SUMMARY

1. Experiments with brain and liver mitochondria have demonstrated that the three phosphorylations linked to the electron-transport chain are not equally susceptible to the uncoupling action of cobra venom.

2. The phosphorylation in the region of cytochrome oxidase is preferentially inactivated by heated venom. These observations have been made in direct experiments *in vitro* and with mitochondria obtained from animals injected with venom.

3. Evidence obtained with mitochondria of animals injected with heated venom (phospholipase A) suggests that the phospholipids concerned with phosphorylations in the reduced diphosphopyridine nucleotide–cytochrome *c* region are considerably resistant to the action of phospholipase A.

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Studies on Phospholipid Structures in Mitochondria of Animals Injected with Cobra Venom or Phospholipase A

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Considerable evidence has been obtained that venoms containing phospholipase A bring about inactivation of respiratory activity and uncouple oxidative phosphorylation when added to isolated tissue preparations (Braganca & Quastel, 1952, 1953; Nygaard, 1953*a*, *b*; Nygaard & Sumner, 1953; Petrushka, Quastel & Scholefield, 1957). It has been suggested that these effects are produced through the destructive action of phospholipase A on mitochondrial phospholipids. Nygaard (1953*a*) has shown that the inactivation of succinic oxidase by phospholipase A from *Crotalus terrificus* venom is dependent on the extent of phospholipid hydrolysed. Results of investigations on the changes produced on oxidative phosphorylation in the mitochondria of animals injected with cobra venom have shown remarkable agreement with findings of experiments *in vitro*. The enzyme systems concerned with esterification of inorganic phosphate appear to be particularly sensitive to the action of cobra venom (Aravindakshan & Braganca, 1959). The present investigation was undertaken to study in greater detail the mechanism underlying the uncoupling action of cobra venom on oxidative phosphorylation in mitochondria. Data from studies on submitochondrial particles as well as on the swelling properties of mitochondria show that cobra venom brings about pronounced changes in mitochondrial structure. These effects have been observed *in vitro* as well as in animals injected with cobra venom or with crystalline phospholipase A.

Several reports have appeared on the uncoupling effect of lysolecithin on oxidative phosphorylation

(Habermann, 1954; Witter, Morrison & Shepardson, 1957). Lysolecithin is also known to enhance the swelling of mitochondria (Witter & Cottone, 1956). Quantitative studies designed to explore to what extent the changes produced by cobra venom may be attributed to the effect of lysolecithin indicate that the lysolecithin formed from the quantities of venom added is too small to be effective. It appears from these findings that the destructive effects of cobra venom on mitochondrial metabolism arise primarily through direct hydrolysis of essential mitochondrial phospholipids by the phospholipase A.

EXPERIMENTAL

Rat-liver submitochondrial fragments were prepared according to Cooper & Lehninger (1956). They were characterized by determination of the P:O ratio and by some of the properties in which they differ from intact mitochondria as shown in Table 1. It is seen that Ca^{2+} ions, which uncouple oxidative phosphorylation and also activate the adenosine-triphosphatase activity in fresh mitochondria, did not affect these properties in the submitochondrial fragments. Mg^{2+} ions, which neither inhibit the P:O ratio nor activate the adenosine triphosphatase in fresh mitochondria, completely inhibited phosphorylations as well as stimulated the adenosine triphosphatase in the fragments. 2:4-Dinitrophenol inhibited phosphorylations and also activated the adenosine triphosphatase in the fragments, as it does in the fresh intact mitochondria. These results showed that the submitochondrial particles employed in these studies had properties identical with those described by Cooper & Lehninger (1956) for the original preparation of these fragments.

Table 1. *Properties of submitochondrial fragments prepared from rat liver*

(*a*) *Oxidative phosphorylation*

The system contained 0·01 M-DL-sodium β-hydroxybutyrate, 2·4 mM-adenosinetriphosphate (ATP), 5 mM-Na_2HPO_4–NaH_2PO_4 buffer, pH 6·0, 0·1 mM-$MgSO_4$, 0·01 M-NaF, submitochondrial fragments containing 2·5–3·0 mg. of protein, 0·03 M-glucose, and 30 units of hexokinase (Berger, Slein, Colowick & Cori, 1946) in a total volume of 3·0 ml. The centre well contained 0·2 ml. of 5 N-NaOH. Gas phase, air; temp., 30°; period of incubation, 30 min.

