SYMPOSIUM: DRUGS IN LIPID METABOLISM. PART I

conducted by The American Oil Chemists' Society at its 37th Fall Meeting, Minneapolis, Minnesota September 30-October 2, 1963

E. C. HORNING AND W. L. HOLMES, PRESIDING
D. H. WHEELER, PROGRAM CHAIRMAN

Drugs and Lipid Transport

MARJORIE G. HORNING, KAY L. KNOX and LYNDA MANI, Lipid Research Center, Department of Biochemistry, Baylor University College of Medicine, Houston, Texas

Abstract

Drugs such as ethanol, carbon tetrachloride, ethionine or ACTH have a profound influence on lipid metabolism. The enzymatic synthesis of triglycerides and phospholipids by the liver, the release of triglycerides from liver to plasma and the transport of free fatty acids from adipose tissue are affected. These changes in lipid metabolism may be considered as resulting from chemically induced stress. In order to study the transport of lipids during a stress not induced by drugs, cold stress was also investigated. The changes in lipid transport following ethanol or ACTH administration were quite different from those observed during cold stress where body temp must be maintained by mobilization of energy producing substrates. However, the alterations in lipid transport during both cold and drug induced stress are dependent on the sex of the rat. The observed effects apparently involve a sex-dependent transport of triglycerides from liver to plasma and also a sex-dependent mobilization of energy-producing substrates.

FOLLOWING THE ADMINISTRATION of drugs such as ethanol, carbon tetrachloride, ethionine, ACTH or morphine, fatty acids mobilized from adipose tissue are deposited in the liver as triglycerides. Treatment with these drugs also affects the enzymatic synthesis of triglycerides, phospholipids and lipoproteins in the liver. The effect of these drugs on lipid transport, and particularly on the transfer of triglycerides from the liver to plasma, is a matter of even greater interest.

The transport of free fatty acids following drug administration has been studied extensively (1–5). However, very little attention has been paid to the effect of drugs on triglyceride transport although the change in plasma concn is usually much greater for triglycerides than for free fatty acids. For example, following ethanol ingestion in the rat, the serum triglyceride level may increase to ca. 1200 γ/ml, while free fatty acids increase at the same time to 250 γ/ml (1). Plasma levels of triglycerides are very responsive to drug administration and can be increased or decreased by treatment with the appropriate drug. Ethanol and ACTH raise the plasma concn of triglycerides by stimulating their transport from liver to plasma; carbon tetrachloride (6) and ethionine (7) lower the plasma concn by repressing triglyceride transfer out of the liver.

The sex of the rat has a profound influence on these changes in plasma triglycerides. It has been known for many years that the increase in liver triglycerides after ethanol or carbon tetrachloride ingestion was dependent on the sex of the rat. The experiments to be discussed show that the response of plasma triglyceride levels to drug administration is also dependent on the sex of the rat. Changes in concn of plasma and liver triglycerides are not due simply to the androgen-estrogen ratio in male and female rats. What is involved in these experiments is a sex-dependent transport of triglycerides from liver to plasma.

The administration of ethanol (8), carbon tetrachloride or ethionine (9) increases the triglyceride content of rat liver. Since stimulation of fatty acid transport to the liver or fatty acid synthesis by the liver would result in an increased liver lipid content, it was necessary to investigate the origin of the fatty acids found in liver following drug administration. The development of gas chromatographic procedures made it possible to prove that the increase in liver triglycerides was paralleled by an increase in liver triglyceride linoleic acid (Fig. 1). Following administration of carbon tetrachloride, for example, the liver triglyceride content increased from a control value of 4 mg/g liver to 78 mg/g liver (Fig. 1). No decrease in phospholipid linoleic acid was observed (Fig. 1). Therefore, the linoleic acid present in liver triglycerides had not been transferred enzymatically from liver phospholipids. Since linoleic acid is not synthesized by the rat, this acid must have been transported to the liver from adipose tissue. From these and related experiments (8) it is now believed that most of the fatty acids, deposited in liver as triglycerides after drug administration, have been transported from the depots and did not arise from *de novo* synthesis in the liver.

