tivity was not extracted by water or toluene, but remained in the dried residue fraction. These results suggest that the administered hydrocarbon is rapidly converted to radioactive metabolites that are firmly bound to tissue. This type of protein binding has been established with other carcinogenic agents (5,6). Studies to determine the chemical nature of the binding of H^3-3-methylcholanthrene or its metabolites with tissue constituents are being undertaken.

The prolonged excretion of radioactive material in feces indicates that the carcinogen or its metabolites have a relatively long sojourn in the organism. The observations of Dao, *et al.*(4) and of Dauben and Mabee(7) support this interpretation. Failure to demonstrate significant quantities of radioactivity in tissues 25 days after administration of the hydrocarbon does not preclude its presence in trace amounts. It must be emphasized that the present technics for determination of tritium in biological material are not sensitive enough to detect with certainty concentrations below 5 dpm/mg. The small and variable quantities found in the feces after the first week may be due in part to the limitations inherent in the technic and in part to biological variation.

Summary. 1) Sprague-Dawley female rats, age 50 days, were given 10 mg H^3-3-methylcholanthrene dissolved in sesame oil by gastric tube. 2) In the first experiment selected tissues, urine and feces were analyzed for radioactivity after 24 hours. Sixty to 90% of administered radioactivity was found in gastric and intestinal contents or in feces; 4.4% in urine. Less than 2% was recovered in the tissues analyzed. Results obtained by combustion technic for measurement of total radioactivity were compared with those obtained by toluene extraction. Data indicate that most radioactivity in fat and mammary tissue could be extracted with toluene, whereas negligible quantities could be obtained by this method for liver, kidney, brain, uterus and skeletal muscle. 3) In the second experiment, 24-hour samples of urine and feces were analyzed for total radioactivity for 24-25 days. Significant quantities of tritium-labeled material appeared in urine for 13-17 days and in feces for 7-11 days. Thereafter, fecal and urinary excretion of radioactivity occurred intermittently in trace amounts.

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1. Huggins, C., Briziarelli, G., Sutton, H., *J. Exp. Med.*, 1959, v109, 25.

2. Wilzbach, K. E., *J. Am. Chem. Soc.*, 1957, v79, 1013.

3. Jacobson, H. I., Gupta, G. N., Fernandez, C., Hennix, S., Jensen, E. V., *Arch. Biochem. Biophys.*, 1960, v86, 89.

4. Dao, T. L., Bock, F. G., Crouch, S., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 635.

5. Miller, E. C., *Cancer Res.*, 1951, v11, 100.

6. Heidelberger, C., Weiss, S. M., *ibid.*, 1951, v11, 885.

7. Dauben, W. G., Mabee, D., *ibid.*, 1951, v11, 216.

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Action of Desoxycorticosterone Acetate* on Glycogen. I. Relationship of Liver Glycogen Formation to Potassium Metabolism. (25985)

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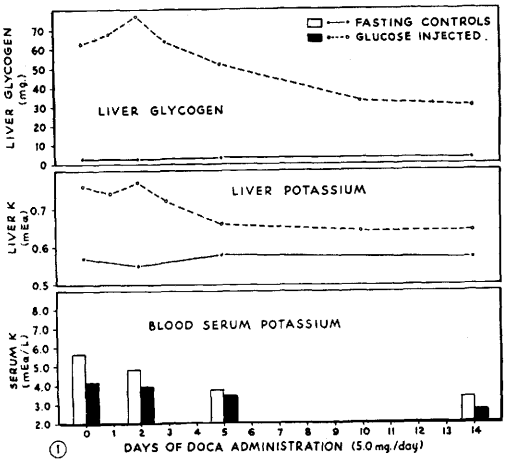
It has been established that a relationship exists between potassium and carbohydrate metabolism in the intact animal. Harrop and Benedict(1) were first to describe a decrease in blood serum potassium concentration following administration of insulin. They believed that this decrease was the result of potassium entering cells of tissue along with glucose during glycogenesis. This idea was supported by Fenn(2) who observed that potassium content of liver of intact rats approximately paralleled glycogen content. Gardner (3) reported that rats depleted of potassium

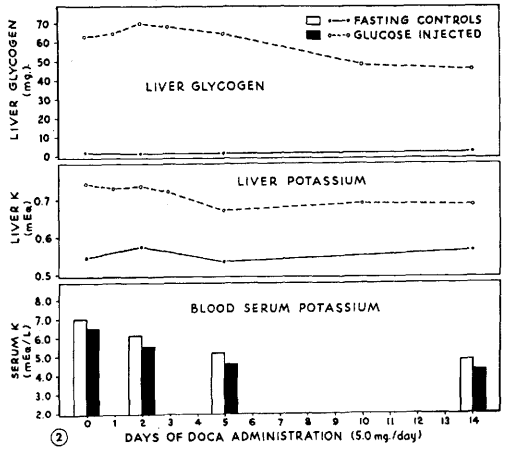
* Generously supplied by Organon, Orange, N. J.