(*b*) *Adenosine-triphosphatase activity*

The system contained 4 mM-ATP, 0·02 M-2-amino-2-hydroxymethylpropane-1:3-diol (tris) buffer, pH 7·4, and submitochondrial fragments containing 0·4–0·5 mg. of protein in a total volume of 1·0 ml. Temp., 30°; period of incubation, 10 min.

Substance added	Concn. of the substance (mM)	P:O ratio	Relative adenosine-triphosphatase activity (μmoles of P liberated)
None	—	2·1	0·33
2:4-Dinitrophenol	0·05	0·2	1·12
Ca^{2+} ions	3	2·2	0·35
Mg^{2+} ions	3	Nil	1·71

Heated cobra (*Naja naja*) venom was prepared by heating solutions of crude venom at 100° for 15 min. as described by Braganca & Quastel (1953). The amount of heated venom employed in experiments is expressed in terms of the amount of crude venom from which it was obtained. Crystalline phospholipase A was prepared from crude cobra venom according to Suzuki, Iwanaga & Kawachi (1958).

Mouse-liver mitochondria used for studying the swelling properties were prepared according to Tapley (1956). For other experiments mouse-brain mitochondria were prepared by the method of Brody & Bain (1952) and liver mitochondria were isolated according to Schneider (1948). The amount of mitochondria used in experiments is expressed as equivalent mg. (the wet weight of the tissue in mg., from which the mitochondria are obtained).

Lysolecithin was prepared by a modification of the procedure employed by Hanahan, Rodbell & Turner (1954). In this preparation 5 g. of commercial egg lecithin was dissolved in 500 ml. of peroxide-free ethyl ether and mixed with 5 ml. of an aqueous solution of 30 mg. of heated cobra venom. The precipitate so formed was removed after 3 hr. by centrifuging and dissolved in the minimum amount of chloroform. The turbid solution obtained was again centrifuged at 15 000 *g* for 30 min. and the residue discarded. To the clear solution 6 vol. of ether was added, and the precipitate was recovered and washed with ether. This procedure was repeated four times. The product obtained was characterized by analysis for phosphorus, nitrogen and fatty acid content. Nitrogen was determined by the micro-Kjeldahl procedure of Johnson (1941). Phosphorus was determined according to King (1930). The amount of fatty acid was estimated by the saponification method (Vogel, 1951). The results given below show that the analytical results obtained are in close agreement with theoretical values and the data reported by Hanahan *et al.* (1954). Theoretical values (%): N, 2·76; P, 6·07; fatty acid, 49·7. Observed values (%): N, 2·80; P, 5·91; fatty acid, 47·1. The absence of any contamination of lysolecithin by phospholipase was demonstrated by the Warburg manometric method described by Braganca & Quastel (1953). The results showed that 10 mg. of lysolecithin produced no carbon dioxide within a period of 2 hr. The minimum lethal dose (MLD) is defined as the minimum dose of the venom preparation expressed in terms of protein content which when injected subcutaneously into 2–6-months-old mice (weighing 20–25 g.) killed all the mice within 18 hr.

RESULTS

Effect of cobra venom on oxidative phosphorylation in submitochondrial fragments

Recent work on the mechanism of oxidative phosphorylation has shown that uncoupling agents in general may be classified into two types: (1) those which uncouple by direct interference with the phosphorylation process; (2) those which inhibit the phosphorylation process indirectly through their action on the structural organization of the mitochondria. Uncoupling agents of the first type have been found effective both in intact mitochondria as well as in submitochondrial fragments

capable of oxidative phosphorylation. Agents belonging to the second type produced uncoupling only in intact mitochondria (Lehninger, Wadkins, Cooper, Devlin & Gamble, 1958). Since the structural organization of mitochondria is already disrupted during the preparation of the submitochondrial fragments, uncoupling agents of this class do not produce any effect in this system. These preparations therefore serve as a tool for studying the mechanism of the uncoupling action of any agent.