Changes in the enzymatic synthesis of triglycerides and phospholipids also occur following the administration of ethanol, carbon tetrachloride or ethionine (10). The enzymatic changes were studied in homogenates prepared from livers of control and drug treated rats using 1-C^{14}-palmitate. This incorporation of radioactive palmitate into triglycerides and phospholipids is shown in Figure 2. In all three examples, drug

INDEX

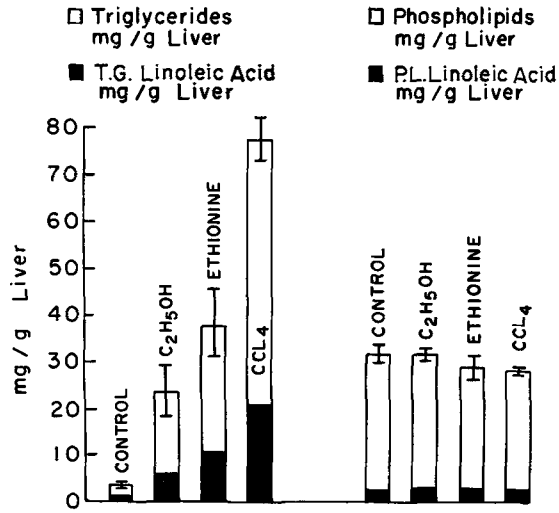

FIG. 1. Mg of linoleic acid/g liver present in the triglycerides and phospholipids isolated from livers of control rats and rats treated with ethanol, carbon tetrachloride or ethionine. Ethanol (6.0 g/kg) was administered by stomach tube as a 50% aqueous solution; carbon tetrachloride (1.5 ml/kg) was administered by stomach tube as a 50% solution in mineral oil, and ethionine was administered intraperitoneally (750 mg/kg).

treatment increased the enzymatic incorporation of fatty acids into triglycerides and decreased the incorporation of fatty acids into phospholipids. When the liver homogenates were prepared six hours after the administration of the drug, the changes in enzymatic incorporation were quite striking (Fig. 2), but small changes were observed in homogenates prepared as early as two hours after ingestion of carbon tetrachloride. If these changes in enzymatic activity were to occur *in vivo*, an increase in liver triglycerides would result.

These enzymatic changes, however, are not great enough to explain the ten to twenty-fold increase in liver triglycerides induced by administration of carbon tetrachloride or ethionine to rats. If, in addition to the enzymatic effect, the release of triglycerides from the liver were blocked, the liver triglyceride content should increase sharply. Therefore, experiments were undertaken to study the transport of triglycerides

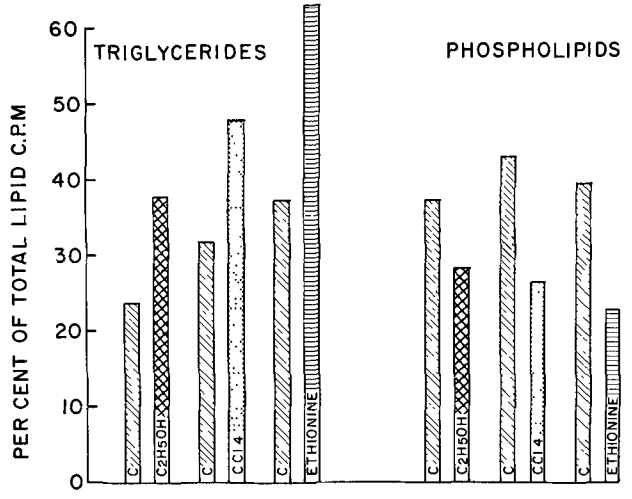

FIG. 2. *In vitro* incorporation of 1-C^{14}-palmitate into triglycerides and phospholipids by homogenates from livers of control (C), ethanol, carbon tetrachloride and ethionine treated rats. Homogenates were prepared six hours after the administration of the drugs in the doses listed in Figure 1. Conditions of incubation have been described previously (10). Results are expressed as percentage of total lipid radioactivity (c.p.m.) incorporated into triglycerides and phospholipids.

