EFFECTS OF AFLATOXIN B_1 ON THE SWELLING AND ADENOSINE TRIPHOSPHATASE ACTIVITIES OF MITOCHONDRIA ISOLATED FROM DIFFERENT TISSUES OF THE RAT

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1. Introduction

Although the cells most affected in aflatoxin poisoning are those around the portal vein and bile duct [1] it has also been reported [2, 3] that the mitochondria of rat liver cells are vulnerable to aflatoxin. Not all tissues respond in the same degree to the *in vivo* administration of aflatoxin. Wogan and his coworkers [4, 5] investigated the distribution of a single-dose injection of [^{14}C]aflatoxin in the rat and found that high concentrations occurred in the liver, considerably smaller amount in the kidney and negligible amounts in the heart and the testis. There seemed to be no published quantitative data on the biochemical functions of mitochondria in the presence of aflatoxin. Therefore, it appeared to us desirable to obtain information on the *in vitro* osmotic behaviour of mitochondria from various tissues. In view of the involvement of adenosine triphosphatase (ATPase) in processes of energy conservation, we have also investigated the effect of aflatoxin on mitochondrial ATPase activities in the liver, kidney, heart and testis.

2. Materials and methods

Mitochondria were isolated essentially according to the method of Tapley [6]. Homogenates of ice-cold 0.25 M sucrose (prepared in glass-distilled water) in which the total volume of the tissue suspension was made up to 10 times the weight of an organ which was removed from a freshly killed 300 g male, albino rat were prepared in a Potter-Elvejhem homogenizer at 0°. Subsequently, all steps were carried out at 4°. The nuclear fraction together with intact cells and cell debris were isolated by centrifuging the homogenate for 10 min at 600 *g* in an MSE angle 13 refrigerated centrifuge. The mitochondria were then sedimented at 5,000 *g* for 20 min and the opalescent supernatant was removed. The mitochondrial pellet was washed by resuspending in 0.25 M sucrose and centrifuging for 10 min at 24,000 *g* and was finally suspended in a volume of 0.34 M sucrose containing 0.02 M Tris buffer of pH 7.4 so that 1 ml of the suspension contained mitochondria from 0.3 g of fresh organ.

Mitochondrial swelling was followed by the method described by Lehninger [7]. 0.1 ml of the mitochondrial suspension was added to 2.4 ml of 0.34 M sucrose-Tris at zero time, and 0.1 ml of aflatoxin B_1 (obtained from Makor Biochemicals Ltd., Jerusalem and dissolved in a minimum quantity of *N,N*-dimethylformamide) solution in 0.34 M sucrose-Tris. This aflatoxin solution was made by shaking a mixture of 1 vol of aflatoxin in *N,N*-dimethylformamide with 5 vols of the 0.34 M sucrose-Tris for 30 min. The changes in light absorption was measured in a Carl-Zeiss spectrophotometer, at 520 nm, against a blank containing 0.1 ml of 0.34 M sucrose-Tris instead of the mitochondrial suspension. The solution of ATP (Na-salt), obtained from the Sigma Chemical Co., St. Louis, USA, which was used to reverse swelling contained: ATP, 0.15 M; bovine serum albumin, 5 mg/ml; $MgCl_2$, 0.15 M.

The assay of ATPase was, essentially, carried out as described by McMurray and Begg [8]. The standard incubation medium, containing final concentrations,

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was 0.05 M Tris buffer, pH 7.4, 0.075 M KCl, 0.108 M sucrose, 0.01 ml of *N,N*-dimethylformamide or aflatoxin B_1 in 0.01 ml of *N,N*-dimethylformamide, and mitochondria isolated from 0.06 g of the organ in a total volume of 1.0 ml. The reaction was started by the addition of 0.002 M ATP. Incubation was carried out, in air, for 20 min, at 20°. The reaction was stopped by adding 2.0 ml of ice-cold 0.725 M perchloric acid. For the determination of inorganic phosphate, the method of Fiske and Subbarow [9] was used. The protein was centrifuged off and the protein-free solution containing perchloric acid was added to the ice-cold molybdate reagent. After diluting to the required volume, the colour was developed for 30 min at 17°.