In the present studies submitochondrial fragments were prepared according to Cooper & Lehninger (1956) and characterized as described above. Studies with intact mitochondria have shown that the uncoupling action of cobra venom on the phosphorylation process is dependent on the relative proportion of venom added to mitochondrial protein. The ratio of venom to mitochondrial protein in the experiments with submitochondrial fragments described in Table 2 is the same as the ratio of venom to mitochondrial protein reported in the earlier findings with intact mitochondria (Aravindakshan & Braganca, 1959). Table 2 shows that 15μg. of heated venom had no effect on the respiration or the P:O ratio in this system. This amount of venom had been found to produce 30% inhibition on the phosphorylation process in the system containing intact mitochondria. When 100μg. of heated venom was employed both the oxygen absorption and the uptake of inorganic phosphate were equally inhibited. It is apparent from these results that cobra venom does not directly inhibit the phosphorylating enzymes.

Effect of cobra venom on the swelling properties of liver mitochondria

Studies on phosphorylation accompanying oxidation, carried out with intact mitochondria and also with submitochondrial particles, suggest that venom affects this process indirectly through its action on the mitochondrial structure. Direct evidence to show that cobra venom produces structural changes in the mitochondria was provided by a study of the effects of the venom on the spontaneous swelling of liver mitochondria. Mouse-liver mitochondria were prepared in 0·44 M-sucrose and were suspended in the test system containing 0·3 M-sucrose. It has been shown by Tapley (1956) that in this concentration of sucrose the mitochondria swell at a moderate rate, and the system is therefore convenient for the study of the effects of agents which either enhance or prevent swelling. Tedeschi & Harris (1955) have demonstrated that $E_{520\,m\mu}$ of suspensions of rat-liver mitochondria in sucrose solutions is inversely related to the mitochondrial volume. In the present studies the decrease in $E_{520\,m\mu}$ is taken as a measure of the extent of swelling.

Fig. 1 shows the changes produced in the swelling properties by heated cobra venom and crystalline phospholipase A. Addition of 0·1μg. of heated venom produced 16% increase in the extent of

Table 2. *Effect of heated cobra venom on oxidative phosphorylation in sub-mitochondrial fragments*

Conditions are as in Table 1. Heated venom was tipped from the side arm after equilibration for 5 min.

Tissue (mg. of protein/ vessel)	Venom (μg./vessel)	ΔP (μmoles)	ΔO (μg.atoms)	P:O
2·74	—	7·5	3·57	2·1
	0·15	6·8	3·39	2·0
	0·75	6·9	3·30	2·1
	3·0	6·8	3·39	2·0
	7·5	6·2	3·12	2·0
2·71	—	7·2	3·12	2·3
	0·15	6·6	2·86	2·3
	0·75	7·2	3·03	2·4
	3·0	7·3	3·20	2·3
	7·5	7·2	3·03	2·4
	15·0	7·5	3·12	2·4

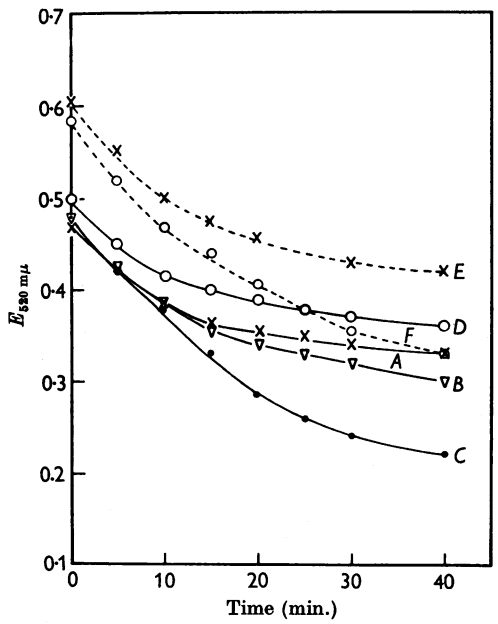

Fig. 1. Effect of addition of phospholipase A and heated venom on the swelling of mouse-liver mitochondria. The test system contained 40 equivalent mg. of mitochondria (mitochondria obtained from 40 mg. wet weight of tissue) in 0·3 M-sucrose, 0·02 M-tris buffer, pH 7·4, in a total volume of 3·0 ml. Temp., 25°. (i) *A*, Control; *B*, 0·1μg. of heated venom; *C*, 0·5μg. of heated venom; *D*, 0·5μg. of heated venom + 1·0 mg. of egg lecithin. (ii) *E*, Control; *F*, 0·026μg. of crystalline phospholipase A.