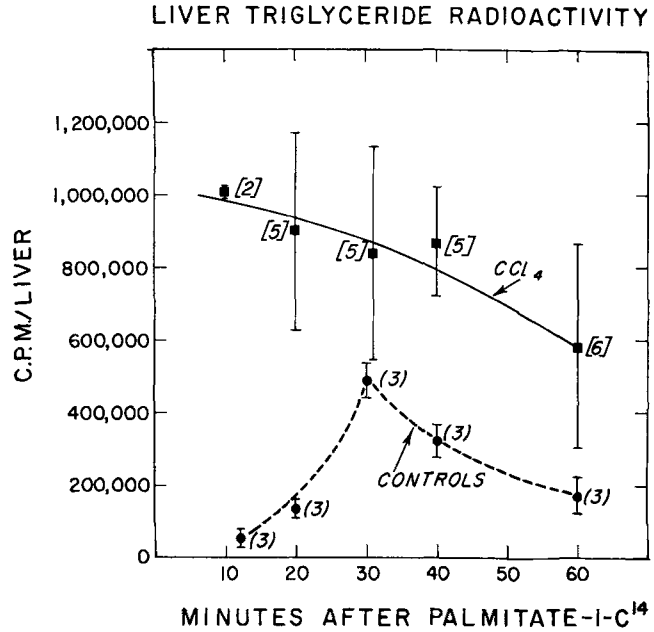

FIG. 3. Changes in liver triglyceride radioactivity in control and carbon tetrachloride treated rats (Sprague-Dawley) during the first hour following the intravenous injection of albumin-bound 1-C^{14}-palmitate. Carbon tetrachloride (1.5 ml/kg) was administered orally four hours before injection of the labeled palmitate.

from liver to plasma. By injecting radioactive palmitate intravenously, it was possible to follow the release of triglycerides from liver and the appearance of labeled lipids in liver and plasma of control and drugs treated rats.

Ten minutes after injection of 1-C^{14}-palmitate into the tail vein, the amount of labeled fatty acid found in liver triglycerides was 24 times greater in treated rats than in control rats (Fig. 3); after 60 min the amount of labeled liver triglycerides was still greater in treated than in control rats (6). In control rats there was a sharp peak in liver triglyceride radioactivity 30 min after injection of 1-C^{14}-palmitate; in carbon tetrachloride treated rats, the peak must have occurred during the first ten minutes following the injection.

Plasma triglyceride radioactivity in control and carbon tetrachloride treated rats is shown in Figure 4. In control rats, the radioactive triglycerides in plasma peak sharply 20 to 30 min after injection of 1-C^{14}-palmitate and this peak corresponds to the peak in liver triglyceride radioactivity (6,11). After carbon tetrachloride ingestion, no peak in the radioactivity of plasma triglycerides was observed; radioactive triglycerides were released from liver to plasma very slowly. It is apparent from a comparison of Figures 3 and 4 that radioactive triglycerides are retained in the liver after carbon tetrachloride ingestion and normal release to the plasma is impaired.

Negligible amounts of labeled free fatty acids were found in plasma and in the liver of control and treated rats in these experiments.

The effect of drugs on triglyceride transport also can be studied by following changes in the concn of liver and plasma triglycerides. Six to eight hours after ingestion of carbon tetrachloride, plasma triglycerides declined to very low levels (50–70 γ/ml) (Fig. 5). During this period, plasma triglycerides increased to from 300–800 γ/ml following ethanol ingestion (Fig. 5) (10). At this time the concn of liver triglycerides was 30–40 mg/g liver in rats receiving carbon tetrachloride and 20–30 mg/g liver in rats