by dietary restriction of this element produced practically no glycogen following intraperitoneal injection of glucose. Desoxycorticosterone acetate (DOCA) has been shown to be a potent mineralocorticoid. When injected into experimental animals, it caused a wasting of potassium resulting in a severe deficiency of that element(4). Low tissue glycogen levels have been reported(5) following administration of large doses of this steroid. *In vitro* studies showed that glycogen formation in liver slices was dependent on a high potassium concentration in the incubating medium (6). Similar studies indicated that even in the presence of high potassium concentration, addition of 1.0 mg % DOCA to the medium completely inhibited glycogen production(7). It was of interest therefore, to study the mechanism of action of DOCA on glycogen formation *in vivo*, and to determine whether the inhibition elicited by this steroid on glycogen production was secondary to, or independent of its action on potassium metabolism. It was postulated that by increasing potassium intake of rats, large decreases in potassium content of tissues and blood serum, normally associated with administration of large doses of DOCA, might be avoided. The action of DOCA on glycogen formation could thus be studied independently of large changes in potassium concentration of both serum and tissues.

Materials and methods. Two hundred and twenty male rats of Wistar origin that weighed 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 for the other 6 subgroups that 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 last injection, food was withdrawn from the cages but the drinking fluid continued to be available *ad libitum*. At time of last injection, 200 mg of glucose per 100 g of body weight were injected intraperitoneally in the form of a 10% solution prepared in isotonic saline. 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 liver was removed, quickly trimmed of fat and connective tissue, and excess blood removed by blotting between sheets of filter paper. Approximately half of each lobe was placed in a tared weighing bottle containing 10 ml of 5.0% trichloroacetic acid and finely minced with scissors for glycogen determination. The remaining portion of liver was placed in a small tared silica dish and ashed for potassium analysis. Glycogen was determined by the method of Carroll *et al.*(8) which consisted of trichloroacetic acid extraction of the homogenized tissue, and alcohol precipitation of the glycogen, followed by the anthrone reaction. Potassium was determined with a Beckman Model DU Flame Spectrophotometer(9) following the analytical procedure of Bills *et al.*(10). The results from the analytical determinations were averaged for each subgroup of animals. Mean liver glycogen and potassium values were expressed in milligrams per liver and meq per liver respectively. Mean blood serum potassium values were expressed in meq/l. Group II consisted of 80 rats subdivided into 4 subgroups of 20 rats each. Rats in the first subgroup received daily injections of sesame oil and served as controls for the other 3 subgroups that received 5.0 mg of DOCA per day for 2, 5 and 14 days respectively. Half of the animals in each subgroup were given tap water to drink and the other half 1.0% potassium chloride solution. These rats served as fasting-controls for the glucose injected rats of Group I, and were treated identically except that 0.9% sodium chloride solution was substituted for the 10% glucose solution injected intraperitoneally 4 hours before sacrifice.

Results. The results obtained with animals that received tap water are presented in Fig. 1. In the fasting control rats, administration





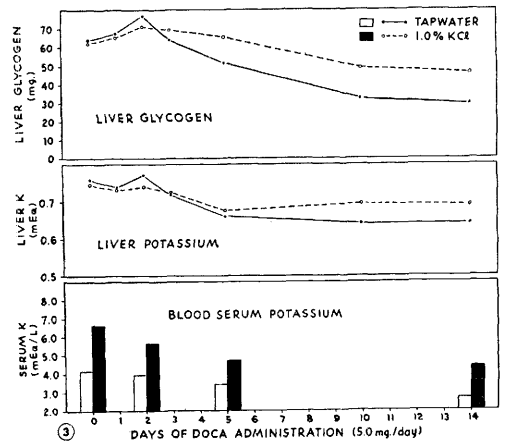


FIG. 1. Effect of 11-desoxycorticosterone acetate (DOCA) on glycogen and potassium content of liver 4 hr after the I.P. inj. of glucose into intact rats.

FIG. 2. Effect of 11-desoxycorticosterone acetate (DOCA) on glycogen and potassium content of liver 4 hr after the I.P. inj. of glucose into intact rats drinking 1.0% KCl solution.

FIG. 3. Effect of 11-desoxycorticosterone acetate (DOCA) on glycogen and potassium content of liver 4 hr after the I.P. inj. of glucose into intact rats drinking 1.0% KCl solution compared with those drinking tapwater.

of DOCA had essentially no effect on either mean glycogen or mean potassium content of the liver. There was however, a marked decrease in blood serum potassium concentration from a mean of 5.67 meq/l for the control group to a mean of 3.31 meq/l for the group of rats treated with 5.0 mg of DOCA for 14 days.

