(4). In the uterus of the rat glycogen synthesis is catalyzed by phosphorylase from G-1-P and not through the UDPG pathway. Since the morphology and physiology of smooth muscle and skeletal muscle are not the same the difference in the metabolic pathway for synthesis of glycogen is not surprising. It is possible that smooth muscle cells of organs that are not stimulated by ovarian hormones can behave in a different way from the smooth muscle cells of the uterus.

Summary. UDPG-glycogen synthetase was not demonstrated in the uterus of the ovariectomized or in the ovariectomized-hormone treated rats. However, in the tongue the enzyme was present. The results show that glycogen of the rat uterus is not synthesized through the UDPG pathway. The results also demonstrate a difference in glycogen synthesis between the smooth muscle of the uterus and skeletal muscle of the tongue.

The authors are grateful to Dr. Warren N. Dannenburg, Dept. of Obstetrics and Gynecology Research, for helpful discussions during the investigation.

1. Stetten, D., Stetten, M., *Physiol. Rev.*, 1960, v40, 505.

2. Bo, W. J., PROC. SOC. EXP. BIOL. AND MED., 1962, v111, 186.

3. Leloir, L. F., Goldenberg, S. H., *Methods in Enzymology,* Colcwick and Kaplan, Academic Press, New York, 1962, 5, 145.

4. Bueding, E., *Fed. Proc.*, 1962, v21, 1039.

5. Kaplan, N. O., Ciotti, M., Hamolsky, M., Bieber, P. E., *Science,* 1960, v131, 392.

6. Hess R., Pearse, A. G. E., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 569.

Received March 27, 1963. P.S.E.B.M., 1963, v113.

Role of Hepatic L-α-Glycerophosphate and Triglyceride Synthesis in Production of Fatty Liver by Ethanol.* (28499)

ESKO A. NIKKILA AND KAARINA OJALA (Introduced by D. Y. Hsia)
Department of Medical Chemistry, University of Helsinki, Finland

Ethanol is known to interfere with lipid metabolism in such a way that a prompt increase in the triglyceride content of the liver follows a large single dose(1). A more prolonged ethanol administration causes, in addition, a hypertriglyceridemia in animals(2) and man(3,4).

The mechanism of these metabolic alterations has not been definitely established. Evidence for an increased mobilization of fat from depots after ethanol has been presented by Horning *et al.*(5) in demonstrating that the fatty acid composition of liver triglycerides of ethanol-treated rats resembles that of adipose tissue. In further support of this concept is the elevated level of plasma free fatty acids reported to occur after intoxicating doses of ethanol in rat(6,7). However, others have not been able to confirm the last finding, and have therefore not accepted the fat mobilization theory(8,9). On the other hand, Lieber and Schmid(10) have demonstrated that ethanol *in vivo* and *in vitro* increases the synthesis of fatty acids and inhibits their oxidation in rat liver slices. Similar results were obtained also by Reboucas and Isselbacher(11), who showed, however, that these effects of ethanol do not adequately explain the development of fatty liver. Ethanol has also been reported to increase the incorporation of palmitic acid into triglycerides *in vitro* by cell-free homogenates of rat liver(12), and, in a perfusion experiment, to decrease the release of triglyceride from the liver(13).

In addition to the above mechanism there remains the possibility that ethanol could increase the rate of hepatic uptake of fatty acids from circulation and their esterification with α-glycerophosphate, *i.e.*, the synthesis of triglycerides. This point has been investi-

* This investigation was supported by grants from Sigrid Jusslius Foundation, Helsinki, and the Finnish State Medical Commission.

gated in the experiments reported herein.

Materials and methods. The experiments were carried out on male albino rats weighing between 240 and 280 g and maintained on usual laboratory chow. No fasting period preceded the experiments. In the studies concerning the effect of ethanol on the liver α-glycerophosphate (α-GP) content the animals were anesthetized with Nembutal, and a piece of liver weighing from 100 to 200 mg was removed for analysis. Thereafter, 5 g of ethanol per kg body weight was given through a stomach tube as a 50% v/v solution. Control rats received an isocaloric amount of glucose in an equal volume. Pieces of liver were removed at 1, 2, and 3 hours after the intubation. The L-α-glycerophosphate content of the tissues was determined by the enzymatic method of Bublitz and Kennedy(14) as modified in this laboratory(15). The completeness of absorption was controlled at the end of the experiment by opening the stomach.

