Summary. Lipids extracted from tubercle bacilli and purified by Anderson's classic procedure, Choucroun's Pmko, and methanol extracts of the bacilli were tested for immunogenicity in mice. Pmko and Anderson's Wax B were the only fractions found capable of eliciting statistically significant immunity against challenge tuberculosis.

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Activity of Succinic Dehydrogenase and Cytochrome Oxydase of Liver Mitochondria in Rats Treated with Cortisone and with an Anabolic Androgen.* (27394)

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Activity of enzyme systems may be modified by hormones, and hormonal action on cellular metabolism may be mediated by enzymes. Enzyme-hormone interaction probably plays an important role in the processes of anabolism and catabolism. An attempt was made previously to elucidate the mechanism of known metabolic antagonism between anabolic steroids and cortisone. Tissue respiration, a function dependant upon enzyme activity, was studied in animals treated with some C^{19} and C^{21} steroids(1). Expected antagonism between these 2 groups of hormones was not found as far as their effect on Qo_2 of various tissues is concerned because both anabolic androgens and cortisone significantly depressed tissue Qo_2 in rats(2,3). It became evident that further studies were indicated to localize the site of enzymatic impairment of respiration, and that the activity of some respiratory enzymes should be determined in mitochondria. Mitochondria are known to metabolize steroid hormones(4). Several individual enzymes of the Krebs cycle are associated with mitochondria but succinic dehydrogenase and cytochrome oxydase, constituting an integral system of hydrogen transferring enzymes, were found almost exclusively in these granules(5,6).

In the present study rats were treated with cortisone and/or an anabolic androgen. Succinic dehydrogenase and cytochrome oxydase activity in liver mitochondria of these animals was determined.

Methods. Male Sprague-Dawley rats, weighing 250-275 g, kept in individual cages and fed special grain diet(7), were used for this study. Cortisone Acetate (Merck) was injected intramuscularly. Methandrostenolone (Ciba), an anabolic androgen(8), was given *per os,* in the form of crushed tablet mixed with food(9). Six groups of rats, 20

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TABLE I. Reaction Mixtures Used for Study of 2 Respiratory Enzymes.

Composition	Succinic dehydrogenase	Cytochrome oxydase
	ml	ml
H_2O (to make 3.0 ml)	.5	.3
.1 M PO_4 pH 7.4 with NaOH	1.0	1.0
.5 M Na-Succinate pH 7.4	.3	—
1 x 10^{-4} M Cytochrome C	.4	—
2.4 x 10^{-4} M Cytochrome C	—	1.0
4 x 10^{-3} M $CaCl_2$	.3	—
4 x 10^{-3} M $AlCl_3$	.3	.3
0.114 M Na-Ascorbate pH 7.0	—	.3
Mitochondria from 2 g of wet tissue in 12 ml of 0.25 M sucrose solution	—	.1
Mitochondria from 2 g of wet tissue in 4 ml of 0.25 M sucrose solution	.2	—

per group, were treated for 14 days as follows: 1—Normal controls, no treatment. 2—Given cortisone, 5 mg daily/rat. 3—Given cortisone, 10 mg daily/rat. 4—Given cortisone, 10 mg daily/rat, and then the treatment discontinued for 14 days. 5—Given Methandrostenolone, 5 mg daily/rat. 6—Treated simultaneously with cortisone and Methandrostenolone, each steroid 5 mg daily/rat.

Rats were killed at the end of experiment (2), livers dissected, rinsed in ice cold physiological saline and placed in ice cold 0.25 M sucrose solution. Separation of mitochondria was done immediately(10,11).

For the study of each enzyme 2 g of wet liver tissue was homogenized with 0.25 M sucrose solution (10 × weight of sample) and centrifuged in Spinco L-Model Ultracentrifuge at speed 700 × *g* for 10 minutes. The supernatant was decanted and the residue washed twice with 0.25 M sucrose solution (5 × weight of sample) and recentrifuged as previously. Supernatant and washing combined were then centrifuged again at speed 8000 × *g* for 10 minutes. Supernatant was decanted, sediment washed twice and recentrifuged again at the same speed. Mitochondria were suspended in 0.25 M sucrose solution in proportions shown in Table I. Manometric determination of enzyme activity was then performed(12) using reaction mixtures described in Table I. Equilibrium time was 10 minutes and the readings were taken at 10-minute intervals. Results were expressed in ml O_2 uptake in mitochondria from 10 mg dry liver tissue per hour (Table II).

