1. Dreisbach, R. H., *J. Pharm. Exp. Therap.,* 1961, v131, 257.
2. ———, *Am. J. Physiol.,* in press.
3. ———, *ibid.,* 1959, v196, 645.
4. Neuman, W. F., Neuman, M. W., *Chemical Dynamics of Bone Mineral,* Univ. of Chicago Press, 1958, 11.
5. Järnefeldt, J., *Biochem. Biophys. Res. Comm.,* 1961, v6, 285.
6. Gerlach, E., Bader, M., Schwoerer, W., *Pflügers Arch. Ges. Physiol.,* 1961, v272, 807.
7. Janke, J., Gerlach, E., Fleckenstein, A., *ibid.,* 1960, v270, 286.
8. Berenblum, I., Chain, E., *Biochem. J.,* 1938, v32, 295.
9. Martin, J. B., Doty, D. M., *Anal. Chem.,* 1949, v21, 965.
10. Northrup, D., *Am. J. Physiol.,* 1935, v114, 46.
11. Bergonzi, M., *Arch. di Sci. Biol.,* 1931, v16, 235.

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Effect of Deoxycorticosterone on Soluble Beef Heart Mitochondrial ATPase.* (27844)

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We have previously reported(1) that the latent ATPase activity of fresh, untreated rat lymphosarcoma and liver mitochondria is released by addition of deoxycorticosterone (DOC) *in vitro* or *in vivo* and that, in contrast, ATPase activities of particulate and solubilized fractions obtained by sonication of lymphosarcoma and liver mitochondria are inhibited by this steroid(2).

In an effort to determine whether or not DOC inhibition of solubilized, mitochondrial ATPase is a general phenomenon, we have examined the effect of DOC upon a preparation of soluble ATPase isolated by Pullman *et al.*(3) from beef heart mitochondria and have found that the activity of this enzyme, as determined by 2 different assay methods, was inhibited by low concentrations of DOC.

Experimental. Doubly glass-distilled water was used for preparation of solutions. Phosphoenolpyruvate (PEP), rabbit muscle lactic dehydrogenase (LDH) and rabbit muscle pyruvic kinase (PK) were products of Boehringer and Soehne, Mannheim, Germany. ATP, ADP, potassium pyruvate, and NADH were purchased from the Sigma Chemical Co., St. Louis; DOC was a gift of Dr. P. Perlman, Schering Corp., Bloomfield.

LDH was assayed spectrophotometrically in a Beckman DU spectrophotometer at 30° using 1.5 ml capacity silica cells of 10 mm light path. Incubation mixtures contained, in μmoles, 0.65 KCl, 50 Tris-acetate buffer (pH 7.4), 0.1 NADH, 3.0 pyruvate, and water and LDH in a total volume of 1.0 ml. When present, 0.025 ml of either propylene glycol (PG) or a 0.02M solution of DOC in PG were substituted for a portion of the water. Reaction was initiated by addition of LDH and the decrease in optical density at 340 mμ followed for 4 minutes. LDH assayed at 453 units per mg protein, as calculated from the straight-line portion (first 2 minutes) of the curve.

PK was assayed spectrophotometrically in a system containing, in μmoles, 70 KCl, 50 Tris-acetate buffer (pH 7.4), 10 $MgCl_2$, 0.1 NADH, 1.0 PEP, 0.24 ADP, and water, 0.05 ml of LDH (14.1 units per ml) and PK in a total volume of 1.0 ml; PG and DOC, where present, were added as described above. Reaction was initiated by addition of LDH and, after the optical density at 340 mμ had fallen to an equilibrium value, 0.05 ml of PK was added and the optical density decrease fol-

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TABLE I. Effect of Deoxycorticosterone upon Enzyme Activities.*

Additions	LDH	PK	ATPase†	
			Spectrophotometric	Release of Pi
	Units of activity/mg enzyme protein‡			
Control	453	55.7	3.68	2.55
PG	430	54.3	3.16	2.57
DOC	427	46.4	2.40	1.49

* Assay conditions are given in *Experimental* section.

† Spectrophotometric assay and assay by Pi release were performed on 2 different preparations of ATPase.