The incorporation of circulating palmitic acid-1-C^{14} into hepatic lipids was determined as follows. Rats received 6 g of ethanol per kg of body weight or an isocaloric amount of glucose through a stomach tube. Four hours later the animals were anesthetized with Nembutal, 1.0 ml of blood was withdrawn for analysis of plasma FFA(16), and immediately thereafter 0.5 ml of rat plasma containing 5 μC of palmitic acid-1-C^{14} (Radiochemical Center, Amersham, England) was injected into a jugular vein. After 10 minutes a piece of liver (about 200 mg) was removed, and the lipids were extracted from it with chloroform-methanol according to Folch *et al.*(17). Part of the extract was taken into ether, evaporated in planchets and the radioactivity present in total liver lipid counted in a Tracerlab windowless flow counter. Another aliquot of the lipid extract was fractionated with thin layer chromatography on silica gel G (Merck, Germany) using n-hexane-diethyl ether-acetic acid-methanol(18) as developing solvent. The triglyceride, FFA, and phospholipid fractions were eluted from the medium and their radioactivities and amount of the ester fractions(19) determined. Assay of plasma lipid radioactivity at 10 minutes after injection of the label showed it to be negligible as compared to that of the liver. Therefore, a correction of hepatic radioactivity for counts provided by the blood present in the tissue was not found necessary. The radioactivity present in the FFA fraction of the liver amounted to only 2 to 4% of total lipid radioactivity.

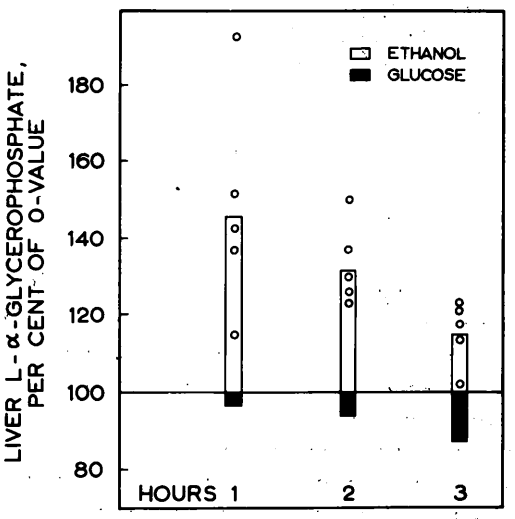

FIG. 1. Change of liver L-α-glycerophosphate content after administration of ethanol or glucose to rats at zero-time.

Results. The basic liver L-α-glycerophosphate concentrations were identical in the ethanol and control group animals (mean 4.3 and 4.5 μM/g). Ethanol administration caused a very distinct increase of the hepatic α-GP content, the change being apparent in the one-hour sample (Fig. 1). The decrease noted in control animals probably reflects the effect of anesthesia and operation. In other experiments, in which ethanol was given to unanesthetized animals, a high liver α-GP level persisted at least 4 hours.

The experimental data in the animals receiving palmitate-1-C^{14} are shown in Table I. The plasma FFA level immediately before injection of the label showed a maximal difference of 105 μEq/l between ethanol-treated animal and its paired glucose-fed control. Thus, the circulating FFA pool, into which the injected label was diluted, was practically identical in both groups. In ani-

TABLE I. Plasma Free Fatty Acid (FFA, μEq/l) Before, and Liver Triglyceride (TG, mg/g Fresh Weight), Radioactivity in Hepatic Total Lipid (TL), Triglyceride, and Phospholipid (PL) 10 Minutes After Intravenous Injection of Palmitic Acid-1-C^{14} into Ethanol-Treated and Control (Glucose) Rats. Mean $\pm$ S.D.

