EFFECT OF DEOXYCORTICOSTERONE ON THE ATPase ACTIVITY OF FRACTIONS OBTAINED AFTER SONICATION OF MITOCHONDRIA*

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Received October 18, 1960

It has been reported (Blecher and White, 1960) that the in vitro addition of certain steroids releases the latent ATPase activity of freshly prepared mitochondria from rat lymphosarcoma and liver; after aging of mitochondria the latent ATPase activity is released and steroids have no further influence. This report describes the effect of deoxycorticosterone (DOC) on the ATPase activity of fractions obtained by differential centrifugation of lymphosarcoma and liver mitochondrial sonicates.

Mitochondria were isolated as in other studies (Blecher and White, 1960). Mitochondria from 12 g tumor or 2 g liver , suspended in 2 ml water, were placed in a tightly capped, 2 ml cellulose nitrate tube and the tube packed in ice in the treatment chamber of a Raytheon 10 kc/sec sonic oscillator. Tumor mitochondria were sonicated at maximum intensity (1.3 amps) for two 2.5-minute periods, replacing the ice between periods; liver mitochondria were treated for a single 2-minute period. Three fractions were obtained by differential sedimentation (Spinco Model L preparative centrifuge, rotor 40): fraction R (brown) sedimented at 25,000 x g for 20 minutes; fraction P (red-brown) sedimented at 105,400 x g for 50 minutes; and, fraction S (clear yellow), the supernatant fluid from fraction P. Particulate fractions were suspended in, and soluble fractions diluted with, 0.35 M sucrose in the case of lymphosarcoma,

* This work was supported in part by grants from the National Institutes of Health, U. S. Public Health Service, the National Science Foundation, and the American Cancer Society.

and water for liver, preparations. Particulate fractions were examined by phase contrast microscopy: R fraction consisted chiefly of "intact" mitochondria, mostly rod-shaped but also containing many small, round mitochondria, only a few of which were swollen and crescented; fraction P, which represents particles derived from mitochondrial membranes, consisted chieflyof very small particles, difficult to resolve under the light microscope, and contained no mitochondria.

The data (Table I) reveal that, following sonication, ATPase activities of tumor or liver mitochondria (fraction R) were from 3 to 4 times that of non-sonicated, aged or DOC-treated mitochondria (cf., Blecher and White, 1960). ATPase activities of mitochondrial membranes (fractions P) are even greater than those observed for sonicated mitochondria; high ATPase activity in a mitochondrial membrane fraction obtained after sonication of liver mitochondria has been observed by others (McMurray, Maley and Lardy, 1958). The soluble mitochondrial matrix (fraction S) was low in ATPase activity for both tissues, particularly liver.

The ATPase activities of fractions R, P, and S were unchanged by aging (60 minutes at 30°) or by the presence of bovine serum albumin (12 mg/ml) in the assay medium.

The latent ATPase activity of fresh, untreated lymphosarcoma or liver mitochondria is released by the addition of DOC (Blecher and White, 1960). In contrast, ATPase activities present in the 3 fractions obtained following sonication of mitochondria were inhibited by DOC (Table I). Since DOC does not cause lysis of mitochondria or leakage of any protein to the medium from mitochondria (Blecher and White, 1960), the release of latency of ATPase activity in intact mitochondria by DOC cannot be due to disruption of mitochondria with concomitantly increased ATPase activity as is seen, for example, following sonication. The inhibiting action of DOC on the ATPase activity of fractions from disrupted mitochondria, contrasted with its releasing of latent ATPase activity of intact mitochondria, is similar to the behavior of mitochondrial ATPase with triiodothyronine.

Triiodothyronine enhances ATPase activity of liver mitochondria (Maley and Johnson, 1957) and inhibits the activity of solubilized ATPase of beef heart mitochondria (Penefsky, Pullman, Datta and Racker, 1960).

TABLE I

ATPase ACTIVITY OF LYMPHOSARCOMA AND LIVER MITOCHONDRIAL FRACTIONS AFTER TREATMENT WITH SONIC VIBRATION

Fraction*	DOC, 0.6 mM	ATPase specific activity**	
		Lymphosarcoma	Liver
R	-	11.9	8.45
	+	7.77	5.24
P	-	16.2	27.3
	+	8.18	12.5
S	-	2.05	0.27
	+	1.48	0.20

* See text for description of fractions.

** μmoles P_i formed in 20 minutes at 30° per mg protein in an assay medium containing Tris buffer, pH 7.4, 50 mM; KCl, 40 mM; $MgCl_2$, 2 mM; and ATP, 2.4 mM.

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