3. Results and discussion

As shown in fig. 1 the optical density of a liver mitochondrial suspension in 0.34 M sucrose decreases with time when aflatoxin is present and the degree of swelling increases with increasing concentration of the toxin. For each concentration, swelling was complete in about 5 min. With 56×10^{-6} mM, which is known to prolong the thrombotest of a 300 g rat [10] there was no effect on mitochondrial swelling. This could be interpreted to mean that the intimate mechanism of anticoagulant action of sublethal doses of aflatoxin might be unrelated to its action on cell organelles. When we compared this effect of aflatoxin with that of carbon tetrachloride, an equally potent hepatocarcinogen, our observation (fig. 1) of liver mitochondrial swelling in the presence of carbon tetrachloride confirmed the results of Recknagel and Malamed [11]. We found that aflatoxin, 3×10^{-3} M, increased the swelling to a lesser extent than did the same concentration of carbon tetrachloride.

Reversible changes in the permeability of mitochondrial membranes are important in the regulation of the metabolic activity of the cell [12]. In the present experiments, the addition of ATP completely reversed the swelling of the mitochondria to which aflatoxin has been added (fig. 1). This finding suggests that toxic doses of aflatoxin may uncouple oxidative phosphorylation and studies to investigate this property are in progress. We have, also, found differences among the various tissues in the degree of mitochondrial swelling produced *in vitro* by aflatoxin. Table 1 illustrates the markedly different susceptibilities of mitochondria from liver, kidney, heart and testis. Each value represents the percentage decrease in optical density of a suspension containing aflatoxin (3×10^{-3} M) less that observed in a control sample without

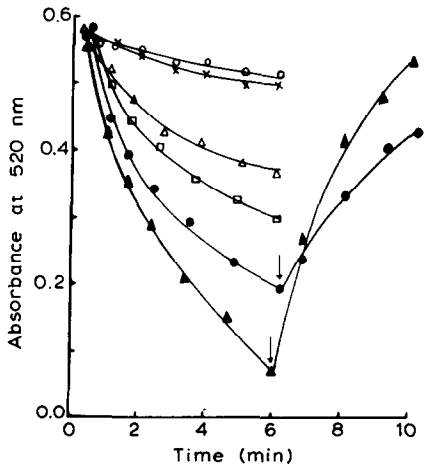

Fig. 1. The reversal action of ATP on rat-liver mitochondrial swelling induced by aflatoxin B_1 and carbon tetrachloride. This system contained 2.4 ml of 0.34 sucrose-0.02 M Tris, 0.1 ml of mitochondrial suspension and 0.1 ml of compound in 0.34 M sucrose–0.02 M Tris. 0.1 ml of ATP solution (as described in the text) was added where shown (↓). Temperature, 19° ± 1°. (o—o—o) Control; (x—x—x) 56×10^{-6} mM aflatoxin; (△—△—△) 3×10^{-6} M aflatoxin; (□—□—□) 3×10^{-4} M aflatoxin; (●—●—●) 3×10^{-3} M aflatoxin; (▲—▲—▲) 3×10^{-3} M carbon tetrachloride.

Table 1

Tissue	Degree of mitochondrial swelling*	ATPase activity: inorganic P released (μmoles)	
		Control	Aflatoxin (20×10^{-5} M)
Liver	42	3.37	6.25
Kidney	18	3.49	5.58
Heart	3	2.56	2.60
Testis	2	2.38	2.31

* Percentage decrease in optical density of the mitochondrial suspension containing aflatoxin B_1 (3×10^{-3} M) less that observed in the control without aflatoxin.

aflatoxin. These values were taken as a measure of the swelling produced by aflatoxin. Swelling produced is striking with mitochondria from liver and kidney. In accordance with the low distribution of aflatoxin in the heart and testis after intraperitoneal injection of ring-labelled [^{14}C]aflatoxin B_1 [13] essentially no swelling was produced by aflatoxin with the mitochondria from these tissues.

Fresh mitochondria exhibit a low ATPase activity which may be enhanced by dinitrophenol [14]. Our studies demonstrate that aflatoxin produces an activation of DNP-induced ATPase in rat liver mitochondria at a concentration of 20×10^{-5} M, but produces no further stimulation with increasing concentration of the toxin. No effect was, however, noticeable in the mitochondria obtained from kidney, heart and testis. Toxicity studies [15, 16] showed an LD_{50} of the order of 6.0 mg/kg body weight for male rat when injected intraperitoneally. But since the distribution through the body fluids is not uniform it is not possible to suggest the concentrations of aflatoxin which may affect the processes of energy conservation in the tissues. However, this work needs to be extended to other tissues and species.

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