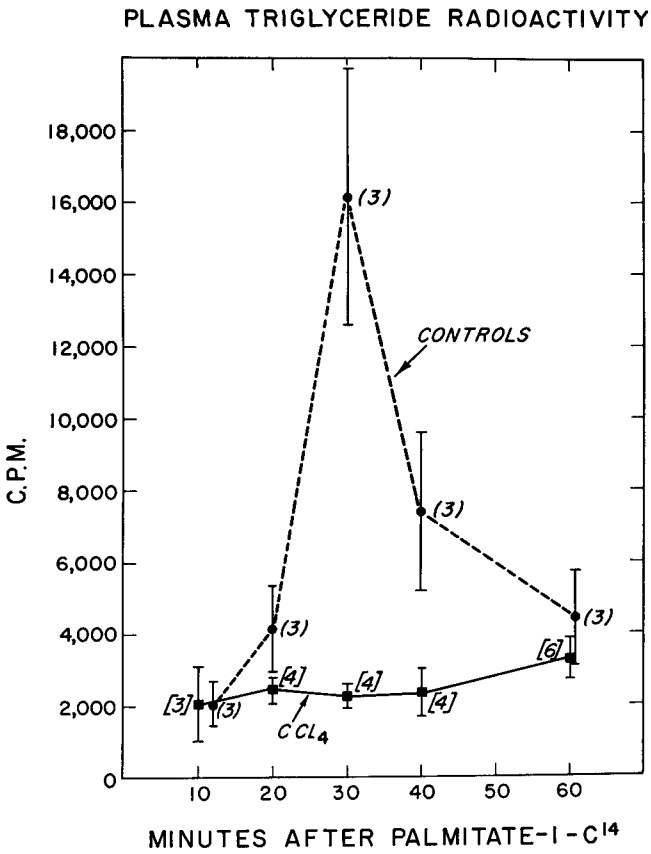

FIG. 4. Changes in plasma triglyceride radioactivity in the same control and carbon tetrachloride treated rats shown in Figure 3.

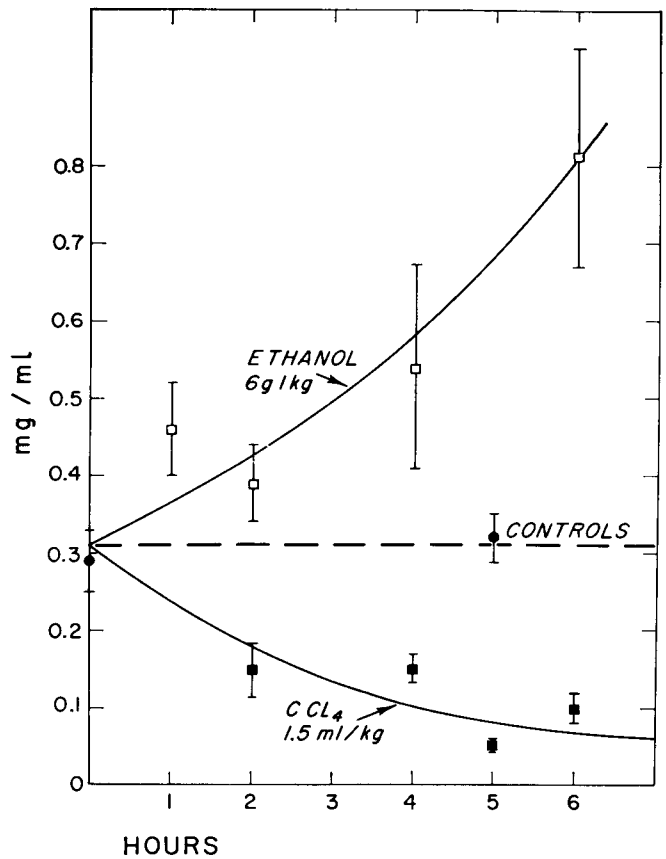

FIG. 5. Changes in plasma triglyceride levels (mg/ml plasma) in fasting female rats (Sprague-Dawley) during the first six hours after oral administration of ethanol (6.0 g/kg) or carbon tetrachloride (1.5 ml/kg).

treated with ethanol. However, 18–24 hr after carbon tetrachloride ingestion, the liver triglyceride concentration reached 80–100 mg/g liver (9); the liver concn after ethanol administration rarely exceeded 25–35 mg/g liver. The stimulation of triglyceride release from liver to plasma by ethanol apparently prevents the excessive accumulation of triglycerides in the liver observed 18–24 hr after treatment with carbon tetrachloride.

In these experiments female rats were employed because the increase in liver triglycerides was greater in female than in male rats. Since the liver triglyceride content is dependent on the sex of the rat and liver is the source of plasma triglycerides (12), plasma triglyceride levels were measured to see if the sex of the rat also influenced the concn of plasma triglycerides.

The effect of ethanol administration on the plasma triglycerides in male and female rats is shown in Figure 6. The plasma triglyceride concn increased from 140–325 γ/ml in female rats 6–8 hr after ethanol ingestion; in the same experiment, however, the plasma triglyceride in male rats increased from 200–1240 γ/ml. The plasma from male rats was lipemic. Plasma cholesterol levels were also higher in the male rat. No significant change in plasma glucose occurred in male rats, but in female rats the plasma glucose level rose from 114–163 mg% after ethanol administration.

When liver triglycerides were measured in the same animals, it was found that the liver triglycerides increased 20 mg/g liver in female rats; in male rats the increase was only 11 mg/g liver. The very high plasma levels observed in male rats in these experiments suggests that triglycerides are transported out of the liver so rapidly that the liver concn never attains the higher levels observed in female rats. The liver triglyceride concn after ethanol ingestion reflects the difference in the rate of release of triglycerides from the livers of male and female rats.