Group	N	FFA	TG	Counts/min/liver $\times 10^{-3}$ TL	TG	PL	S.a. of TG (cpm/μg)
Glucose	5	670 $\pm$ 55	7.2 $\pm$ 1.9	1360 $\pm$ 90	920 $\pm$ 35	330 $\pm$ 40	10.1 $\pm$ 1.8
Ethanol	5	710 $\pm$ 60	10.2 $\pm$ 2.7	2450 $\pm$ 120	1710 $\pm$ 60	420 $\pm$ 55	18.2 $\pm$ 5.7

mals receiving ethanol there was an almost 2-fold increase in the incorporation of injected palmitic acid-C^{14} into liver lipids as compared to controls. The increment of hepatic radioactivity was mostly accounted for by the triglyceride fraction whereas the incorporation of the label into phospholipids was increased only slightly. The labeling of hepatic FFA fraction was identical in both groups. The liver triglyceride concentration of ethanol-treated rats was slightly elevated but nevertheless the specific activity of this fraction was higher than in control animals. The newly synthesized triglyceride thus had derived its fatty acid mainly from circulation.

Discussion. The rapid increase of the α-glycerophosphate content of the liver after ethanol administration apparently is mainly due to its increased production from dihydroxy-acetone phosphate (DHAP), a reaction stimulated by excess DPNH, which generates on oxidation of ethanol. An increase of liver DPNH/DPN ratio after ethanol administration has been conclusively demonstrated by several authors (20, 21, 11). This change presumably causes a shift in the DHAP $\rightleftarrows$ α-GP equilibrium to the side of α-GP. On the other hand, if ethanol also causes an increased lipolysis in adipose tissue, as is possible, part of the excess hepatic α-GP might be derived from plasma glycerol, which is rapidly esterified in the liver.

The factors controlling the rate of hepatic triglyceride synthesis are largely unknown, but several observations suggest that the concentration of FFA in plasma is one factor (22,23). Although the present results do not exclude the possibility that the plasma FFA level was raised at some point after administration of ethanol, they clearly suggest that this mechanism does not solely explain the increase of liver triglyceride by ethanol. Another important factor in the hepatic triglyceride synthesis might be rate of production and concentration of α-glycerophosphate in the liver. The data reported here have demonstrated a parallelism between liver content of α-GP and hepatic uptake and esterification of circulating palmitic acid in ethanol-treated and control rats, but this, of course, forms no definite evidence for a causal relationship between the two phenomena.

At any rate, the present experiments suggest that an increased triglyceride synthesis is an important factor in the pathogenesis of the ethanol-induced fatty liver. It may be that an accelerated mobilization of fatty acids from adipose tissue is another necessary condition. The increased hepatic extraction of plasma palmitic acid after ethanol can hardly be explained by changes in hepatic blood flow as this has been reported to be uninfluenced by ethanol(24).

The decrease of plasma FFA level after ethanol administration observed by Lieber *et al.*(9) is possibly explained by an increased hepatic utilization, and it does not necessarily exclude the possibility of an increased production. The hypertriglyceridemia which follows prolonged ingestion of ethanol results possibly from an equilibration between the liver and plasma triglyceride pools.

The results suggest that insight into the pathogenesis of ethanol-induced fatty liver requires observations in an early phase of its development. None of the changes found herein is demonstrable when liver triglyceride content has attained its maximum.

Summary. Administration of ethanol to rats as a single dose caused a significant in-

crease in liver L-α-glycerophosphate concentration and in incorporation of intravenously injected palmitic acid-1-C^{14} into liver triglycerides. It is suggested that these metabolic alterations may play an important role in the pathogenesis of fatty liver and of hypertriglyceridemia produced by ethanol. The changes could be observed only during the first few hours following ingestion of ethanol.