Following intraperitoneal injection of glucose, mean glycogen and mean potassium contents of liver from the rats that did not receive DOCA increased 59.1 mg and 0.187 meq respectively. Glycogen production was facilitated by administration of DOCA for either one or 2 days, but was inhibited in the rats that received the steroid for 5 days or more. Inhibition of glycogen formation was approximately paralleled by a decrease in potassium content of both liver and blood serum. Statistical studies showed that the decreases in both potassium and glycogen content were significant in the rats that received DOCA for 5 days or more.

The results obtained with animals that received 1.0% potassium chloride solution were presented in Fig. 2. As in the studies with fasting rats that received tap water, DOCA had essentially no effect on either glycogen or potassium content of liver. However, there was a decrease in blood serum potassium concentration of 2.09 meq/l for the rats that received DOCA for 14 days.

In rats that received DOCA for less than 3 days, the glycogenic response and increase in liver potassium content following glucose administration was essentially the same as that observed in those that received tap water as shown in Fig. 3. For the glucose injected rats treated with DOCA for 5 days or more, those that received potassium chloride solution had a higher blood serum potassium concentration and produced more glycogen than those that received tap water. These differences were statistically significant.

Discussion. Conflicting reports have ap-

peared concerning the action of DOCA on glycogen formation in intact animals. Koch and Bohlander(11) found that administration of 1.0 mg DOCA per day for 6 days to intact rats resulted in a one-third increase in liver glycogen concentration. Winter and Thompson(12) reported that implantation of 5 mg pellets of DOCA subcutaneously in intact rats was without effect on glycogen concentration. Verzar and Wang(5) showed that administration of a single 20 mg injection of DOCA intraperitoneally completely inhibited glycogen formation in the liver of intact rats. These conflicting reports can be explained in part on the basis of the results of the present studies. These studies showed that DOCA administered daily for short periods of time resulted in increased liver glycogen production, but when administered daily for longer periods of time, there was a decrease in glycogen production.

The observation that potassium entered the liver during glycogenesis was in agreement with the report by Fenn(2). Since decrease in glycogen formation approximately paralleled decrease in potassium content of liver and blood serum, these data suggested that DOCA induced inhibition of glycogen formation was secondary to potassium depletion. The observation that glycogen formation was essentially normal in DOCA treated rats that had an increased intake of potassium chloride further supported this mechanism of action of DOCA on glycogen formation. These data also suggested that it was the availability of potassium from extracellular fluid that limited production of glycogen in the livers of DOCA treated rats rather than potassium content of the liver.

Summary. 1. Daily administration of DOCA for short periods caused an increased glycogenic response in liver of rats following intraperitoneal injection of glucose. Daily administration of DOCA for longer periods resulted in decreased glycogenic response. 2. DOCA induced inhibition of glycogen formation was accompanied by decrease in potassium content of both liver and blood serum. 3. By forcing an increase in potassium intake, the potassium content of both liver and blood serum was maintained at essentially normal levels and liver glycogen production was not significantly inhibited in rats that received DOCA. 4. These data indicated that DOCA induced inhibition of glycogen formation was secondary to potassium depletion.

1. Harrop, G. A., Benedict, E. M., PROC. SOC. EXP. BIOL. AND MED., 1923, v20, 4030.

2. Fenn, W. D., *J. Biol. Chem.*, 1939, v128, 297.

3. Gardner, L. I., Talbot, N. B., Cook, C. D., Berman, H., Uribe, C., *J. Lab. Clin. Med.*, 1950, v35, 592.

4. Harrison, H. E., Harrison, H. C., PROC. SOC. EXP. BIOL. AND MED., 1939, v42, 506.

5. Verzar, F., Wang, F. C., *Nature*, 1950, v165, 114.

6. Hastings, A. B., Teng, C. T., Nesbett, F. B., Sinex, F. M., *J. Biol. Chem.*, 1952, v194, 69.

7. Verzar, F., Wenner, W., *Biochem. J.*, 1948, v42, 35.

8. Carroll, N. V., Longley, R. W., Roe, J. N., *J. Biol. Chem.*, 1956, v220, 583.

9. Gilbert, P. T., Hames, R. C., Beckman, A. O., *Anal. Chem.*, 1950, v22, 772.

10. Bills, C. E., McDonald, F. G., Niedermeier, W., Schwartz, M. C., *Anal. Chem.*, 1949, v21, 1076.

11. Koch, R., Bohlander, A., *Internat. Z. Vitaminforsch.*, 1951, v23, 129.

12. Winter, C. A., Thompson, J. D., PROC. SOC. EXP. BIOL. AND MED., 1944, v55, 95.

Received June 27, 1960. P.S.E.B.M., 1960, v104.