‡ A unit of activity is defined as that amount of enzyme which catalyzes the turnover of 1 μmole of substrate per min under the specified assay conditions. Specific activity is defined as the number of units per mg enzyme protein.

lowed over 6 minutes, over which period zero order kinetics were followed. PK assayed at 56 units per mg protein.

The beef heart mitochondrial ATPase preparation studied was kindly supplied by Dr. M. E. Pullman. Stock material (about 12 units per mg protein) was stored at 4° as a suspension in 50% ammonium sulfate. For assay, aliquots of the stock suspension were dissolved in 24 volumes of 0.01 M Tris-acetate buffer (pH 7.4) for the spectrophotometric assay and in 5 volumes of buffer for the assay by Pi release. As recommended by Pullman *et al.*(3), working solutions of ATPase were prepared just before use and were kept at room temperature.

Assay of ATPase activity by release of Pi in the presence of an ATP regenerating system was performed essentially as described by Pullman *et al.*(3), employing 1.83 units (32.8 μg) of PK and approximately 0.2 unit of ATPase activity. Where present, 0.025 ml of PG or of 0.02 M DOC in PG were substituted for an equal volume of water. Pi was determined by a modification(4) of the Lowry and Lopez procedure(5) following deproteinization of incubation mixtures with trichloroacetic acid (5%, final concentration).

The spectrophotometric assay of ATPase in the presence of an ATP regenerating system was performed essentially as described by Pullman *et al.*(3) in the presence of excess PK (28.6 μg, 1.63 units) and LDH (10.9 μg, 4.95 units). PG and DOC, where present, were added as noted in the previous assay. The reaction was followed for 3 minutes following addition of ATPase; zero order kinetics were followed during this period.

Enzyme protein determinations were performed by the method of Lowry *et al.*(6) using standardized crystalline albumin (Armour Pharmaceutical Co.) as reference.

Results and discussion. Representative data (Table I) indicate that propylene glycol, the solvent for DOC, was without effect upon the specific activities of purified preparations of LDH, PK, and ATPase. DOC (0.5 mM) exerted no significant effect upon the activities of LDH or PK, but resulted in an inhibition of ATPase of between 35 and 42%, as assayed by 2 different methods.

Present results with a solubilized and purified beef heart mitochondrial ATPase are similar to those previously reported(2) with solubilized ATPase obtained from sonically-disrupted mitochondria obtained from rat lymphosarcoma and liver, insofar as the inhibitory effect of DOC is concerned. Since doubts have been expressed(3,7,8) regarding the necessity for invoking the existence of 2 separate ATPases in mitochondria, it is tempting to consider that the inhibitory effect of DOC upon solubilized ATPase represents a direct action upon the enzyme. Therefore, the action of DOC in releasing the latent ATPase activity of intact mitochondria might be viewed as an indirect effect, the primary action of the steroid being exerted upon the mitochondrial membranes to "release" ATPase activity. Evidence in support of this concept is provided by previous studies from this laboratory(1) which demonstrated a correlation between release of latency of mitochondrial ATPase by DOC and the entry of water through mitochondrial membranes. The mechanisms of those actions of DOC remain unknown.

Summary. The activity of solubilized, beef heart mitochondrial ATPase, as assayed both spectrophotometrically and by release of Pi from ATP, was decreased by over 35% in the presence of deoxycorticosterone.

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1. Blecher, M., White, A., *J. Biol. Chem.,* 1960, v235, 3404.

2. ———, *Biochem. Biophys. Res. Comm.,* 1960, v3, 471.

3. Pullman, M. E., Penefsky, H. S., Datta, A., Racker, E., *J. Biol. Chem.,* 1960, v235, 3322.

4. Potter, V. R., *ibid.,* 1947, v169, 17.

5. Lowry, O. H., Lopez, J. A., *ibid.,* 1946, v162, 421.

6. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., *ibid.,* 1951, v195, 265.

7. Cooper, C., *Biochim. Biophys. Acta,* 1958, v30, 484.

8. Siekevitz, P., Löw, H., Ernster, L., Lindberg, O., *ibid.,* 1958, v29, 378.

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