Rat liver and plasma lipids after carbon tetrachloride administration

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SUMMARY Oral administration of CCl_4 to rats produced (*a*) decreased cholesterol, phospholipid, and triglyceride plasma concentrations and elevated triglyceride levels in liver, (*b*) several hours later, a growing increase in plasma FFA, and (*c*) still later, increased plasma cholesterol, triglyceride, and phospholipid concentrations and continually rising liver triglyceride.

The accumulation of hepatic triglyceride induced by CCl_4 was smaller after administration of sympathetic anti-release agents; larger after administering a ganglion-blocking agent; smaller after the animals had been cord-sectioned; reduced by adrenalectomy and (to a much lesser extent) by bilateral splanchnicectomy. Corticoid treatment partially restored the response to CCl_4 in adrenalectomized animals. Cord section prevented the plasma FFA increase induced by CCl_4, although it did not modify the decrease in plasma phospholipid; both adrenalectomy and hypophysectomy also prevented the CCl_4-induced plasma FFA increase.

The results are interpreted as indicating that both adrenal corticoids and an intact sympathetic nervous system are necessary for fatty liver induction by CCl_4.

KEY WORDS liver · plasma · lipids · carbon tetrachloride · fatty liver · rat · catecholamines · corticoids · ganglion blockage · cord section · splanchnicectomy · adrenalectomy

ADMINISTRATION OF CCl_4 to animals produces, among other alterations, an increased liver fat content. The early evidence for this effect was derived from histological studies, and did not indicate the specific lipid class or classes involved in the fat increase. Later chemical investigations revealed that the rise in liver lipid following administration of CCl_4 to rats was due to increased liver triglyceride (1, 2).

The underlying mechanism responsible for this triglyceride increase has recently been the object of considerable speculation (3–8). Calvert and Brody (4) suggested that the sympathetic nervous system is involved. Their investigations revealed that certain treatments which would be expected to decrease the effects of endogenous catecholamine secretion (i.e., adrenergic blockade, spinal cord transection, and bilateral adrenalectomy) partially or completely prevented the CCl_4-induced increase in total liver lipid (4, 9). It has been shown recently that much larger amounts of catecholamines are excreted after CCl_4 administration (10). Hepatic lipid levels increase after epinephrine administration (11, 12); triglycerides increase most (13). In the present study, the lipid changes in plasma and liver following CCl_4 administration were investigated in order to determine whether they would be consistent with those expected from a catecholamine-induced effect. Also, the influence of several treatments on the lipid alterations is presented and discussed in reference to the previously proposed mediation by catecholamines. Since the adrenal cortex is involved in fatty liver induction by catecholamines and other agents (14–18), the role of the corticoids in CCl_4-induced lipid accumulation is also considered.

METHODS

Female albino Holtzman or Sprague-Dawley rats, 180–220 g, were used. They were maintained on an ad lib. diet of Purina laboratory pellets and tap water prior to the administration of CCl_4–peanut oil 1 : 1 or peanut oil alone. All animals were fasted from the time they were given CCl_4 or peanut oil until they were sacrificed.

Surgical Procedures

For surgical manipulations, animals were anesthetized with diethyl ether. Bilateral dorsal incisions provided the

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most convenient approach for adrenalectomy and splanchnicectomy. The greater splanchnic nerves were sectioned a short distance below the diaphragm. Adrenalectomized and splanchnicectomized animals were used 1–4 days after the operations. In the adrenalectomized animals, 0.9% NaCl replaced regular drinking water. The completeness of the splanchnicectomy was assessed by measurement of urinary epinephrine. The catecholamine assay used has been previously described (9). Only animals with markedly decreased epinephrine excretion after the operation were used for further studies. Hypophysectomy: the pituitary gland was removed through the opening made by puncturing the tympanic bulla with a 15 gauge needle. Operated animals having plasma corticosterone levels >5 μg/100 ml were discarded. Spinal cord transection was performed at the C-7 level; 1–2 mm of the spinal cord was removed. From the time of operation until the administration of CCl_4 (usually 1 day), the animals were fed finely crumbled food pellets, and water was periodically injected subcutaneously. The bladder was emptied at intervals by means of externally applied pressure.

Rats were sacrificed under hexobarbital anesthesia (100 mg/kg, intraperitoneally).

Analysis

Blood samples were collected and centrifuged at 700 × *g* for 20 min. Plasma and liver samples were frozen at −20° until assayed. Plasma free fatty acid was measured by the method of Dole (19); total cholesterol in plasma and liver by the Trinder procedure (20); plasma triglyceride by the Van Handel and Zilversmit method (21), and liver triglyceride by the modification of Butler et al. (22).

Lipids were extracted from liver and plasma by the procedure of Folch et al. (23), using a total of 9 ml of CH_3OH and 18 ml of $CHCl_3$. An aliquot of a water extract of the CH_3OH–$CHCl_3$ extract was taken for phospholipid analysis.

Inorganic phosphate was determined by the method of Fiske and Subbarow (24), and corticosterone by the Zenker and Bernstein procedure (25).