Since large doses of ethanol are known to stimulate the pituitary-adrenal axis (13), the response observed with ethanol could be due to release of ACTH from the pituitary. Therefore comparable experiments were carried out with male and female rats receiving depot ACTH subcutaneously (40 USP units, Armour Acthar

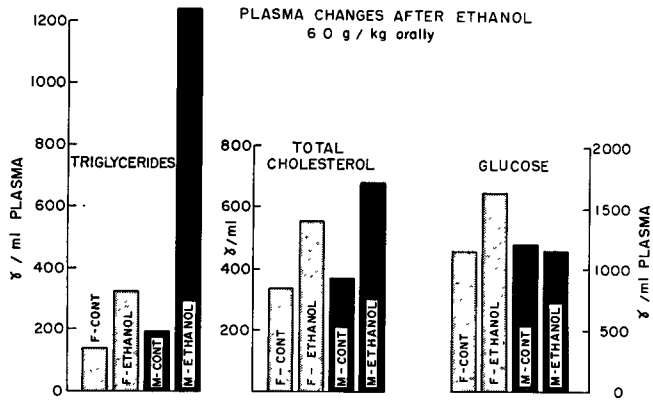

Fig. 6. Changes in concn of plasma triglycerides, cholesterol and glucose in fasting male and female rats (Sprague-Dawley, 180–200 g) 7–8 hr after the oral administration of ethanol (6.0 g/kg) as a 50% aqueous solution. The animals were anesthetized with Nembutal and then bled from the heart 7–8 hr after administration of the ethanol. Triglycerides were determined by a modification of the method of Butler et al. (18), cholesterol by a modification of the method of Hanel and Dam (19), and glucose by the glucose oxidase procedure. Results are expressed as μg/ml plasma.

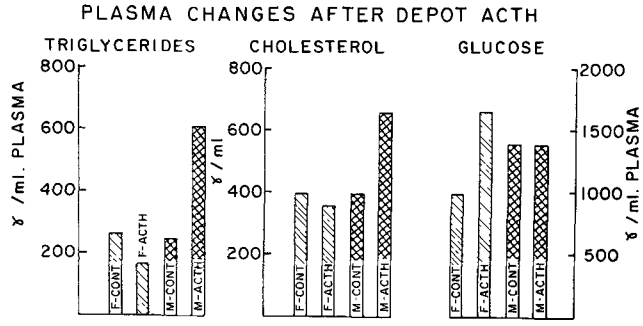

FIG. 7. Changes in the concn of plasma triglycerides, cholesterol and glucose in fasting male and female rats (Sprague-Dawley, 180–200 g) 7–8 hr after the subcutaneous injection of 40 USP units of depot ACTH (Armour Acthar Gel). Experimental procedure was the same as described for Figure 6. Results are expressed as μg/ml plasma.

Gel, a dose well tolerated by rats).

Eight hours after injection of ACTH into male rats, the concn of plasma triglycerides was twice as high in treated as in control rats; there was also an increase in the plasma cholesterol concentration in treated rats (Fig. 7). Very little change in either plasma triglycerides or cholesterol occurred in female rats following subcutaneous injection of depot ACTH.

However, in female rats the plasma glucose levels increased from 106–165 mg% seven to eight hours after injection of ACTH. In male rats, no change in plasma glucose occurred.

Thus ACTH stimulation did not provoke the same metabolic changes observed with ethanol. ACTH stimulated the release of triglycerides to the plasma only in male rats; no release of triglycerides occurred in female rats.

These experimental conditions (ethanol, ACTH) may be used to study stress in laboratory animals (1). In order to study the transport of lipids during a stress not induced by drugs, cold stress was investigated. When male rats were maintained at 4C for 6–8 hr, the plasma triglyceride concn dropped to half the level of the control rats maintained at room temp (Fig. 8) and the plasma glucose levels increased significantly from 110–170 mg%. In female rats maintained at 4C, no significant change occurred in either plasma triglycerides or glucose.

The response of plasma cholesterol to cold stress was variable and is under investigation. It was found that exposure to cold could result in a rise in plasma cholesterol levels in both male and female rats, but the rise does not always occur. In the experiment shown in Figure 8, a marked increase occurred only in female rats.

