated for 15 min at 25° with either control or toxin preparations.

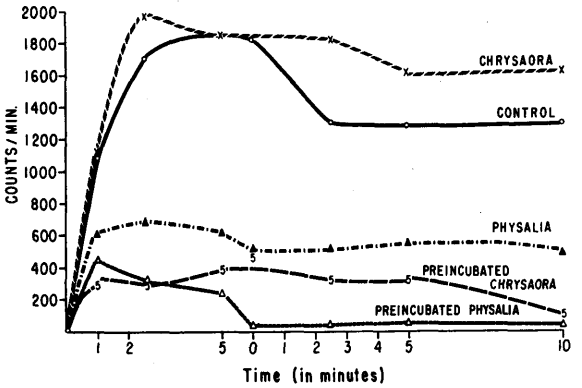


FIG. 1. Uptake and release of ^{45}Ca by mitochondria incubated with *Chrysaora* venoms at 4°. Dosage was 5 LD_{100} of C venom (3.0 mg protein) and 0.5 LD_{100} P venom (1.8 mg protein). At time 0 the venoms were removed from the mixture by sedimentation of the mitochondria and resuspension in nonradioactive buffer.

A mitochondrial suspension (3 ml) containing 0.5 mg protein placed in a cuvette was inoculated with C or P venom (less than 0.15 ml volume). The rate of mitochondrial lysis was determined as the decrease in optical density at 520 nm (Fig. 2). Both toxins caused a rapid decrease in optical density.

Electron microscopic examination of venom-treated mitochondria revealed relaxed membranes, loss of mitochondrial ground substance and swelling of the cristae in the remaining intact organelles. The alterations produced by *Physalia* toxin appeared to be more severe than those by *Chrysaora* (Fig. 3).

Rat liver mitochondria were preincubated for 7 min in the presence or absence of varying amounts of P toxin or C toxin. Respiratory control indices (RCI) were obtained after the addition of substrate and ADP. The results are shown in Fig. 4. Increasing dosages of either C or P toxin lead to progressive declines in RCI. With 0.5 LD_{100} of C toxin, the mitochondria were incapable of oxidizing glutamate and the rate of succinate oxidation did not increase upon the addition of ADP. With P toxin at 1 LD_{100} (50 μl) stimulation by ADP but not respiratory control was obtained with both substrates. Two LD_{100} (100 μl) of P toxin resulted in the inhibition of substrate oxidation and visible decrease in optical density of the mitochondrial suspension. The effect of 1 LD_{100} of P toxin or C toxin could be overcome by the addition of bovine serum albumin (4 mg/ml) to the reaction mixture (Fig. 4). The mitochondria treated with either toxin did not oxidize exogenous NADH.

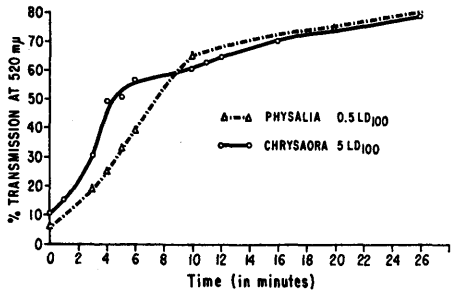


FIG. 2. Changes in optical density of a mitochondrial suspension incubated with jellyfish venom as a function of time.

Jellyfish toxins have been reported to affect the calcium binding by sarcoplasmic reticulum (5, 6). This action was thought to be due to a direct action on the organelle and not a result of calcium chelation or an ATPase activity of the venom (6). The present study confirms the fact that mitochondrial function is significantly impaired by both venoms.

The fact that *Physalia* venom is more active in inhibiting mitochondrial function than *Chrysaora* venom, even though it has lower concentrations of protein and mouse lethal potency, indicates that the lethal factor is not