swelling in 40 min. (curve *B*) and 0·026μg. of phospholipase A increased the swelling by 45% in the same period (curves *E* and *F*). Higher concentration of heated venom produced greater effects (curve *C*). Increased swelling of mitochondria has been observed on addition of venom from *Agkistridon piscivorus* (Petrushka *et al.* 1957). These findings indicate that changes produced by cobra venom on the swelling of mitochondria are brought about through the action of phospholipase A on phospholipids of mitochondrial membrane. It was of interest to find that 1·0 mg. of egg lecithin completely reversed the increase in swelling brought about by 0·5μg. of heated venom (curves *C* and *D*). This quantity of lecithin alone had no effect on the swelling process. The protective effect of lecithin on the uncoupling of oxidative phosphorylation by *A. piscivorus* venom has been demonstrated by Petrushka *et al.* (1957). Presumably lecithin is able to compete with the phospholipids of the mitochondria for the added phospholipase.

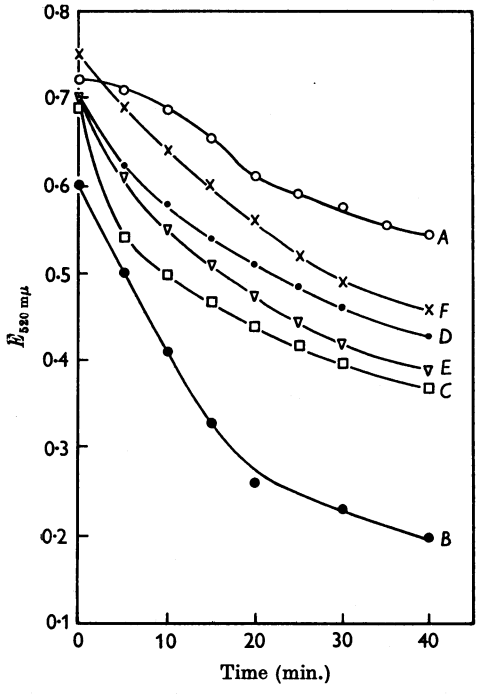

Fig. 2. Effect of injected cobra venom and phospholipase A on the swelling properties of liver mitochondria. Assay system was as in Fig. 1. *A*, control; *B*, mice injected with 50μg. of crude venom and killed after 30 min.; *C*, mice injected with 75μg. of heated venom and killed after 30 min.; *D*, mice injected with 50μg. of crude venom and killed after 10 min.; *E*, mice injected with 9μg. of crystalline phospholipase A and killed after 30 min.; *F*, mice injected with 22·5μg. (MLD) of crude venom and killed after 30 min. The results are representative of at least six experiments.

In order to explore the changes produced by cobra venom in the intact animal, the rate of swelling of mitochondria isolated from mice injected with cobra venom was investigated. In the first series of experiments mice were injected with 50μg. of crude venom, 75μg. of heated venom or 9μg. of crystalline phospholipase A. These doses were lethal in 2·5 hr. The mice were then killed after 10 or 30 min. As seen in Fig. 2, mitochondria of the experimental animals (curves *B* and *C*) swelled at a faster rate compared with those of control animals (curve *A*). Changes in mitochondrial swelling could be detected within an interval of 10 min. after the animals were injected with crude venom (curve *D*). Injections of crystalline phospholipase produced changes in mitochondria similar to those brought about by crude venom and heated venom, the extent of swelling being 37% higher than in the controls (curve *E*). In another series of experiments animals were injected with a minimum lethal dose of crude venom (this dose was lethal in 8–10 hr.). Mitochondria of such animals killed 30 min. after the injection of the minimum lethal dose were also considerably damaged as shown by the greater extent of swelling (curve *F*) as compared with the controls.