Administration of CCl_4 and Drugs

When CCl_4 was administered by stomach tube, the dose, unless otherwise indicated, was 2.5 ml of CCl_4 (in 2.5 ml of peanut oil) per kg body weight. Control animals received 2.5 ml of peanut oil per kg. For studies with inhaled CCl_4, a chamber with a dual syringe mechanism was employed.[1] The dual syringe permitted CCl_4 to be fed into a vaporizer at a constant calculated rate. The vaporized CCl_4 was blown into the chamber along with a known volume of air, thus maintaining a constant CCl_4 concentration. Periodic UV spectroscopic analysis of the CCl_4 concentration indicated that its variability was not greater than 5%. The ganglionic-blocking agent trimethidinium bismethosulfate[2] was given in two subcutaneous doses, 50 mg/kg simultaneously with CCl_4, and another 50 mg/kg 6 hr later. The anti-release agent guanethidine[3] was administered intraperitoneally 48 and 24 hr prior to CCl_4, 50 mg/kg each time. Another anti-release agent, β-TM10[4], was given in two subcutaneous doses of 100 mg/kg each, one 6 hr prior to and the other simultaneously with CCl_4. Hydrocortisone was administered intraperitoneally to adrenalectomized animals, 2.5 mg/kg twice daily from the time of operation. An additional 12 mg/kg was given 30 min before CCl_4, and 6 mg/kg 10 hr later. The schedule for administration of epinephrine[5] to adrenalectomized rats consisted of subcutaneous injections of 0.15 mg/kg twice daily following adrenalectomy, supplemented with 1 mg/kg 30 min before CCl_4 and an additional 0.5 mg/kg 10 hr later.

Results were analyzed by the "*t*" test (26) and the criterion used for significance was $P < 0.05$.

RESULTS

Changes Induced by CCl_4

Fig. 1 shows the changes in plasma lipid concentrations following oral administration of CCl_4. Phospholipid, cholesterol, and triglyceride concentrations decreased during the first 6 hr and stayed low for at least 16 hr. Plasma triglyceride concentrations subsequently rose and were higher than control values during the 18–26 hr period. Plasma cholesterol and phospholipid concentrations likewise rose, but did not exceed control levels either at the 18–26 hr period or 48 hr after CCl_4 administration. By 72 hr all three plasma lipid values were markedly higher in animals receiving CCl_4 than in animals receiving only peanut oil.

Free fatty acid (FFA) concentrations in plasma did not differ from control values during the 2–6 hr interval, but rose during the 8–16 hr period and continued to rise throughout the times studied.

A more detailed picture of the plasma triglyceride change is presented in Fig. 2. The graph clearly illus-

[1] Kindly supplied by Dr. Herbert Cornish, Dept. of Industrial Health, School of Public Health, University of Michigan.

[2] Trimethidinium bismethosulfate (Ostensin) kindly supplied by Wyeth Institute for Medical Research, Radnor, Pa.

[3] Guanethidine, [2-(octahydro-1-azocinyl) ethyl] guanidine sulfate (Ismelin), kindly supplied by Dr. F. F. Yonkman, Ciba Laboratories, Summit, N.J.

[4] β-TM10, [2-(2,6-dimethylphenoxy) propyl] trimethyl ammonium chloride (SKF 6890a), kindly supplied by Dr. Edwin J. Fellows of Smith, Kline and French Laboratories, Philadelphia, Pa.

[5] Epinephrine in oil (Adrenalin in Oil) kindly supplied by Mr. Richard Kolb of Parke, Davis and Co., Detroit, Mich.