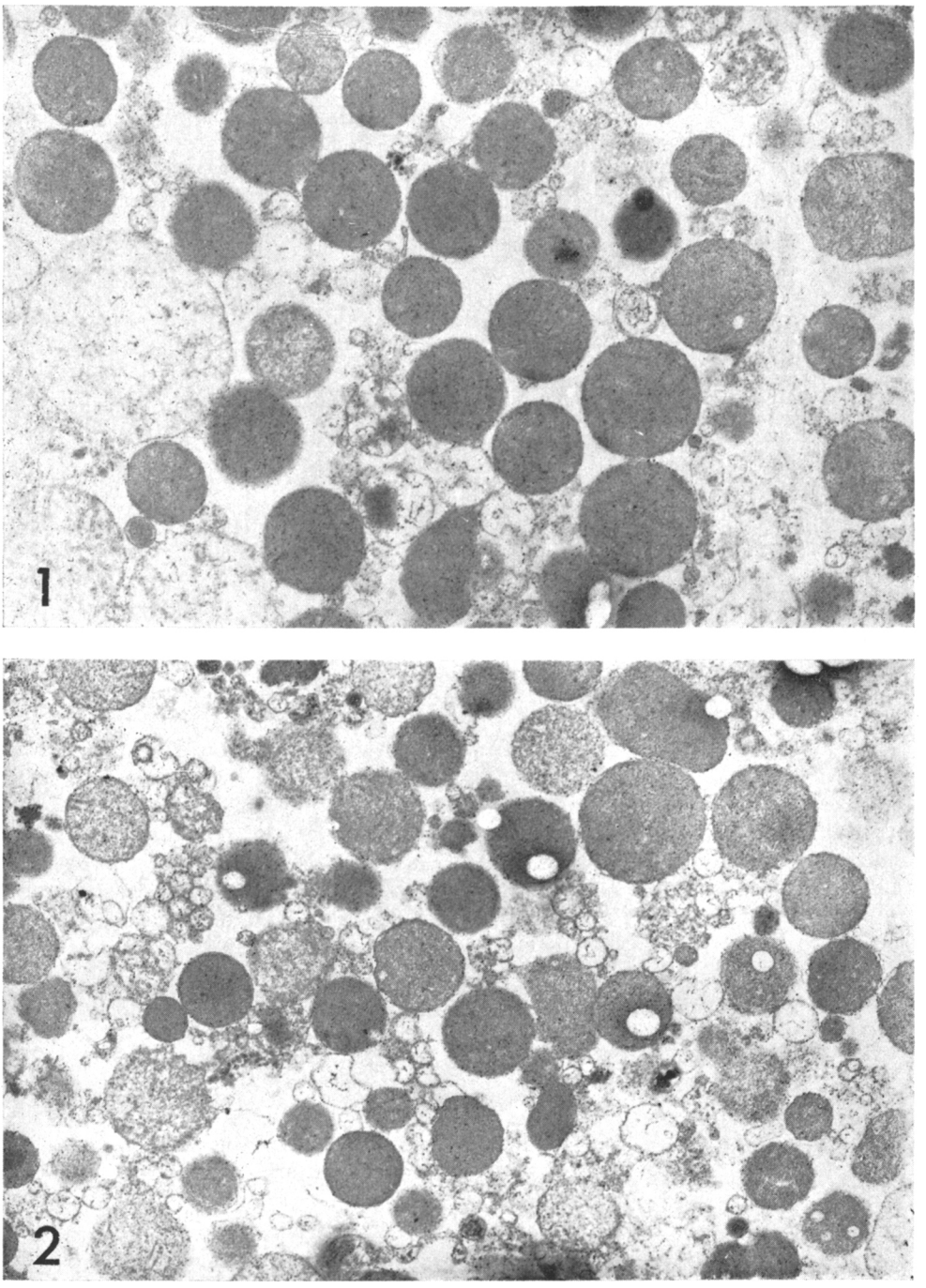
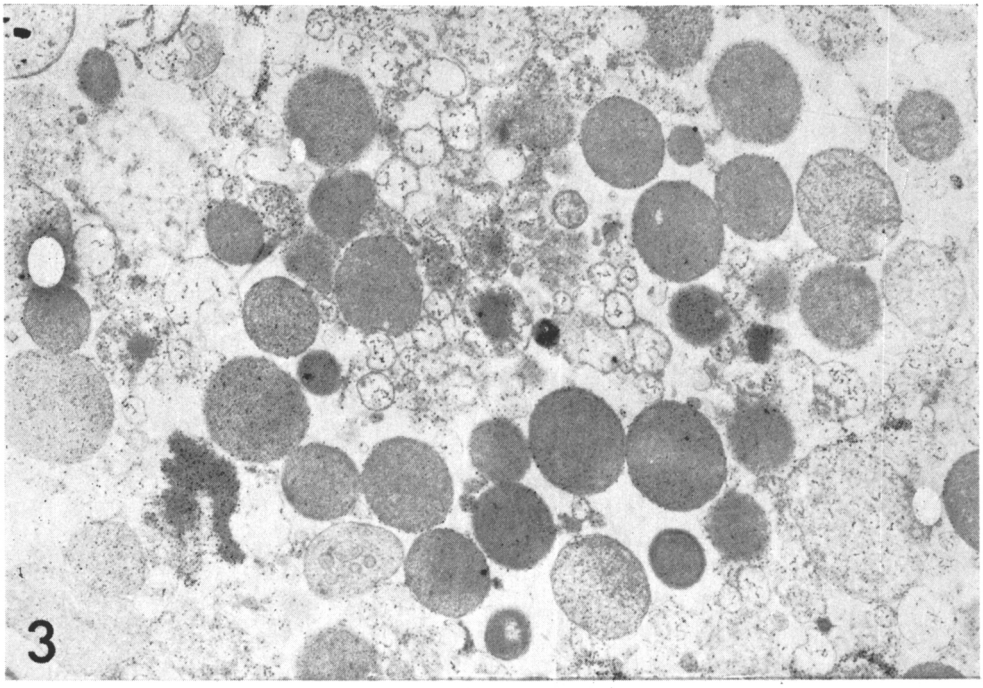