Effect of lysolecithin on oxidative phosphorylation and on the swelling of mitochondria

Since lysolecithin is known to uncouple oxidative phosphorylation and produce swelling of liver mitochondria it was necessary to determine whether the destructive effects produced by phospholipase A on mitochondrial metabolism were due to the lysolecithin produced. This question was explored earlier by Petrushka, Quastel & Scholefield (1959). They observed that phospholipase C from *Clostridium welchii*, which does not give rise to lysolecithin, behaved like phospholipase A in inhibiting mitochondrial respiration, from which they concluded that the destructive effects of phospholipase are not due to the lysolecithin formed. In view of these observations, a quantitative study was carried out to determine to what extent lysolecithin might be implicated in the uncoupling action of cobra venom on oxidative phosphorylation and its effects on the swelling of mitochondria under the experimental conditions employed.

In these experiments 50μg. of heated venom were incubated with brain and liver mitochondria under similar conditions to those employed for the study of oxidative phosphorylation. As reported earlier this quantity of venom produced complete uncoupling of oxidative phosphorylation (Aravindakshan & Braganca, 1959). After incubation for

20 min. the lipids were extracted according to Marinetti, Scaramuzzino & Stotz (1957), and the lysophospholipids estimated by their haemolytic action on a standard system of human erythrocytes (Bernheimer, 1947). The quantities of lysophospholipids are expressed in terms of lysolecithin. These experiments showed that 200 equivalent mg. of brain mitochondria or 300 equivalent mg. of liver mitochondria gave rise to 50–75μg. of lysolecithin. Under similar conditions mitochondria incubated without the venom did not produce detectable amounts of lysophospholipids. Witter & Cottone (1956) also did not detect lysolecithin in fresh liver mitochondria.

Effects of graded amounts of lysolecithin on the P:O ratio and on the swelling properties of mitochondria are shown in Table 4 and Fig. 3 respectively. It is evident from Table 4 that 0·25 and 0·5 mg. of lysolecithin increased the respiration, whereas 2·0 mg. of lysolecithin inhibited the oxygen uptake by about 40%. There was no effect on oxidative phosphorylation by 0·25 mg. of lysolecithin. Quantities higher than this (0·5–2·0 mg.) depressed the P:O ratio progressively to nil. It is therefore clear that lysolecithin in amounts which are produced by the action of venom on the mitochondria does not have any effect on the respiration or on the phosphorylation process.

Fig. 3 shows the effect of lysolecithin on the swelling of mouse-liver mitochondria in 0·3M-sucrose. There was no effect on the swelling by 1·0μg. of lysolecithin, whereas 50μg. enhanced swelling by 78%. An estimate of the quantity of lysolecithin that could be produced by the action of heated venom on the amounts of mitochondria employed in these experiments may be extrapolated from the data given in Table 3. It is evident that no more than 1μg. of lysolecithin could be produced under these conditions. Thus it is apparent from these studies that lysolecithin could

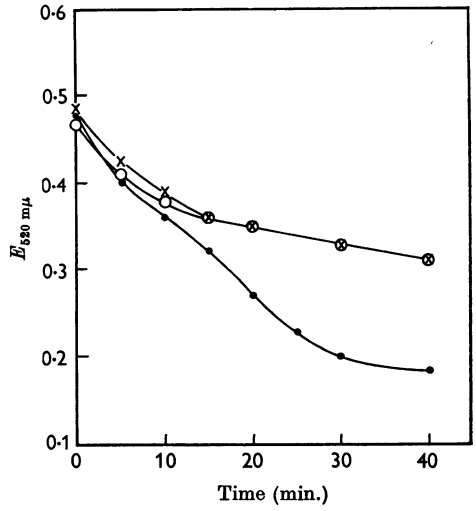

Fig. 3. Effect of lysolecithin on the swelling properties of mouse-liver mitochondria. Assay system was as in Fig. 1. ×, Control; ○, 1·0μg. of lysolecithin; ●, 50μg. of lysolecithin.

