and the inhibitor is characterized as immunoglobulin M (IgM) which does not incorporate complement in its union with antigen.

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Influence of Carbon Tetrachloride, Vitamin E, and Protein Upon Liver Slice Respiratory Activity (33436)

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The relation of nutritional state to certain toxic manifestations caused by carbon tetrachloride (CCl_4) has been the subject of several investigations. For instance, lowering dietary vitamin E or protein has been shown to increase the susceptibility of rats to the lethal or necrogenic effects of CCl_4 (1–3), but a protein-free diet was demonstrated by McLean and McLean (4, 5) to have the reverse effect.

Although several studies have been concerned with the effects of CCl_4 intoxication on *in vitro* liver carbohydrate metabolism (6–8), little attention has been paid to the influence of diet therein. Therefore, studies were initiated to determine how different dietary treatments, particularly those known to afford some protection against the gross effects of CCl_4, influence the animal's response to this toxic chemical at the cellular level. The influence of vitamin E and of protein level on some aspects of *in vitro* liver carbohydrate metabolism as affected by CCl_4 administered *in vivo* was investigated.

Materials and Methods. Animal treatments. Weanling male albino rats of the Osborne-Mendel strain[1] were maintained on a purified diet of the following composition (%): vitamin-free casein,[2] 18; sucrose, 63.8; stripped lard, 9; cod liver oil, 1; Jones-Foster salt mixture, 4; vitamin E-free vitamin mixture (9), 4; and choline chloride, 0.2. Usually, half of the rats were fed this vitamin E-deficient diet and the other half received the same diet supplemented with 100 mg of DL-alpha-tocopherol acetate/kg of diet. In an-

[1] Division of Nutrition colony, Food and Drug Administration, Washington, D.C.

[2] General Biochemicals, Chagrin Falls, Ohio.

[3] New England Nuclear Corporation, Boston, Massachusetts. The specific activity of D-glucose-^{14}C (uniformly labeled) was 8.8 mCi/mmole.

[4] Reagents obtained in Biochemica Test Combinations (C. F. Boehringer & Soehne GmbH., Mannheim, Germany).

other experiment, the casein level of the diet was lowered to 10% and the sucrose content was increased proportionately, while the concentration of all other dietary constituents remained the same. Feeding the vitamin E-deficient diets for 5 weeks produced a vitamin E-deficient state in the appropriate test animals according to the following criteria: (a) a positive dialuric acid test on tail blood samples (10); and (b) a consistent decline in oxygen uptake ("respiratory decline") by liver slices (11).

After 5 weeks of feeding, both groups of rats were fasted for 24 hr and then given either 2 ml of CCl_4–olive oil (1:1)/kg of body weight, or 1 ml of olive oil/kg of body weight, by gastric intubation. The rats were fasted for another 24 hr, then were decapitated and exsanguinated. Livers were rapidly excised and placed in ice-cold 0.9% saline for washing. A portion of liver was preserved in buffered formalin for histological examination.

In vitro *procedures.* Slices of 0.5 mm thickness from chilled livers were equally distributed among Warburg flasks so that each flask contained 100–200 mg of tissue. The incubation medium was Krebs–Ringer phosphate solution (12), the $CaCl_2$ content of which was reduced by 20% to prevent precipitate formation in the chilled medium; the pH was 7.05–7.10. Half of the flasks also contained 0.011 *M* D-glucose with one μCi of uniformly labeled D-glucose-$^{14}C^3$ added from the sidearm just prior to oxygen uptake measurements. Oxygen uptake from an air atmosphere at 37° was observed during 25 and 50 min of incubation. Evolved $^{14}CO_2$ was trapped in 0.2 ml of 30% NaOH placed in the center well. The final volume of the incubation medium per flask was 3.0 ml.

After 50 min of incubation, the reaction in each flask was terminated by acidifying the medium with 0.25 ml of 6*N* H_2SO_4. When the flasks had been shaken for an additional hour in the Warburg bath to insure complete absorption of evolved CO_2, the center well contents were removed, diluted with carrier bicarbonate, and converted to $Ba^{14}CO_3$, which was washed until neutral, plated, and counted in a Nuclear-Chicago Omniguard

TABLE I. Mortality, Liver Size, and Liver Fat of Rats Given a Single Dose of CCl_4.[a]