Figure 3

the agent causing mitochondrial impairment. This fact is also supported by the observation that heated venoms which are no longer lethal still inhibit mitochondrial function.

Previous experiments with C toxin failed to demonstrate an inhibition of oxidative phosphorylation with specimens containing 25 LD_{50} (15). These experiments were performed utilizing mitochondrial suspensions which contained EDTA whereas the present



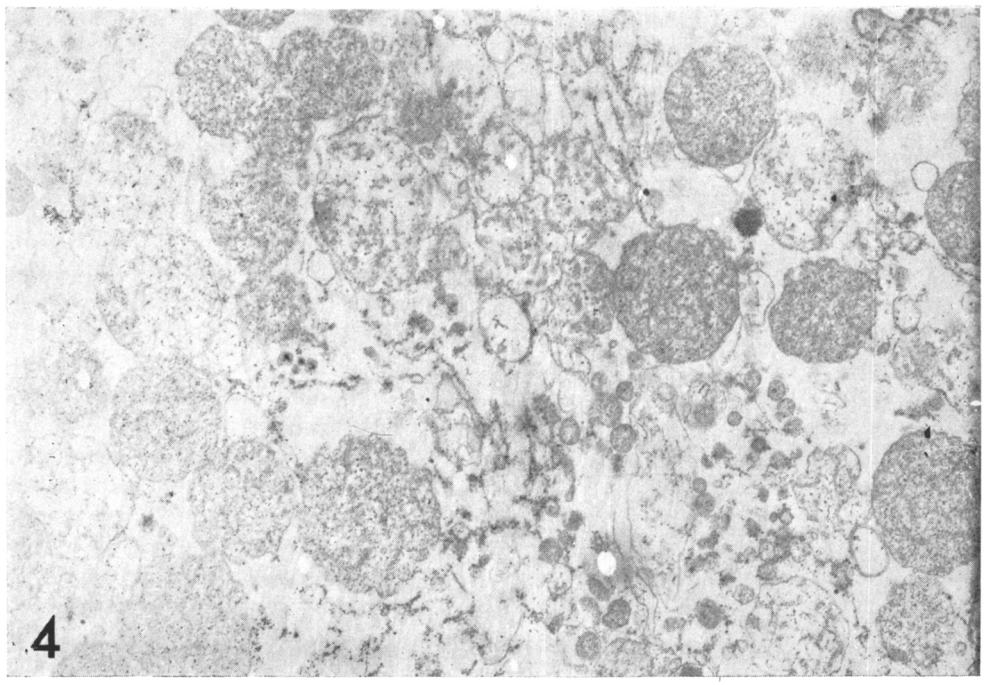


Figure 3 (continued)

investigations were conducted with media lacking that compound.

The results obtained with jellyfish toxin on the functional activity of mitochondria resemble those seen when mitochondria are exposed to snake venoms and purified phospholipases and proteinases (17–19). The points of similarity are the decline in RCI,

