EXP. BIOL. AND MED., 1959, v102, 744.
2. Wada, H., Turner, C. W., *J. Dairy Sci.*, 1959, v42, 1198.
3. Anderson, R. R., Brcokreson, A. D., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 567.
4. Damm, H. C., Turner, C. W., *ibid.*, 1961, v107, 893.

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Activity of Malic Dehydrogenase and DPN-Cytochrome-C-Reductase of Liver Mitochondria in Rats Treated with Cortisone and with an Anabolic Androgen.* (28350)

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It is apparent that hormones may modify the activity of intracellular enzyme systems. Because of known metabolic antagonism between cortisone and some anabolic steroids, it was considered possible that these two groups of hormones may differ in their action on respiratory enzymes(1). It was found previously that both C^{21} and C^{19} steroids significantly depressed Qo_2 of various tissues in rats *in vivo*(2,3). To localize the site of enzymatic impairment of respiration, further studies were made on liver mitochondria which are known to contain respiratory enzymes(4,5). It was found that the succinic dehydrogenase activity of these mitochondria was depressed in cortisone treated rats, but was not affected by anabolic steroid Methandrostenolone. Cytochrome oxidase activity was, however, not affected by the C^{21} and C^{19} steroids studied(1). It was then evident that a difference between cortisone and an anabolizer does exist as far as their effect on the activity of a respiratory enzyme of Krebs cycle is concerned. We were, therefore, interested to explore this problem further.

In the present study, rats were treated with cortisone and/or an anabolic androgen. Malic dehydrogenase and DPN-cytochrome-c-reductase of these animals were determined.

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Methods. Male Sprague-Dawley rats, weighing 250-275 g, kept in individual cages and fed special grain diet(6), were used for this experiment. Animals were treated with Cortisone Acetate (Merck) and Methandrostenolone (Ciba) *per os,* in the form of crushed tablets mixed with food(7). Five groups of rats were treated for 14 days as follows: 1. Normal controls, no treatment. 2. Given cortisone, 5 mg daily/rat. 3. Given cortisone, 5 mg daily/rat and then the treatment discontinued for 14 days. 4. Given Methandrostenolone, 5 mg daily/rat. 5. Treated stimultaneously with cortisone and Methandrostenolone, each steroid 5 mg daily/rat.

At the end of the experiment the rats were lightly anaesthetized with ether. Their livers, *in situ,* were perfused with physiological saline, dissected, rinsed in ice cold saline and placed in ice cold 0.25 M sucrose solution. Mitochondria were separated immediately(8,9). Two grams of liver were homogenized with 0.25 M sucrose solution (5 × weight of sample) in a Virtis "45" homogenizer, and after decantation the flask was washed with the same volume of sucrose solution. The combined solutions were centrifuged in a Spinco Model-L ultracentrifuge at 700 × *g* for 10 minutes. The supernatant was decanted and the residue washed twice with 0.25 M sucrose solution (2½ × weight of sample) and recentrifuged as previously. Supernatant and washings combined were then centrifuged again at 10,000 × *g* for 10 minutes. The supernatant was de-

TABLE I. Reaction Mixtures Used for the Study of Two Respiratory Enzymes.

Composition	Malic dehydrogenase (ml)	DPN-cytochrome-c-reductase (ml)
H_2O	0.6	—
0.1 M PO_4 pH 7.4	0.8	0.8
0.1 M nicotinamide	0.3	0.3
0.5 M Na-malate	0.3	0.3
0.5 M Na-glutamate	0.3	0.3
4×10^{-4} M cytochrome c	0.3	0.3
Meyerhof extract, excess	—	0.6
Suspension of mitochondria	0.2	0.2
0.5% DPN (sidearm; added after equilibration)	0.2	0.2

canted, and the residue washed twice and recentrifuged again at the same speed. Mitochondria were suspended in 2 ml of 0.25 M sucrose solution and manometric determination of enzyme activities carried out using the reaction mixture described in Table I (10). All determinations were done in triplicate. Equilibrium time was 10 minutes and the readings were taken at 5 minute intervals. Results were expressed in μl of O_2 uptake in mitochondria from 10 mg of dry liver tissue per hour (Table II).

Results. Treatment of rats with cortisone significantly depressed the activities of malic dehydrogenase and DPN-cytochrome-c-reductase of liver mitochondria. This depression was reversible and normal values were found after treatment was discontinued for 14 days. The activities of both enzymes were increased in rats treated with anabolic steroid Methandrostenolone. This anabolizer, when administered to rats simultaneously with cortisone, did not modify the inhibitory action of cortisone.

Discussion. Glucocorticoids are known to produce negative nitrogen balance and to affect gluconeogenesis. These anti-anabolic steroids affect various liver enzymes *in vitro* and *in vivo*(11,12,13). They also may inhibit oxidative phosphorylation in liver mitochondria(14). It has been stated that enzymatic impairment found in tissues of cortisone treated animals may be localized at the level of the Krebs cycle(12). Our previous (1) and present results agree with this statement. Metabolic antagonism between cortisone and anabolic androgens is well known and we recently reviewed this problem in detail(15). Such an antagonism might also be expected in the hormonal action of anti-anabolic and anabolic steroids on some enzyme systems.