When the livers of animals subjected to cold stress

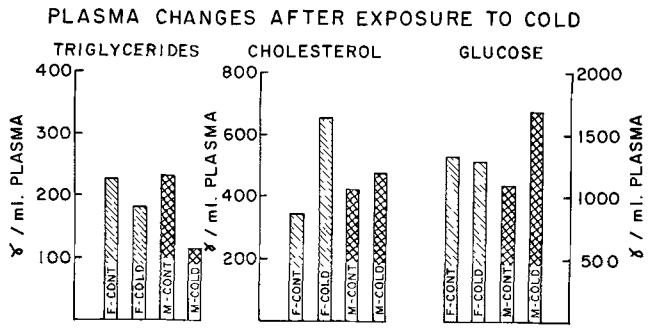

FIG. 8. Changes in concn of plasma triglycerides, cholesterol and glucose in fasting male and female rats (Sprague-Dawley, 180–200 g) after 7–8 hr exposure to cold (4C). Experimental procedure was the same as described for Figure 6. Results are expressed as μg/ml plasma.

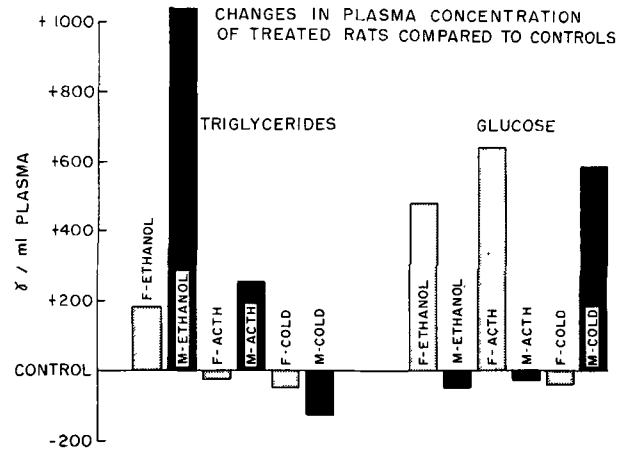

FIG. 9. Comparison of the changes in plasma concn of triglycerides and glucose after administration of ethanol (6.0 g/kg), depot ACTH (40 USP units) and exposure to cold (4C) for 7–8 hr. Results are expressed as μg/ml plasma change from control levels.

were analyzed, it was found that the triglyceride content of the livers of female rats had increased from 3 mg/g liver to 12–20 mg/g liver. In male rats subjected to cold stress there was no increase in the liver triglyceride content from the control level of 2 mg/g liver.

The results of these experiments with ethanol, ACTH and cold, summarized in Figures 9 and 10, show that the transport of cholesterol, triglycerides and glucose depends not only on the type of stress, but also on the sex of the rat. After ethanol or ACTH administration the increase in plasma triglycerides was greater in male rats than in female rats, and no change in plasma glucose levels occurred. In female rats there was a significant increase in the plasma glucose level. If plasma triglycerides and plasma glucose represent the available or mobilized energy-producing substrates needed in emergency situations, then during ethanol or ACTH induced stress the male rat mobilizes triglycerides more easily than glucose and the situation is reversed in the female rat.

During cold stress the rat must maintain its body temp at 37C ± 1C in an external environment at 4C. The male rat responds to this type of experimental stress by mobilizing glucose instead of triglycerides. The plasma and liver triglycerides fall suggesting that in cold stress free fatty acids are either not released from the depots or that triglycerides are oxidized in the liver as rapidly as they are synthesized.

This rather simple explanation of the mobilization of either glucose or triglycerides for energy during pituitary-adrenal stimulation does not apply to female rats subjected to cold stress. No change in either plasma triglycerides or glucose occurred, although triglycerides accumulated in the liver.

It is obvious from these initial experiments that cold stress induces quite different metabolic changes from either ethanol- or ACTH-induced stress. These sex-dependent changes may provide a new experimental approach for the investigation of the biochemical regulation of temperature.