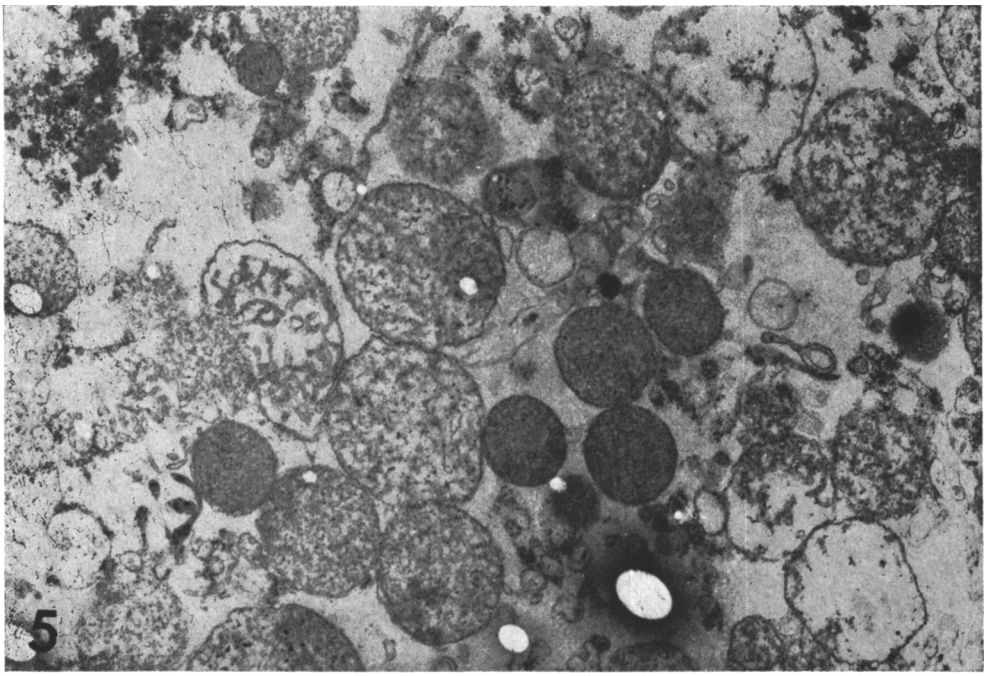


FIG. 3. Electron microscopic examination of control and treated mitochondrial suspensions. Mitochondria were incubated for 15 min in radioactive buffer with toxins or controls. Control mitochondrial suspensions (1); mitochondria incubated with distilled water, 0.15 ml (2); mitochondria incubated with bovine serum albumin, 3.6 mg (3); mitochondria incubated with *Physalia* venom, 0.5 LD_{100}, 1.65 mg protein (4); mitochondria incubated with *Chrysaora* venom, 5 LD_{100}, 3.6 mg protein (5). (The magnification of the photographs is 14,600×).

the inhibition of oxidation at higher concentrations of toxin, the decrease in optical density of the mitochondrial solution and the protection afforded by bovine serum albumin. This would suggest that the effects of jellyfish toxins upon mitochondrial function are mediated through lipases and proteinases in a manner analogous to the effects of snake venoms.

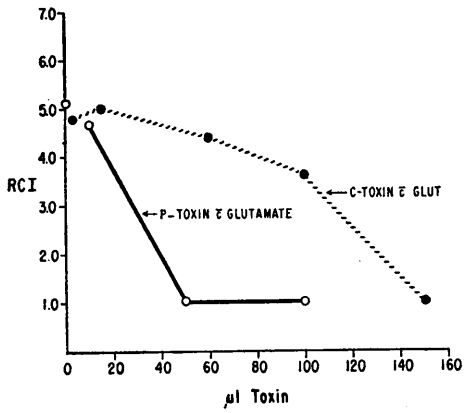


FIG. 4. Effect of jellyfish toxins on the respiratory control index (RCI) of rat liver mitochondria. (—) *Physalia* toxin (1 LD_{100}, 3.2 mg protein) and (.....) *Chrysaora* toxin (0.5 LD_{100}, 0.32 mg protein). Mitochondria were preincubated with toxins for 7 min.

Summary. A study of the effects of *Chrysaora quinquecirrha* and *Physalia physalis* venoms on the uptake and binding of calcium by mitochondria revealed a decrease in these functions. Electron microscopy revealed damage to the mitochondria. Experiments on oxidative phosphorylation revealed that these jellyfish venoms possessed an action on mitochondria similar to that of snake venoms.

This study was supported in part by grants-in-aid from the National Marine Fisheries Service and the Maryland Department of Natural Resources, (PL 89), the Frank C. Bressler Research Fund of the

University of Maryland, the U.S. Department of Health, Education and Welfare, Grants Nos. 1R01 ES EC 00599 and GM 15700 and National Science Foundation, RANN Grant No. 29906. The authors gratefully acknowledge Dr. Dennis Brown of the University of Maryland School of Medicine who performed the electron microscopy.

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Received Apr. 5, 1973. P.S.E.B.M., 1973, Vol. 143.