Results. Treatment of rats with cortisone significantly depressed the activity of succinic dehydrogenase (Table II). This depression was reversible, and normal values were found after treatment was discontinued for 14 days. Methandrostenolone, anabolic steroid used in this experiment, did not alter mitochondrial activity of succinic dehydrogenase, nor did the combined treatment with this steroid and cortisone improve the inhibitory action of cortisone on succinic dehydrogenase. None of the steroids studied significantly altered cytochrome oxydase activity under the described experimental conditions (Table II).

Discussion. Examples of hormonal action mediated by enzymes are now many and the hypothesis that each hormone affects particular enzyme systems may be verified in the near future. Studies on estrogen-stimulable transhydrogenase probably present the best known milestone on the way to understand hormone-enzyme interaction(13). Cortisone is known to alter some enzymes involved in protein and glucose metabolism(14,15,16). Direct action of this steroid on some mito-

TABLE II. Succinic Dehydrogenase and Cytochrome Oxydase Activity Expressed in ml of O_2 Taken up by Mitochondria from 10 mg of Dry Liver Tissue Per Hour. 20 rats in each group. Means, S.E.

Group	Treatment	Succinic dehydrogenase	Cytochrome oxydase
1	None	77.3 ± 2.0	298.4 ± 4.3
2	Cortisone, 5 mg/day for 14 days	33.1 ± 2.2*	305.4 ± 4.5
3	Cortisone, 10 mg/day for 14 days	39.6 ± 2.7*	343.8 ± 5.8
4	*Idem*, then no treatment for 14 days	70.9 ± 5.3	297.6 ± 6.3
5	Methandrostenolone, 5 mg/day for 14 days	74.5 ± 3.0	349.8 ± 6.0
6	Cortisone + Methandrostenolone both 5 mg/day for 14 days	40.3 ± 3.2*	311.5 ± 6.6

* P less than 1% : cortisone treated *vs* other groups.

chondrial enzymes was demonstrated previously(15). It was recently reported that cortisone inhibits oxydative phosphorylation in liver mitochondria(17) and that the enzymatic impairment observed in tissue of cortisone treated animals may be localized at the level of the Krebs cycle(15). Our results generally agree with this statement. We were able to show that under described experimental conditions the activity of succinic dehydrogenase in liver mitochondria of cortisone treated rats was depressed. This depression was a reversible phenomenon.

It may, therefore, be concluded that in the liver mitochondria at least one site for the impairment of respiratory metabolism, after cortisone, would be located at the level of Krebs cycle between the succinate and fumarate. This specific effect of cortisone cannot be altered by an anabolic steroid, under the conditions described. Activity of cytochrome oxydase was not affected by the treatment of rats with C^{19} and C^{21} steroids.

In this study, one site of enzymatic depression, apparently specific for cortisone and unaffected by an anabolic steroid, was observed. This constitutes at least one difference between these 2 groups of steroids as far as their action on Krebs cycle is concerned.

Summary. Two respiratory enzymes in liver mitochondria of rats treated with cortisone and an anabolic androgen were studied. The activity of succinic dehydrogenase of cortisone treated animals was depressed. This depression was reversible and the interruption of treatment resulted in normalization of activity of this enzyme. Anabolic steroid Methandrostenolone did not affect this enzyme nor did combined treatment with cortisone and anabolizer prevent the action of cortisone on succinic dehydrogenase. Cytochrome oxydase activity of liver mitochondria was not affected by C^{21} and C^{19} steroids used in this study. It is apparent that at least one site for the impairment of respiratory metabolism after cortisone is located at the level of Krebs cycle between the succinic and fumarate.

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