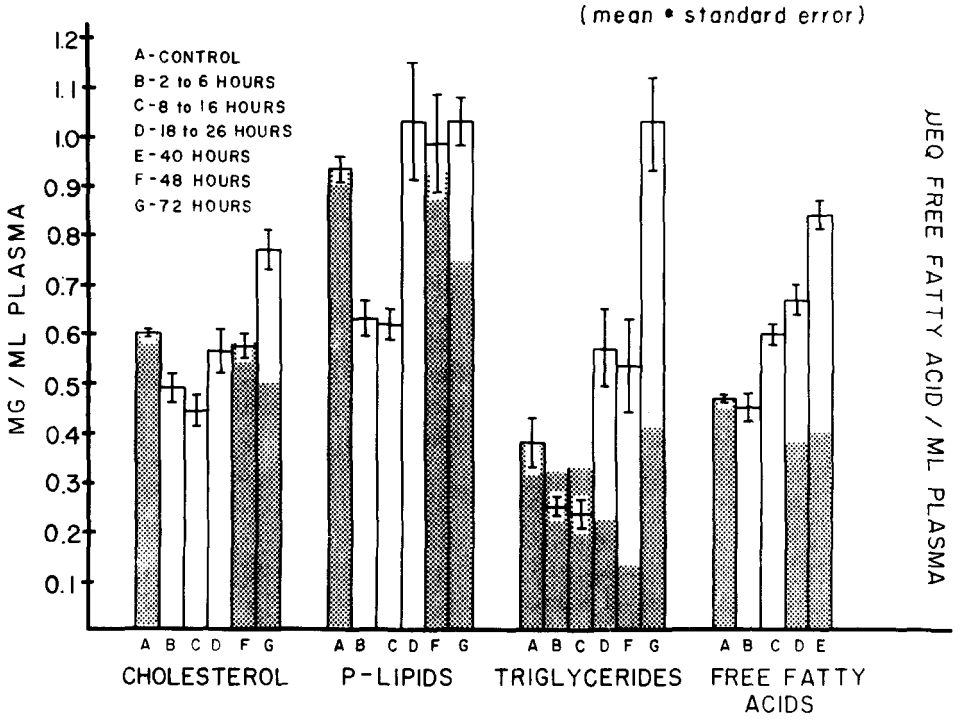

FIG. 1. Changes in lipid fractions of rat plasma following CCl_4 administration. The stippled areas represent control values. Time *A* values were determined using fed animals. Stippled areas at other times indicate the effects of peanut oil and starvation. CCl_4 administered orally, 2.5 ml/kg in peanut oil. Cholesterol: *B*, *C*, and *G* significantly different from control, $P < 0.01$. Phospholipids: *C* and *D* significantly different, $P < 0.001$. Triglycerides: all times significantly different, $P < 0.05$. Free fatty acids: *C*, *D*, and *E* significantly different, $P < 0.001$. In each group $n \geq 6$. Free fatty acids estimated as microequivalents per milliliter of plasma; all other parameters as milligrams per milliliter.

trates that neither the early decreases nor the subsequent increases are due to effects of peanut oil or starvation.

Liver lipid changes are shown in Fig. 3. Cholesterol levels exhibited a minor progressive increase. The apparent decreases in liver phospholipid concentration may reflect the increased liver weight due to the greater triglyceride content after CCl_4. Triglyceride levels were elevated at the earliest time measured (<1 hr, see Fig. 4), and continued to increase for at least 2 days; although they were lower at 72 hr than at 48 hr, they were still above the levels in starved, peanut oil controls. Total lipid reflected the triglyceride changes. Fig. 4 illustrates in greater detail the dissociation of the effects of CCl_4 and peanut oil from those of peanut oil alone.

A dose–response relationship of the liver triglyceride accumulation at 20 hr is presented in Fig. 5. Doses of 0.31, 0.62, 1.25, 2.5, and 5.0 ml/kg CCl_4 were administered. It can be seen from the graph that 0.31 ml/kg, or one-eighth of the usual dose, nearly doubled the liver triglyceride compared with the control. A dose of 5.0 ml/kg elicited no further increase over the levels obtained with 2.5 ml/kg.

TABLE 1 EFFECT OF DRUGS ON THE CCl_4-INDUCED HEPATIC TRIGLYCERIDE INCREASE

Drug	Regimen	
	Peanut Oil	CCl_4
None	11.1 ± 0.07 (33)	69.5 ± 2.1 (61)
Guanethidine*	7.0 ± 1.0 (5)	39.2 ± 3.7 (6)
β-TM10	8.1 ± 1.0 (7)	51.4 ± 1.7 (5)
Trimethidinium†	11.5 ± 2.8 (10)	79.5 ± 4.6 (14)

Values are expressed as milligrams of triglyceride per gram liver (wet weight), mean ± standard error. Animals were sacrificed 20 hr after administration of CCl_4 or peanut oil. No. of animals in parentheses.

* Blocks release of catecholamines.

† Ganglion-blocking agent.

Effects of Various Treatments on Response to CCl_4

Twenty hours after a dose of CCl_4, 2.5 ml/kg, liver triglyceride was 69.5 compared with 11.1 mg/g in the controls (Table 1). Pretreatment of the rat with guanethidine or with β-TM10 reduced the response ($P < 0.001$). Both agents reduced the liver triglyceride concentration in the controls ($P < 0.001$). Pretreatment with trimethidinium bismethosulfate, however, enhanced the