Table 3. *Liberation of lysophospholipids from mouse-brain and liver mitochondria by heated cobra venom*

Brain mitochondria (200 equivalent mg.) or 300 equivalent mg. of liver mitochondria (mitochondria obtained from mg. wet weight of tissue) suspended in 0·15M-KCl in a total volume of 3·0 ml. were incubated with venom at 30° for 20 min. $E_{540\ m\mu}$ was taken as a measure of haemolysis.

Venom added (μg.)	Lysolecithin formed (μg.)	
	Brain	Liver
Nil	Nil	Nil
50	70·6	63·0
50	61·6	53·2
50	68·4	56·6

Table 4. *Effect of added lysolecithin on oxidative phosphorylation in mouse-brain and liver mitochondria*

The system contained 15 mM-sodium glycylglycine, pH 7·4, 0·01M-NaF, 0·01M-Na_2HPO_4–NaH_2PO_4 buffer, pH 7·4, 0·03M-glucose, 0·15M-KCl, 8 mM-$MgSO_4$, 2·5 mM-ATP, 13 mM-sodium pyruvate, 2 mM-sodium fumarate, 30 units of hexokinase and 200 equivalent mg. of brain mitochondria or 300 equivalent mg. of liver mitochondria (mitochondria obtained from mg. wet weight of tissue) in a total volume of 3·0 ml. The centre well contained 0·2 ml. of 5N-NaOH. Temp., 30°; gas phase, air; period of incubation, 20 min. Lysolecithin was tipped from the side arm after equilibration for 5 min.

Lysolecithin (mg./vessel)	Brain			Liver		
	ΔP (μmoles)	ΔO (μg.atoms)	P:O	ΔP (μmoles)	ΔO (μg.atoms)	P:O
—	12·2	4·37	2·8	14·5	5·80	2·5
0·25	12·3	4·64	2·8	18·0	6·96	2·6
0·50	11·1	5·36	2·1	12·2	6·42	1·9
1·00	8·0	4·01	1·5	5·5	4·60	1·2
1·50	2·6	2·41	1·1	1·9	2·77	0·7
2·00	1·0	2·63	0·3	Nil	2·41	Nil

Table 5. *Effect of lysolecithin on oxidative phosphorylation in rat-liver submitochondrial fragments*

The system was as in Table 1.

Tissue (mg. of protein/ vessel)	Lysolecithin added (μg.)	ΔP (μmoles)	ΔO (μg.atoms)	P:O
2·62	—	4·9	2·32	2·1
	75	4·2	2·23	1·9
	150	5·0	2·50	2·0
	225	5·2	2·59	2·0
	300	5·1	2·41	2·1
2·67	—	6·4	3·21	2·0
	75	6·2	2·94	2·1
	150	6·9	3·20	2·1
	225	6·9	3·30	2·1
	300	6·1	3·03	2·0

not be responsible for the various effects produced by cobra venom on mitochondrial functions.

To study the mechanism of the uncoupling action of lysolecithin, the effect of this compound on oxidative phosphorylation in submitochondrial fragments was investigated. Table 5 shows that 300μg. of lysolecithin had no effect on the respiration or on the P:O ratio in this system. Table 4 shows that this concentration of lysolecithin in relation to the mitochondrial protein produced almost complete uncoupling in intact mitochondria. These findings demonstrate that lysolecithin has no effect on the phosphorylation process, and, like venom, uncouples oxidative phosphorylation in intact mitochondria through its action on the mitochondrial structure.