In the present study Methandrostenolone had the opposite action to cortisone on 2 respiratory enzymes of liver mitochondria studied. The activities of malic dehydrogenase and DPN-cytochrome-c-reductase were decreased in cortisone treated rats but increased in animals treated with Methandrostenolone. The depressive action of cortisone on the above enzymes was a reversible phenomenon. It is apparent that the enzymatic depression by an anti-anabolic steroid and the enzymatic stimulation by an anabolic steroid, may occur at the level of Krebs cycle. This means that an antagonism between some C^{21} and C^{19} steroids may be observed *in vivo*, as far as the action of these steroids on the activity of some respiratory enzymes in mitochondria of rat's liver is concerned. The significance of the enzymatic effect observed in this study on hormonal

TABLE II. Malic Dehydrogenase and DPN-cytochrome-c-reductase Activity Expressed in μl of O_2 Taken up by Mitochondria from 10 mg of Dry Liver Tissue per Hour. Mean and S.E.

Group	Treatment	No. of rats	Malic dehydrogenase	No. of rats	DPN-cytochrome-c-reductase
1	None	37	8.11 ± .08	31	10.01 ± .21
2	Cortisone, 5 mg/day for 14 days	20	6.46 ± .28*	18	7.74 ± .34*
3	*Idem*, then no treatm't for 14 days	10	9.18 ± .55†	10	11.25 ± .56†
4	Methandrostenolone, 5 mg/day for 14 days	20	10.46 ± .23*	18	12.09 ± .34*
5	Cortisone + Methandrostenolone, both 5 mg/day for 14 days	18	6.48 ± .28*	18	7.78 ± .29*

* P less than 1%, treated *vs* control group.
† No statistical difference from control group.

regulation of energy metabolism, still remains to be found. Steroids may be involved in the energy utilizing steps by which amino acids are converted to proteins. They may affect the oxidation of some essential amino acid and indirectly alter the protein synthesis(16). It may be that the primary effect of steroids is on cellular permeability. It cannot be concluded from this study that the above mentioned antagonism in enzymatic effects is related to the catabolic or anabolic activity of the steroids studied. Further work may show that such a relationship does occur.

Summary. The activities of 2 mitochondrial respiratory enzymes were determined in the liver of normal rats and of rats treated with cortisone and/or anabolic steroid Methandrostenolone. The activities of malic dehydrogenase and DPN-cytochrome-c-reductase were decreased in cortisone treated rats. This depression was reversible and interruption of treatment resulted in normalization of activities of both enzymes. Simultaneous treatment of rats by cortisone and the anabolizer did not, however, prevent this depression. The activities of both enzymes were increased in rats treated with Methandrostenolone alone.

1. Kowalewski, K., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 971.

2. Kowalewski, K., Bekesi, G., *ibid.*, 1961, v106, 300.

3. ———, *Arch. intern. pharmacod. et Ther.*, 1962, v135, 393.

4. Schneider, W. C., van Potter, R., *J. Biol. Chem.*, 1949, v177, 893.

5. Cleland, K. W., Slater, E. C., *Biochem. J.*, 1953, v53, 547.

6. Kowalewski, K., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 448.

7. ———, *Endocrinology*, 1958, v62, 493.

8. Schneider, W. C., *J. Biol. Chem.*, 1948, v176, 259.

9. Schneider, W. C., Hogeboom, G. H., *ibid.*, 1950, v183, 123.

10. Umbreit, W. W., Burris, R. H., Stauffer, J. F., *Manometric Techniques*, Burgess Publ., 3rd ed., 1959, p177.

11. Lacroix, E., Leusen, I., *Acta Endocrin.*, 1959, v31, 324.

12. Lacroix, E., *Arch. Intern. Physiol. et Bioch.*, 1959, v67, 539.

13. Smith, A. L., Koeppe, O. J., Franz, J. M., *Endocrinology*, 1961, v69, 872.

14. Kerppola, W., *ibid.*, 1960, v67, 252.

15. Kowalewski, K., in *Ciba International Symposium on Protein Metabolism*, Springer Verl., 1962, p237.

16. Lardy, H. A., in *Hormonal Regulation of Energy Metabolism*, Ed. by L. W. Kinsell, C. C Thomas, U.S.A., Publ., 1957, p45.

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Comparative Studies in Rous Sarcoma. IV. Glucose Metabolism of Normal and Rous Sarcoma Virus-Infected Cells.* (28351)

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Previous studies have shown that chick embryo fibroblasts infected with Rous sarcoma virus (RSV) *in vitro* acquire certain new morphologic, growth and synthetic properties(1-8) in which they resemble the malignant cells found in Rous sarcomas. It has been observed that when chick embryo cells are infected with RSV *in vitro*, the cultures accumulate lactic acid(2) and it has been assumed that this was due to an increase in the aerobic glycolytic utilization of glucose by the virus-infected cells. This metabolic pattern for glucose utilization has been accepted as one of the characteristics of cancer cells *in vivo*(9) and is exhibited by Rous sarcoma tumor tissue(10) and by chorioallantoic membrane cells infected with RSV *in vivo*(11). It was of interest, therefore, to

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