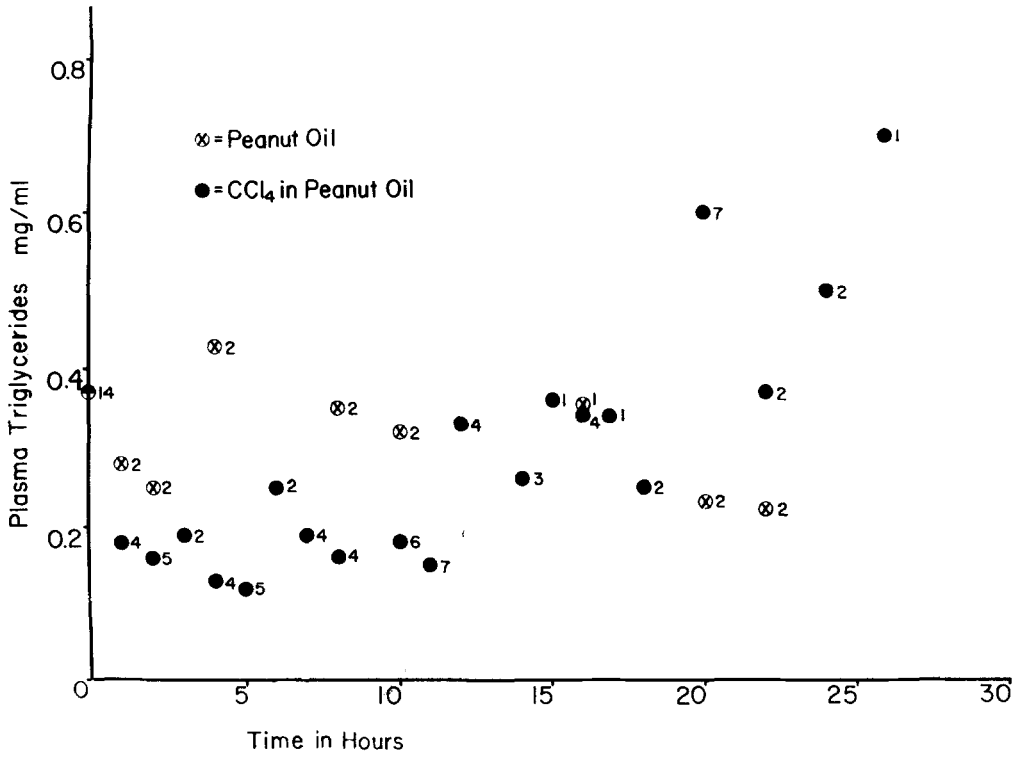

FIG. 2.. Changes in triglyceride levels in rat plasma following oral CCl_4 administration (2.5 ml/kg in peanut oil). The value on the ordinate is the mean of 14 untreated controls. All other values are means for the number of animals indicated.

effect of CCl_4 ($P < 0.05$). Trimethidinium administration did not alter hepatic triglyceride concentrations in control animals.

Spinal cord transection proved more effective than any of the drug treatments in preventing the response to CCl_4 (Table 2). Since cord section was effective to about the same degree against both orally administered and inspired CCl_4, it is assumed that the operation did not reduce lipid accumulation by altering intestinal absorption of the CCl_4. It was noted that cord section did not reduce triglyceride levels of control rats; rather, cord-sectioned animals receiving only peanut oil had higher liver triglyceride contents than did unoperated rats receiving peanut oil. Cord section likewise reduced the extent of total hepatic lipid accumulation in CCl_4-treated animals.

TABLE 2 EFFECT OF SPINAL CORD TRANSECTION ON CCl_4-INDUCED CHANGES IN HEPATIC TRIGLYCERIDE AND TOTAL LIPID

Treatment	Triglyceride*		Total Lipid*	
	Intact	Cord Section	Intact	Cord Section
None	9.6 ± 0.8 (26)	8.2 ± 1.2 (12)	42 ± 2 (5)	39 ± 2 (5)
Inhaled CCl_4†	53.5 ± 4.2 (5)	16.8 ± 2.4‡ (5)		
Peanut oil	11.1 ± 0.7 (33)	15.7 ± 1.5 (11)	38 ± 1 (6)	
Oral CCl_4 (in peanut oil)	69.5 ± 2.1§ (61)	31.3 ± 1.3‡,§ (43)	101 ± 5 (6)	52 ± 4 ‡,‖ (5)

* Milligrams per gram wet weight (mean ± SE).
† Animals were sacrificed 24 hr after the end of a 3 hr exposure to 6,000 ppm CCl_4.
‡ $P < 0.001$ compared to intact, CCl_4-treated group.
§ 20 hr after dose of 2.5 ml/kg.
‖ 20 hr after dose of 1.5 ml/kg.

