cerned directly with esterification of diglyceride.

We feel that the following hypothesis is in keeping with the known blocking action of PEBG on oxidative phosphorylation(9). By blocking oxidative phosphorylation in intact tissue, PEBG decreases the amount of available ATP. Since step I in the formation of glycerides involves ATP, a decrease in ATP will curtail this reaction and less acyl CoA, necessary for steps II and IV, will be produced. The limited amount of acyl CoA produced is competed for by α-glycerophosphate (II) and diglyceride (IV) and since the former is in greater abundance, it has a much greater chance to react with acyl CoA. Accordingly, the α-glycerophosphate is converted to diglyceride, but the latter never accumulates sufficiently to compete successfully for the limited amount of acyl CoA and thus remains as diglyceride instead of being rapidly converted to triglyceride. In the homogenates, however, where excess ATP is supplied, the formation of acyl CoA is not limited. With the interference with oxidative phosphorylation and secondarily with the Kreb's cycle there is a relative accumulation of the 3-carbon fragment lactate(10). It is possible that other 3-carbon fragments also accumulate and there would be more α-glycerophosphate to be converted to triglycerides; thus an increased amount of the latter is formed.

Summary. The effect of PEBG on triglyceride synthesis has been studied *in vitro*. In intact tissue PEBG interferes with the conversion of diglyceride to triglyceride. In homogenates, PEBG increases the incorporation of labeled fatty acid into triglyceride. It thus differs from insulin in its effect on triglyceride synthesis. An explanation for this is presented.

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Action of Deoxycorticosterone Acetate* on Glycogen. II. Relationship of Muscle Glycogen Formation to Potassium Metabolism.† (27920)

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A recent report from this laboratory(1) indicated that inhibition of liver glycogen formation after repeated daily administration of desoxycorticosterone acetate (DOCA) to intact rats was secondary to action of the steroid on potassium metabolism.

It has been reported(2) that the mechanism of glycogen formation in skeletal muscle might not be the same as that in liver. It was therefore of interest to determine if glycogen formation in skeletal muscle of intact rats was also inhibited by DOCA, and if so,

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whether the inhibition was secondary to, or independent of the action of the steroid on potassium metabolism.

Materials and methods. Two hundred and twenty male rats of Wistar origin weighing between 200 and 300 g were divided into 2 groups. Group I consisted of 140 rats which were further divided into 7 subgroups of 20 rats each. Half of the rats in each subgroup were given tap water to drink and the other half were given 1.0% potassium chloride solution. Rats in the first subgroup received daily injections of sesame oil and served as controls. The other 6 subgroups received 5.0 mg of DOCA daily in the form of a 5.0% solution in sesame oil, injected subcutaneously for 1, 2, 3, 5, 10 and 14 days respectively. Twenty-four hours before the last injection, food was withdrawn from the cages but drinking fluid was available *ad libitum*. A 10% glucose solution in isotonic saline was prepared fresh daily. At the time of the last injection of DOCA the rats were injected intraperitoneally with 200 mg of glucose per 100 g of body weight. After 4 hours the animals were anesthetized by injecting 5.0 mg of sodium pentobarbital intraperitoneally per 100 g of body weight.

Fifteen minutes later the abdominal cavity was opened and blood was collected from the inferior vena cava for potassium analysis. The gastrocnemius muscles from both hind legs were removed and quickly trimmed of fat and connective tissue. The muscle from one leg was placed in a tared weighing bottle containing 10 ml of 5.0% trichloroacetic acid and finely minced with scissors for glycogen determination. The muscle from the other leg was placed in a small tared silica dish and ashed for potassium analysis.

Glycogen was determined by the method of Carroll *et al.*(3) which consisted of triple extraction of the homogenized tissue with trichloroacetic acid, and alcohol precipitation of the glycogen from the supernatant liquid, followed by the anthrone reaction. Potassium was determined with a Beckman Model DU Flame Spectrophotometer(4) following the analytical procedure of Bills *et al.*(5).