DISCUSSION

Studies described in this paper have shown that although both heated venom (phospholipase A) and lysolecithin can uncouple oxidative phosphorylation in fresh mitochondria, they have no action on the phosphorylation accompanying respiration in submitochondrial particles. This clearly shows that they do not have direct inhibitory action on the phosphorylating enzyme systems. Observation on the effects of heated venom and lysolecithin on the swelling properties of isolated liver mitochondria of normal animals have also demonstrated that addition of minute quantities of these materials can bring about pronounced changes in the mitochondrial membrane. It is satisfying to find complete correlation between these findings and the observations made on the mitochondria of animals injected with cobra venom. Thus the mitochondria of animals injected with venom showed greater susceptibility to swell than those of controls. The cobra toxin apparently damages the mitochondrial structure very rapidly, as changes in the swelling properties of liver mitochondria could be detected within 10 min. after the injection. These findings suggest that changes in the mitochondrial membrane may be among the early biochemical disturbances produced by cobra venom on the mitochondrial metabolism. Experiments *in vitro* have shown that the swelling of mitochondria is produced with smaller quantities of the toxin than those required to produce changes in the P:O ratio, indicating that changes in the permeability probably precede the effects on other mitochondrial functions. Experiments *in vitro* as well as with injected animals show that the effects produced by crystalline phospholipase *A* on mitochondrial metabolism are similar to changes brought about by cobra venom. Minute quantities can uncouple oxidative phosphorylation and also produce considerable increase in the swelling properties of the mitochondria. It is well established that mitochondria are rich in phospholipids, and these are known to play an important role in the phosphorylation process (Green, 1959; Schneider, 1959). It is evident from the reports of Petrushka *et al.* (1957) as well as the present studies that heated venom can hydrolyse mitochondrial phospholipids. These observations therefore support the conclusion that cobra venom disturbs mitochondrial metabolism through the destructive action of phospholipase on mitochondrial phospholipids. The effects observed in the intact animal are presumably mediated by a similar mechanism. Since it has already been demonstrated that the phospholipase A of cobra venom can penetrate the blood–brain barrier and bring about hydrolysis of brain phospholipids (Aravindakshan & Braganca, 1959) it is clear that phospholipase A can inactivate mitochondrial functions in the brain in the intact animal.

The data regarding the uncoupling action of lysolecithin reported here appear to be in disagreement with the results of Witter *et al.* (1957). Although these authors have employed rat-liver tissue, whereas the present findings relate to mouse tissues, the amounts of mitochondria employed in both investigations are of the same order. Several studies (Marples & Thompson, 1958; Saunders, Thomas & Robinson, 1958) have reported that phospholipase is a contaminant of lysolecithin preparations. The sample of lysolecithin employed in the present study was therefore shown by analysis to be free from phospholipase or lecithin. Witter *et al.* (1957) have analysed their sample of lysolecithin for C, H and P. This analysis, however, could not determine the presence of traces of phospholipase. It is possible therefore that the greater effect of lysolecithin on oxidative phosphorylation reported by these authors may have been due to small quantities of venom present as impurity in the sample of lysolecithin.

Quantitative experiments, carried out to determine to what extent the various changes in the mitochondrial properties result from the effects of lysolecithin, have clearly shown that the quantity of lysolecithin formed from venom under the experimental conditions could not possibly account for the various effects observed. These studies do not exclude the possibility that lysolecithin produced from venom could be more localized on the surface of the mitochondria and as such may have greater destructive effects on mitochondrial metabolism than added lysolecithin. The studies described have, however, shown that lecithin reverses the action of venom on the swelling of mitochondria *in vitro*, presumably owing to a competition of lecithin with mitochondrial phospholipids for the phospholipase. Lecithin also reverses the uncoupling effect of venom on oxidative phosphorylation (Petrushka *et al.* 1957). These observations are in favour of the view that the various effects are brought about by the direct action of venom phospholipase on the phospholipids of the mitochondria. Attempts to reverse, with lecithin, the effect of cobra venom on the swelling of mitochondria in the injected animals were, however, not successful. It is of interest that Nygaard, Dianzani & Bahr (1954) have observed that lysolecithin and phospholipase A produce qualitatively different types of changes in the morphology of the mitochondria as detected by electron microscopy.

SUMMARY

1. Heated cobra venom did not uncouple oxidative phosphorylation in submitochondrial fragments prepared by digitonin extraction. These experiments suggest that venom does not directly inhibit the phosphorylating enzymes.

2. Addition of small quantities of crystalline phospholipase A enhanced spontaneous swelling of fresh mitochondria. The effect could be reversed by lecithin. Mitochondria of animals injected with phospholipase also showed greater swelling than the controls.

3. Evidence presented demonstrates that the various effects produced by cobra venom on mitochondrial metabolism result from hydrolysis of mitochondrial phospholipids by the phospholipase. The quantity of lysolecithin produced could not account for the various effects of cobra venom on mitochondrial metabolism.

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