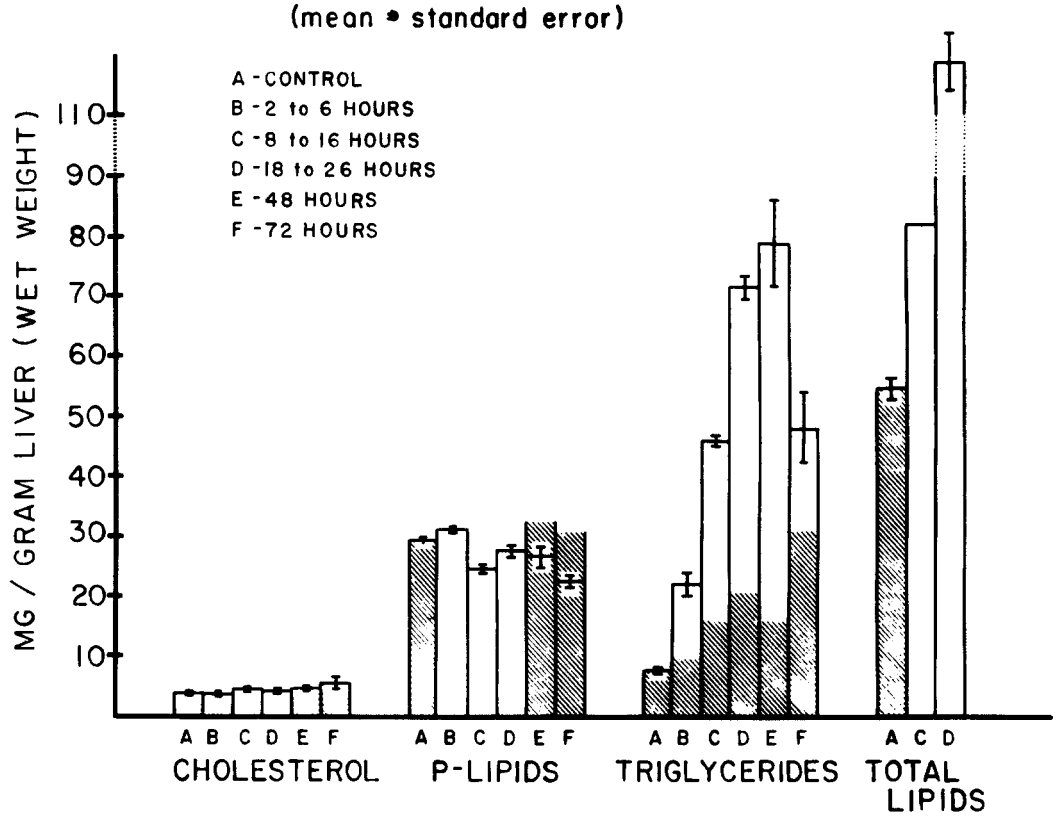

FIG. 3. Changes in lipid fractions of rat liver following oral CCl_4 administration (2.5 ml/kg in peanut oil). The cross-hatched areas indicate the effects of peanut oil alone. Triglycerides significantly different from control, $P < 0.001$, at all times. Total lipids significantly different from control, $P < 0.001$, at *C* and *D*. In each group n $\geq$ 6.

Table 3 shows that spinal cord transection prevented the CCl_4-induced increase in plasma FFA. The operation per se did not lower the plasma FFA levels of rats receiving only peanut oil. Although cord section was ineffective in preventing the early (12 hr) fall in plasma phospholipid, the treatment partially eliminated the CCl_4-induced decline in plasma cholesterol.

Prior bilateral adrenalectomy was the most effective treatment for preventing the CCl_4-induced accumulation of triglycerides and of total lipids (Table 4). Liver triglyceride levels of adrenalectomized control animals were lower than those of unoperated animals; however, total lipid levels were not altered by adrenalectomy. Plasma cholesterol was elevated in adrenalectomized rats and the plasma cholesterol levels decreased in both intact and adrenalectomized animals after CCl_4 administration. The plasma FFA increase seen in intact rats after CCl_4 administration did not occur in adrenalectomized rats. Adrenalectomy failed to prevent the CCl_4-induced fall in plasma phospholipid.

Since the effect of adrenalectomy could be attributed to removal of either cortical or medullary hormones, the relative roles of the two factors were analyzed further. Plasma corticosterone measurements indicated that both hypophysectomized and adrenalectomized animals had markedly reduced circulating corticoid levels and did not show any rise in corticoids after CCl_4 administration (Table 5). Thus hypophysectomized animals could be used to measure the lipid response in the absence of corticoids. There was less total lipid in the liver of hypophysectomized animals treated with CCl_4 than in intact CCl_4-treated rats (Table 6). However, the suppression of lipid accumulation was not as marked as it was following adrenalectomy. The total liver lipid was measured in adrenalectomized animals which had been given 1.5 mg/kg epinephrine (in oil, subcutaneously) prior to CCl_4 administration. The liver lipid after this treatment was less than that found following adrenalectomy alone. Higher epinephrine doses were not tested.

Splanchnicectomy and the administration of hydrocortisone to adrenalectomized animals were the techniques utilized to study the influence of adrenal corticoids in the absence of the medulla. Epinephrine secretion is markedly reduced following transection of the splanchnic nerves. Triglyceride accumulation following CCl_4 was significantly decreased in splanchnicectomized