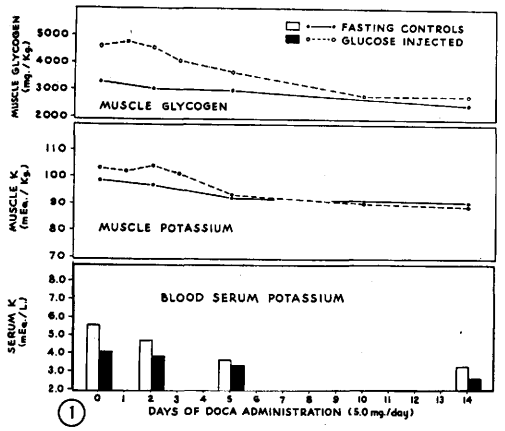
Results from the analytical determinations were averaged for each subgroup of animals. The mean muscle glycogen and potassium values were expressed in milligrams per kg and meq/kg of tissue respectively. The mean blood serum potassium values were expressed in meq/l.

Group II consisted of 80 rats, divided into 4 subgroups of 20 rats each. Rats in the first subgroup received daily injections of sesame oil and served as controls. The other 3 subgroups received 5.0 mg of DOCA per day for 2, 5 and 14 days respectively. Half of the animals in each subgroup received tap water and the other half 1.0% potassium chloride solution to drink. These rats served as fasting controls for the glucose injected rats of group I, and were treated identically with the exception that 0.9% sodium chloride solution was substituted for 10% glucose solution 4 hours before sacrifice.

Results. The results obtained with rats that received tap water are presented in Fig. 1. In the fasting control rats, daily administration of DOCA caused a slight decrease in both potassium and glycogen concentrations of muscle, and a marked decrease in blood serum potassium concentration. Following intraperitoneal injection of glucose, the mean glycogen and mean potassium concentrations of gastrocnemius muscle of rats that did not receive DOCA increased 1310 mg/kg or 40% and 3.7 meq/kg or 3.9% respectively. Glycogen production following glucose injection was inhibited in rats that received DOCA for 3 days or more. The inhibition was approximately paralleled by a decrease in potassium concentration of both muscle and serum. In rats that received DOCA for 14 days, glycogen concentration increased only 7% and there was no appreciable increase in potassium concentration of the muscle following the intraperitoneal injection of glucose.

Mean blood serum potassium concentration was consistently lower in rats that received glucose than in comparable animals that were sacrificed in the fasting state.

Results obtained with animals that received 1.0% potassium chloride are presented in Fig. 2. In the fasting control rats, administration of DOCA had essentially no effect on either mean glycogen or mean potassium



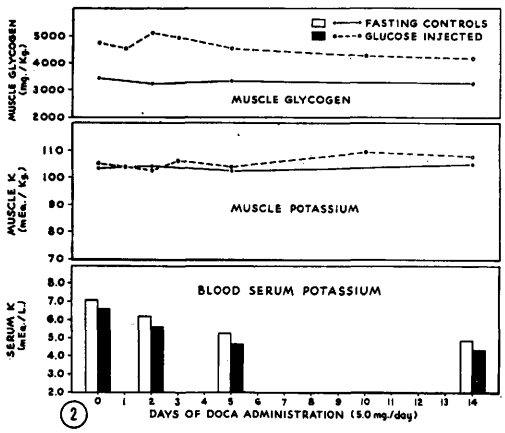


FIG. 1. Effect of II-desoxycorticosterone acetate (DOCA) on glycogen and potassium concentration in the gastrocnemius muscle 4 hr after the I.P. injection of glucose into intact rats.

FIG. 2. Effect of II-desoxycorticosterone acetate (DOCA) on glycogen and potassium concentration in the gastrocnemius muscle 4 hr after the I.P. injection of glucose into intact rats drinking 1.0% KCl solution.

concentration of muscle. Blood serum potassium concentration was markedly elevated in rats that did not receive DOCA, but was essentially normal in those that received DOCA for either 5 or 14 days. The glycogenic response following intraperitoneal injection of glucose into control rats that did not receive DOCA was essentially the same as that observed for the rats that received tap water. Glycogen production was slightly inhibited in the potassium chloride treated rats that received DOCA but as shown by comparison of Fig. 1 with Fig. 2, the inhibition was much less than in those that received tap water. Thus for the glucose injected rats that received DOCA for 5 days or more, those that received 1.0% potassium chloride had a higher mean concentration of potassium in the blood serum and gastrocnemius muscle, and produced more glycogen following glucose injection than those that received tap water.