Stimulation of the pituitary-adrenal axis also releases catecholamines, and these amines are also involved in lipid transport (1,14). It is known that Dibenamine, an adrenergic blocking agent, partially reverses the fatty liver due to carbon tetrachloride (15). Since triglyceride transport is impaired by carbon tetrachloride, the effect of Dibenamine on triglyceride transport was investigated. The results of

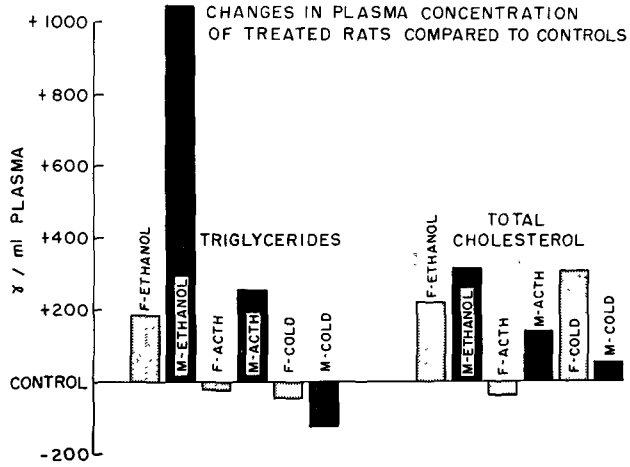

FIG. 10. Comparison of the changes in plasma concn of triglycerides and cholesterol after administration of ethanol, depot ACTH and exposure to cold for 7–8 hr. Results are expressed as μg/ml plasma change from control levels. Figures 9 and 10 summarize the results presented in Figures 6–8.

some experiments with Dibenamine are shown in Figures 11 and 12.

It was found that Dibenamine alone stimulated the release of triglycerides from liver to plasma (16). The plasma triglyceride content increased from 180–400 γ/ml while the liver triglyceride content declined from 5.6–3.8 mg/g liver. When rats receiving carbon tetrachloride were pretreated with Dibenamine, the plasma triglycerides were maintained at normal levels in contrast to the rats receiving only carbon tetrachloride (Fig. 11). In addition, the triglyceride content of the liver (15 mg/g) was half that of rats receiving only carbon tetrachloride (27 mg/g). Thus Dibenamine reversed the carbon tetrachloride fatty liver by maintaining triglyceride transport out of the liver at normal levels, counteracting the blocking effects of carbon tetrachloride on triglyceride transport.

If Dibenamine is acting as an adrenergic blocking agent in these experiments, it may be presumed that the catecholamines are involved in triglyceride transport from liver to plasma.

Sex-dependent changes in plasma lipids are of interest because atherosclerosis is widely believed to be a result of a lipid metabolic disturbance, and up to about the sixth decade of life this vascular disease is seen predominately in males. Similar experiments

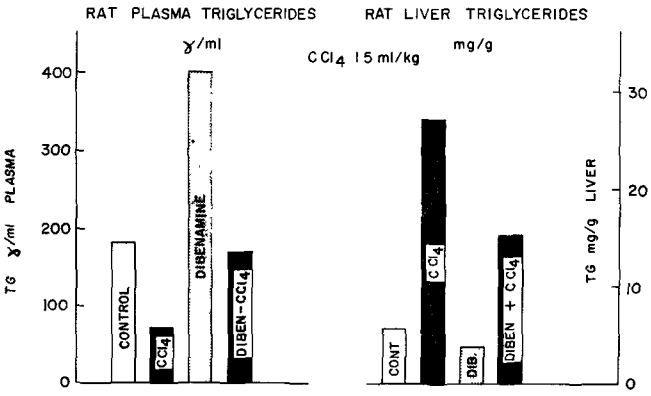

FIG. 11. Changes in plasma and liver triglycerides in control, tetrachloride-, Dibenamine- and carbon tetrachloride + Dibenamine treated rats. Carbon tetrachloride (1.5 ml/kg) was administered orally as a 50% solution in mineral oil. Dibenamine (10 mg/kg) was injected intraperitoneally 18 hr before administration of carbon tetrachloride. Experimental procedure was the same as described for Figure 6. Results are expressed as μg of triglyceride/ml plasma and as mg of triglyceride/g liver.