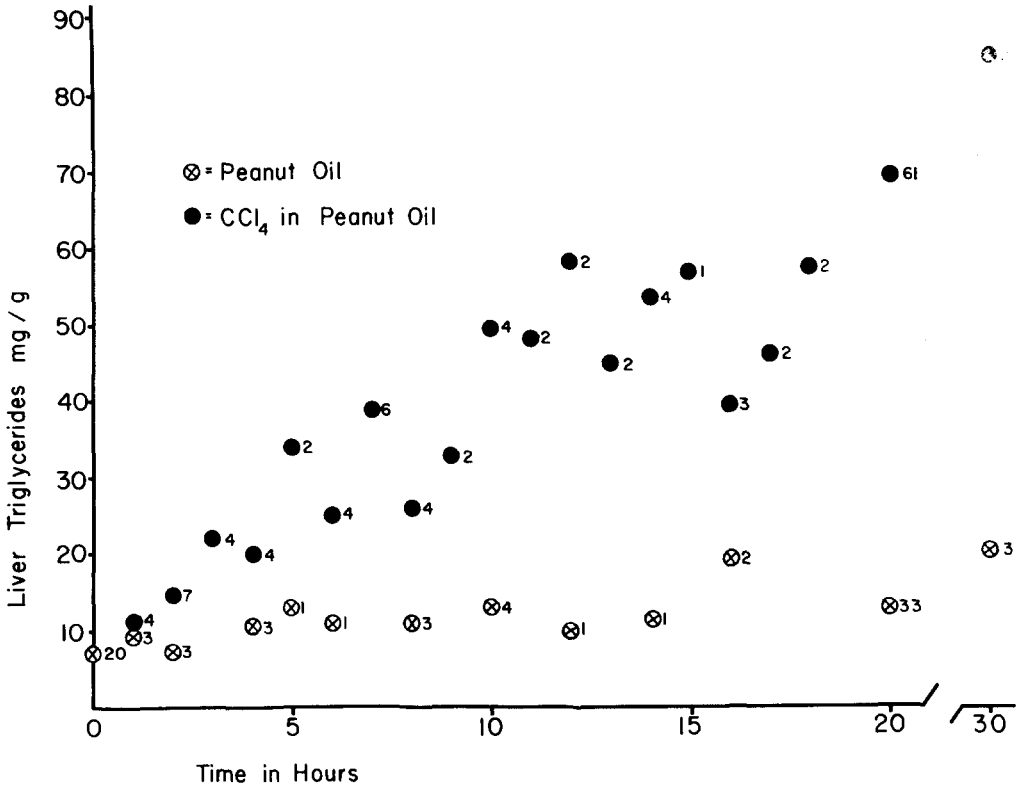

FIG. 4. Changes in triglyceride levels in rat liver following oral CCl_4 administration (2.5 ml/kg in peanut oil). Peanut oil controls at zero time are shown on the ordinate. Values are means for the number of animals indicated.

rats and in adrenalectomized rats which had been given hydrocortisone. The decrease was not as great as in adrenalectomized rats. The total lipid accumulation induced by CCl_4 was somewhat less in adrenalectomized and hydrocortisone-treated rats than in intact animals.

DISCUSSION

The hepatic lipid level is affected by a number of factors. These include the release of FFA from adipose tissue, uptake of FFA by the liver, hepatic FFA metabolism (synthesis, oxidation, incorporation into triglycerides, and phospholipid), and release of lipid from the liver as lipoprotein. Accumulation of abnormal amounts of lipid in the liver could conceivably result from a change in the rate of one or more of these steps. Certain hormones and neurohumoral agents produce adipokinetic effects and can increase liver triglyceride (27–29). Thus when a substance foreign to the body increases liver lipid, the action may be an indirect one through one of the endogenous factors.

In earlier studies it was shown that the CCl_4-induced lipid accumulation could be prevented by interference with the sympathetic nervous system (4, 9). Thus it was postulated that the lipid accumulation might be mediated through the catecholamine release resulting from CCl_4 administration. The present study examines this hypothesis further.

Comparison of Effects of Catecholamines and CCl_4 on Lipid Metabolism

One approach was to compare the changes produced by catecholamines with those resulting from CCl_4. Catecholamines stimulate adipose tissue lipolytic activity (30) and cause release of FFA from adipose tissue (19, 28,31–33. CCl_4, like catecholamines, elicits an increase in plasma FFA. However, the FFA increase following catecholamines is immediate, whereas it is delayed following CCl_4. Our results indicate no increase until 8 hr after CCl_4 administration, although Rees and Shotlander (34) have reported finding an elevation as early as 5 hours after treatment. It should be mentioned that these are all measurements of concentration only, and do not reflect possible changes in turnover rate. Maling et al. (6) have proposed that CCl_4 may increase FFA uptake by the liver. In the presence of increased uptake by the liver, plasma FFA levels might fail to reflect an accelerated release of FFA from adipose tissue. Hypophysectomy, adrenalectomy, and spinal cord transection prevented the FFA increase following CCl_4. This would

TABLE 3 EFFECT OF SPINAL CORD TRANSECTION ON THE CCl_4-INDUCED CHANGES IN PLASMA PHOSPHOLIPIDS, CHOLESTEROL, AND FFA

Treatment	Phospholipid*	Cholesterol*	FFA†
None	0.87 ± 0.08 (9)	0.61 ± 0.02 (9)	0.38 ± 0.01 (4)
Cord section	0.93 ± 0.05 (6)	0.64 ± 0.04 (6)	0.42 ± 0.02 (4)
CCl_4	0.71 ± 0.08‡ (8)	0.48 ± 0.05‡ (8)	0.64 ± 0.04 (6)
Cord section + CCl_4	0.63 ± 0.12 (9)	0.56 ± 0.03 (9)	0.45 ± 0.02§ (5)

* Milligrams per milliliter (mean ± SE). Animals sacrificed 12 hr after CCl_4 administration (2.5 ml/kg).
† Microequivalents per milliliter plasma (mean ± SE). Animals sacrificed 20 hr after CCl_4 administration (1.5 ml/kg).
‡ $P < 0.01$ compared to control group.
§ $P < 0.01$ compared to intact rats receiving CCl_4.