The decrease in mean blood serum potassium concentration following injection of glucose was less than that in rats that received tap water to drink.

Discussion. It was demonstrated that repeated daily administration of DOCA to intact rats resulted in inhibition of muscle glycogen formation from intraperitoneally injected glucose, which approximately paralleled the decrease in potassium concentration of both serum and gastrocnemius muscle. When the concentration of potassium in muscle and serum was maintained at essentially normal values by forcing an increase in potassium intake, glycogen production in gastrocnemius muscle of DOCA treated rats approached that of control rats. These observations suggested that the inhibition of glycogen formation induced by repeated daily injection of DOCA was secondary to the action of the steroid on potassium metabolism. These results are in agreement with those reported earlier for the action of DOCA on glycogen formation in liver(1).

It seems unlikely, however, that the action of DOCA on glycogen formation can be explained entirely in terms of the availability of potassium from the blood or the potassium level of the tissue in which glycogenesis is taking place. Other investigators have demonstrated(6) that potassium concentration of tissues and blood serum is markedly elevated in adrenalectomized rats, but that glycogen formation is inhibited(7). It has also been reported that administration of DOCA to adrenalectomized rats results in a decrease in tissue and serum potassium levels (8) and an increase in glycogen formation (9). It thus appears that other factors are involved. This problem is under investigation.

Many reports have appeared concerning the possible relationship of adrenocortical function to the pathogenesis of rheumatoid

arthritis. The results reported here are of particular interest in this regard since muscle weakness and atrophy are frequently among the early symptoms in patients with this disease.

Summary. 1. Daily administration of DOCA to intact rats resulted in inhibition of muscle glycogen formation, accompanied by an approximately proportional decrease in potassium concentration of both skeletal muscle and blood serum. 2. By forcing an increase in potassium intake of DOCA treated rats, potassium concentrations of both muscle and blood serum were maintained at essentially normal levels, and glycogen production was not significantly inhibited. 3. These data indicated that the DOCA induced inhibition of muscle glycogen formation was secondary to potassium depletion.

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Response of Obese Patients to an 11β-Hydroxylase Inhibitor, Methopyrapone (SU-4885).* (27921)

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We have previously reported that urinary 17-ketosteroid and 17-ketogenic steroid excretion was significantly greater in obese than in normal-weight persons(1,2). One-third of all obese patients of all ages were found to have urinary 17-ketogenic steroid excretion greater than the accepted upper limit of normal of the method used, and 16% had similarly high urinary 17-ketosteroid excretion. This was also independently reported by Eckert, Green and Migeon(3) for urinary 17-hydroxycorticosteroids (Glenn-Nelson method) and by Mlynaryk, Gillies and Pattee(4) for urinary Porter-Silber chromogen excretion in obese patients. Both groups of investigators further found increased cortisol production rates in obese subjects by isotope-dilution technics. Green, Eckert and Migeon (5) also reported that the pituitary-adrenal axis of obese patients responds normally to corticosteroid-suppression and ACTH stimulation tests.

This paper provides further information on pituitary-adrenal function in obesity by reporting the urinary steroid responses of 15 obese patients to an 11β-hydroxylase inhibitor, methopyrapone, better known to clinical investigators as SU-4885. In low concentrations this agent has been found to be a relatively selective inhibitor of 11β-hydroxylation of steroids in the human adrenal cortex (6,7). This leads to a decrease in cortisol secretion, a resultant rise in ACTH secretion and the secretion of large quantities of 11-desoxycorticosteroids such as compound S. Compound S and its metabolites are readily measurable in the urine as either 17-hydroxycorticosteroids or 17-ketogenic steroids. The increase in urinary excretion of 17-hydroxycorticoids or 17-ketogenic steroids after administration of this inhibitor has been employed as an index of the integrity of the hy-

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