1. Mallov, S., Bloch, J. L., *Am. J. Physiol.*, 1956, v184, 29.
2. Klatskin, G., *Gastroenterology*, 1961, v41, 443.
3. Albrink, M. J., Klatskin, G., *Am. J. Med.*, 1957, v23, 26.
4. Jones, D. P., Losowsky, M. S., Davidson, C. S., Lieber, C. S., *J. Lab. Clin. Med.*, 1962, v60, 888.
5. Horning, M. G., Williams, E. A., Maling, H. M., Brodie, B. B., *Biochem. Biophys. Res. Comm.*, 1960, v3, 635.
6. Brodie, B. B., Maling, H. M., Horning, M. G., Maickel, R. P., in *Drugs Affecting Lipid Metabolism*, Elsevier Publ. Co., 1961, p104.
7. Mallov, S., *Quart. J. Stud. Alcoh.*, 1961, v22. 250.
8. Elko, E. E., Wooles, W. R., DiLuzio, N. R., *Am. J. Physiol.*, 1961, v201, 923.
9. Lieber, C. S., Leevy, C. M., Stein, S. W., George, W. S., Cherrick, G. R., Abelmann, W. H., Davidson, C. S., *J. Lab. & Clin. Med.*, 1962, v59, 826.
10. Lieber, C. S., Schmid, R., *J. Clin. Invest.*, 1961, v40, 394.
11. Reboucas, G., Isselbacher, K. J., *ibid.*, 1961, v40, 1355.
12. Wakabayashi, M., Horning, M. G., Maling, H. M., Brodie, B. B., *Fed. Proc.*, 1961, v20, 276.
13. Schapiro, R. H., Shimizu, Y., Drummey, G. D., Isselbacher, K. J., *Clin. Res.*, 1962, v10, 234.
14. Bublitz, C., Kennedy, E. P., *J. Biol. Chem.*, 1954, v211, 951.
15. Nikkilä, E. A., Ojala, K., *Acta Chem. Scand.*, 1963, v17, 554.
16. Trout, D. L., Estes, E. H., Jr., Friedberg, S. J., *J. Lipid Res.*, 1960, v1, 199.
17. Folch, J., Lees, M., Stanley, S. G. H., *J. Biol. Chem.*, 1957, v226, 497.
18. Brown, J. L., Johnston, J. M., *J. Lipid Res.*, 1962, v3, 480.
19. Snyder, F., Stephens, N., *Biochem. Biophys. Acta*, 1959, v34, 244.
20. Forsander, O., Räihä, N., Suomalainen, H., *Z. Physiol. Chem.*, 1958, v312, 243.
21. Smith, N. E., Newman, H. W., *J. Biol. Chem.*, 1959, v234, 1544.
22. Fine, M. B., Williams, R. H., *Am. J. Physiol.*, 1960, v199, 403.
23. Feigelson, E. B., Pfaff, W. W., Karmen, A., Steinberg, D., *J. Clin. Invest.*, 1961, v40, 2171.
24. Castenfors, H., Hultman, E., Josephson, B., *ibid.*, 1960, v39, 776.

Received April 1, 1963. P.S.E.B.M., 1963, v113.

A Behavioral Deficit Associated with Phenylketonuria in Rats.* (28500)

V. J. POLIDORA, D. E. BOGGS, AND HARRY A. WAISMAN
Wisconsin Regional Primate Research Center, and Joseph P. Kennedy, Jr. Memorial Laboratory, Department of Pediatrics, University of Wisconsin, Madison

It has been established in this laboratory that certain conditions of dietary intake of excessive phenylalanine by rats(1) or monkeys(2) result in urinary excretion of phenylketones and elevated blood phenylalanine levels. Since it has been shown that phenylketonuric monkeys have severe and presumably permanent decrements in learning abilities and other complex behaviors(2), it was of interest to determine if rats with similar biochemical evidence of phenylketonuria also have deficits in higher-order behavior. This preliminary report describes a behavioral testing procedure which has been reliable in rapidly detecting and quantifying a decrement in the maze performance of phenylketonuric rats.

The selection of this procedure was dictated in part by several previous experiments which may be described briefly. The initial goal of this research has been to devise a learning task simple enough for rapid mastery by the rat, but difficult enough for re-

* Supported by U. S. Public Health Service grant.