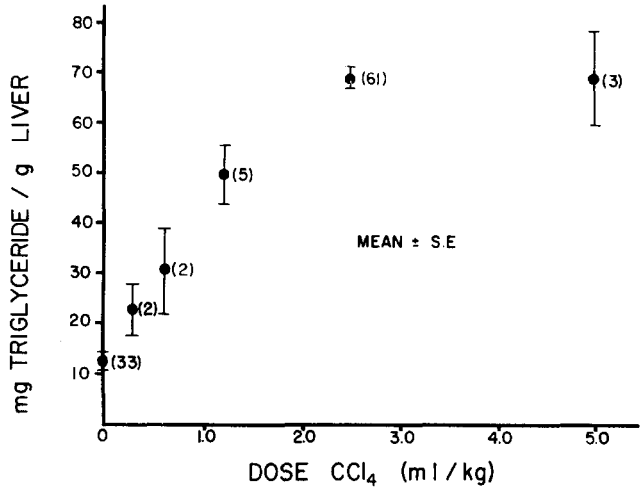

FIG. 5. Effect of various doses of CCl_4 on the triglyceride content of rat liver. Value on ordinate is the mean of the peanut oil controls. All doses of CCl_4 were given orally in 1 : 1 mixture with peanut oil.

be expected if the response is mediated through catecholamines, since adrenal cortical steroids are required for the FFA release produced by catecholamines (35–37). The relative effectiveness of the three procedures for preventing lipid accumulation and blocking the FFA increase was not the same. None of the procedures completely blocked lipid accumulation in the liver. These two factors make it unlikely that the catecholamine-mediated increase in FFA which occurs can account for the entire lipid accumulation.

Previous studies in our laboratory showed that depression of fatty acid oxidation is a late manifestation of CCl_4-induced liver damage (3). Thus we can conclude that depressed oxidation is not a factor in the early lipid changes following CCl_4 administration in the rat. Moreover, epinephrine may stimulate fatty acid oxidation in the liver (38).

There is no convincing evidence for increased triglyceride synthesis resulting from the action of either catecholamines or CCl_4. Depressed secretion of triglyceride from the liver has been shown to develop following CCl_4 (5, 6, 39, 40). The mechanism has not been elucidated, but it may be related to depressed carrier protein synthesis (8, 41). Heimberg and Fizette (42) have shown that norepinephrine can depress release of triglyceride from the liver. The depressed release of triglyceride after CCl_4 could thus be catecholamine-mediated, but it need not be, since depression of triglyceride release can be produced in the isolated liver by CCl_4 (43). It has not been ascertained whether the decreased triglyceride

TABLE 4 EFFECT OF ADRENALECTOMY ON THE CCl_4-INDUCED CHANGES IN LIVER AND PLASMA LIPID

Treatment	Liver		Plasma		
	Triglyceride*	Total Lipid*	Phospholipid†	Cholesterol†	FFA‡
Peanut oil					
Intact	11.1 ± 0.7 (33)	38 ± 1 (6)	0.87 ± 0.08 (7)	0.59 ± 0.02 (7)	0.38 ± 0.01 (4)
Adrenalectomized	5.5 ± 0.7§ (17)	40 ± 1 (4)	1.28 ± 0.20 (6)	0.74 ± 0.05 (6)	0.44 ± 0.02 (3)
CCl_4					
Intact	69.5 ± 2.1 (61)	101 ± 5 (6)	0.71 ± 0.08 (8)	0.44 ± 0.05 (7)	0.64 ± 0.04 (6)
Adrenalectomized	15.1 ± 2.0 (9)	58 ± 2 (6)	0.68 ± 0.07 (9)	0.61 ± 0.02 (9)	0.45 ± 0.02 (4)

* Milligrams per gram wet weight (mean ± SE). Animals sacrificed 20 hr after CCl_4 administration (2.5 ml/kg).
† Milligrams per milliliter plasma (mean ± SE). Animals sacrificed 12 hr after CCl_4 administration (2.5 ml/kg).
‡ Microequivalents per milliliter plasma (mean ± SE). Animals sacrificed 20 hr after CCl_4 administration (2.5 ml/kg).
§ $P < 0.001$ compared with intact rats.

TABLE 5 EFFECTS OF CCl_4, ADRENALECTOMY, AND HYPOPHYSECTOMY ON PLASMA CORTICOSTERONE*

Treatment	Control	CCl_4
None	20.1 ± 7.7 (6)	
Peanut oil	29.4 ± 1.8 (5)	51.6 ± 5.9† (4) 57.3 ± 1.7‡ (4)
Adrenalectomy + peanut oil	2.5 ± 0.5§ (4)	2.5, 1.1‡,§
Hypophysectomy + peanut oil	3.5 ± 0.4§ (3)	3.1 ± 0.9‡,§ (3)

* Micrograms corticosterone per 100 ml plasma (mean ± SE).
† 1.5 ml CCl_4/kg.
‡ 2.5 ml CCl_4/kg.
§ $P < 0.001$ compared with untreated controls.