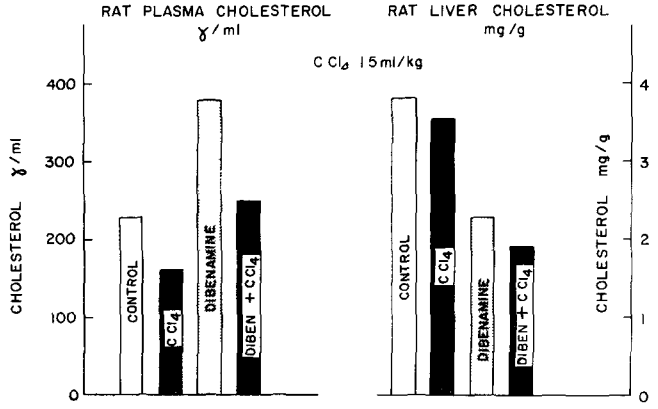

FIG. 12. Changes in plasma and liver cholesterol concn in the same rats presented in Figure 11. Results are expressed as μg of cholesterol/ml plasma and mg cholesterol/g liver.

were carried out with normal young men and women. Changes in plasma lipids and glucose were followed in these subjects after the subcutaneous injection of depot ACTH. However, no sex dependent changes in plasma, triglycerides, cholesterol or glucose were observed. The dose (mg/kg) was, of course, much smaller than that used in rats. However, when the excretion of urinary steroids was followed using gas chromatographic procedures (17), sex dependent changes in the pattern of excretion were observed in preliminary experiments. These experiments are being pursued.

The experimental conditions we have used (ethanol, ACTH and cold) stimulate the pituitary-adrenal axis and in this sense may be used to study experimental stress in the rat. It is apparent from these studies on a closely controlled genetic strain of rats maintained on a uniform diet that the metabolism and transport of energy-producing substrates is different in male and female rats. The same experimental stimulus applied to male and female rats must release different hormones or different amounts of the same hormones to effect the sex-dependent metabolic changes which have been observed.

ACKNOWLEDGMENT

This work was supported by Grant HE 05435-03 from the National Institutes of Health.

REFERENCES

1. Paoletti, R., R. P. Maickel, R. L. Smith and B. B. Brodie, "Drugs as *Tools in Studies of Nervous System Regulation of Release of Free* Fatty Acids from Adipose Tissue," Pergamon Press, London, 1963, p. 29.
2. Steinberg, D., M. Vaughan and S. Margolis, J. Biol. Chem. *235*, 38 (1960).
3. Havel, R. J., and A. Goldfien, J. Lipid Res. *1*, 102 (1959).
4. Schotz, M. C., and I. H. Page, *Ibid. 1*, 466 (1960).
5. Mallov, S., and P. N. Witt, J. Pharmacol. Exp. Ther. *132*, 126 (1961).
6. Maling, H. M., A. Frank and M. G. Horning, Biochim. Biophys. Acta *64*, 540 (1962).
7. Olivecrona, T., "Effect on Drugs on Synthesis and Mobilization of Lipids," Pergamon Press, London, 1963, p. 151.
8. Horning, M. G., E. A. Williams, H. M. Maling and B. B. Brodie, Biochem. Biophys. Res. Comm. *3*, 635 (1960).
9. Horning, M. G., M. Earle and H. M. Maling, Biochim. Biophys. Acta *56*, 175 (1962).
10. Horning, M. G., M. Wakabayashi and H. M. Maling, "Effect of Drugs on Synthesis and Mobilization of Lipids," Pergamon Press, London, 1963, p. 13.
11. Laurell, S., Acta Physiol. Scand. *47*, 218 (1959).
12. Byers, S. O., and M. Friedman, Am. J. Physiol. *198*, 629 (1960).
13. Brodie, B. B., W. M. Butler, Jr., M. G. Horning, R. P. Maickel and H. M. Maling, Am. J. Clin. Nut. *9*, 432 (1961).
14. Paoletti, R., R. L. Smith, R. P. Maickel and B. B. Brodie, Biochem. Biophys. Res. Comm. *5* 424 (1961).
15. Brodie, B. B., H. M. Maling, M. G. Horning and R. P. Maickel, "Drugs Affecting Lipid Metabolism," ed. S. Garattini and R. Paoletti, Elsevier Pub. Co., Amsterdam, 1961, p. 104.
16. Horning, M. G., L. Mani and K. L. Knox, Second International Pharmacological Meeting, Prague, Czechoslovakia, August, 1963.
17. Horning, E. C., W. J. A. VandenHeuvel and B. G. Creech, "Methods of Biochemical Analysis," Vol. XI, ed. D. Glick, Interscience Publishers, New York, 1963, p. 69.
18. Butler, W. M., Jr., H. M. Maling, M. G. Horning and B. B. Brodie, J. Lipid Res. *2*, 95 (1961).
19. Hanel, H. K., and H. Dam, Acta Chem. Scand. *9*, 977 (1955).

[Received April 15, 1964—Accepted July 8, 1964]