TABLE 6 EFFECTS OF CCl_4 ON LIVER AND PLASMA LIPIDS IN VARIOUSLY OPERATED ANIMALS

Treatment	Liver Triglyceride*	Total Liver Lipid*	Plasma FFA†
Peanut oil			
Intact	11.1 ± 0.3 (33)	38 ± 1 (6)	0.38 ± 0.01 (4)
Adrenalectomy	5.5 ± 0.7‡ (17)	41 ± 1 (4)	0.44 ± 0.02 (3)
Hypophysectomy	—	41 ± 1 (5)	0.44 ± 0.03 (4)
Adrenalectomy + epinephrine	7.45	44 ± 5 (4)	—
Splanchnicectomy	3.2, 7.5	—	—
Adrenalectomy + hydrocortisone	9.2, 12.3	41 ± 2 (4)	—
CCl_4§			
Intact	69.5 ± 2.1 (6)	101 ± 5 (6)	0.64 ± 0.04 (6)
Adrenalectomy	15.1 ± 2.0‖ (9)	58 ± 2 (6)	0.45 ± 0.02 (4)
Hypophysectomy	—	81 ± 2 (5)	0.32 ± 0.01 (4)
Adrenalectomy + epinephrine	16.1, 16.1	46 ± 2 (4)	—
Splanchnicectomy	34.4 ± 5.0‖ (6)	—	—
Adrenalectomy + hydrocortisone	43.0 ± 6.2 (3)	73 ± 8¶ (4)	—

* Milligrams per gram wet weight (mean ± SE).
† Microequivalents per milliliter plasma (mean ± SE).
‡ $P < 0.001$ compared with intact animals given peanut oil.
§ 20 hr after CCl_4 administration (2.5 ml/kg).
‖ $P < 0.001$ compared with intact animals given CCl_4.
¶ $P < 0.02$ compared with intact animals given CCl_4.

release following CCl_4 persists in cord-sectioned or adrenalectomized animals.

Epinephrine and norepinephrine eventually elevate plasma phospholipid, cholesterol, and triglyceride levels. After CCl_4 administration, similar changes occur. With both catecholamines and CCl_4, these responses occur later than the increase in FFA and liver triglyceride. CCl_4 elicits an early decrease in plasma triglycerides, phospholipid, and cholesterol which is not seen with catecholamines. These early changes could conceivably result from reduced carrier protein synthesis (8, 41). Although the decrease in plasma cholesterol following CCl_4 could be partially prevented by adrenalectomy, that in plasma phospholipid persisted in both adrenalectomized and cord-sectioned animals.

Evidence from Studies Involving Interference with Sympathetic Nervous System Function

Guanethidine and β-TM10 are known to block release of catecholamines, particularly norepinephrine. The agents reduced the triglyceride accumulation produced by CCl_4 administration. They also slightly decreased the liver triglyceride in control animals. This could be ascribed to blockade of a tonic release of catecholamines which serves to maintain normal FFA and triglyceride levels (44). Interestingly, exogenous epinephrine is less effective in releasing FFA following guanethidine than it is in the untreated animal, suggesting that the normal mechanism of FFA release from adipose tissue is more complex than previously thought, and involves at least an additional synaptic connection and possibly a reflex pathway.

The possibility also exists that these agents may, in some way not involving catecholamine release, block lipid transport or synthesis. The treatments could thus preclude all lipid accumulation, and these experiments would not elucidate actual mechanisms of the CCl_4 effect. The cause of the increased triglyceride levels following CCl_4 + trimethidinium is not readily apparent. No other ganglion-blocking agents were tested; thus it is not known whether the effect on triglyceride would be a property common to this class of agents or one peculiar to the drug employed.

The accumulation of triglyceride and total lipid in the liver, the increase in plasma FFA, and the fall in plasma cholesterol normally seen following CCl_4 were markedly inhibited in spinal cord-sectioned animals. Control hepatic triglyceride levels were not lowered by cord section. These results suggest that catecholamines are playing a more than supportive role in the lipid response to CCl_4. It is unlikely that absorption of CCl_4 was decreased by cord section, since inhaled CCl_4 also produced less lipid increase in cord-sectioned animals.

The Role of the Adrenal Cortex in the CCl_4-Induced Fatty Liver

It has previously been shown that the adrenal cortex is essential for epinephrine-induced lipid accumulation, as well as for fatty livers produced by exogenous agents (14–18). Adrenal cortical steroids may be essential for FFA release (35–37), although other effects of corticoids on lipid metabolism have been proposed (45, 46). The present results suggest that corticoids are essential for the induction of fatty liver by CCl_4 since it does not occur in the adrenalectomized rat and is reduced in the hypophysectomized animal. The adrenalectomized rat in which corticoids are replaced can accumulate lipid in response to CCl_4.

The stress of CCl_4 administration causes ACTH release. Since ACTH can mobilize FFA, the role of this hormone in production of the fatty liver should be considered. However, it does not appear that ACTH is a major factor since hypophysectomy, which would eliminate not only ACTH, but other lipid-mobilizing factors as well, does not markedly reduce the CCl_4-induced lipid accumulation. Greater reduction is seen in splanchnicectomized animals and even more in cord-sectioned animals, suggesting that catecholamines play a more prominent role.

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