	Dietary casein (%)	+ Vitamin E[b]		- Vitamin E	
		Control	CCl_4	Control	CCl_4
Deaths during 24 hr following dosage	18	0/8	1/15	0/21	0/16
	10	0/8	0/13	0/11	4/17
Liver size (g/100 g of body wt.)	18	3.52 ± 0.11 (6)[c]	4.92 ± 0.19[d'] (10)	3.51 ± 0.07 (6)	4.71 ± 0.10[e] (12)
	10	3.58 ± 0.11 (5)	4.68 ± 0.14[e] (11)	3.50 ± 0.10 (8)	5.11 ± 0.15[e'] (13)
Total liver fat (g/100 g dry wt. of liver)	18	22.9 ± 1.0 (6)	35.8 ± 1.3[e] (11)	27.6 ± 2.3 (7)	37.2 ± 2.6[e] (14)
	10	**23.6 ± 2.1 (5)**	**34.7 ± 2.6[e] (10)**	25.2 ± 1.3 (8)	37.2 ± 1.8[e] (13)

[a] Rats fed diet 5 weeks, fasted 24 hr, dosed, fasted another 24 hr, then decapitated. Carbon tetrachloride-dosed rats received 2 ml of CCl_4–olive oil (1:1)/kg of body weight via gastric intubation; others received only olive oil.

[b] One hundred mg of DL-alpha-tocopherol acetate/kg of diet.

[c] Mean ± SE; number of rats/group in parentheses.

[d] Superscripts refer to significant ($p < 0.05$) effects related to CCl_4 dosage (') or dietary casein level (').

TABLE II. Effects of CCl_4, Dietary Vitamin E, and Dietary Casein Level on Oxygen Consumption by Rat Liver Slices.[a]

Dietary casein (%)	Incubation media[b]	Oxygen uptake (μl/mg of dry wt. tissue/50 min)			
		+ Vitamin E[c]		— Vitamin E	
		Control	CCl_4	Control	CCl_4
18	Endogenous	2.53 ± 0.21 (7)[d]	1.52 ± 0.08[fe](6)	2.61 ± 0.29 (6)	1.25 ± 0.22[f](6)
	Glucose	2.67 ± 0.18 (7)	1.57 ± 0.08[f] (6)	2.72 ± 0.27 (6)	1.33 ± 0.20[f](6)
10	Endogenous	1.75 ± 0.10[k](4)	1.18 ± 0.09[fk](4)	2.10 ± 0.14 (6)	1.64 ± 0.16 (6)
	Glucose	1.72 ± 0.07[k](7)	1.29 ± 0.10[f] (7)	1.98 ± 0.16[k](8)	1.75 ± 0.15[e](8)

[a] See Table I.

[b] Modified Krebs–Ringer Phosphate medium ± D-glucose (0.011 *M*).

[c] One hundred mg of DL-alpha-tocopherol acetate/kg of diet.

[d] Mean ± SE; number of rats in parentheses.

[e] Superscripts refer to significant effects ($p < 0.05$) related to CCl_4 dosage ([f]), vitamin E deficiency ([e]), or dietary casein level ([k]).

low-beta counter at an efficiency of 13%. The counts were corrected for self-absorption. The tissue slices from the flasks were dried overnight at 110° and weighed. Several samples of acidified media were stored frozen at —15° until assayed for lactic acid, using lactic dehydrogenase and measuring the increase in UV absorption at 340 mμ due to NAD^+ reduction.[4] Net production of lactic acid was calculated as the difference between values in the absence and presence of added glucose.

Livers were analyzed for dry weight and total fat (13). All results were analyzed for significant differences ($p = < 0.05$) by the Student's *t* test (14).

Results. Gross effects. Rats fed low casein, vitamin E-deficient diets showed an increase in susceptibility to the lethal effects of CCl_4 when compared to those given diets adequate in vitamin E and/or protein content (Table I). Livers from all CCl_4-dosed rats had a mottled appearance due to a combination of fattiness and hemorrhage. Significant increases in liver-to-body weight ratios and in liver fat due to CCl_4 were unaffected by dietary vitamin E or casein levels. Histological examination of liver sections, using hematoxylin and eosin stains, showed moderate necrosis and some fat-filled cells only in livers from CCl_4-dosed rats; no differences due to vitamin E deficiency were noticeable.

Oxygen uptake. Oxygen uptake by rat liver slices was lowered by CCl_4 dosage (Table II); this effect does not appear to be related to the presence or absence of vitamin E in the diet. The decrease due to CCl_4 was of greatest magnitude in the animals on 18% casein, and least in the 10% casein, vitamin E-deficient group, indicating no increased susceptibility due to low dietary protein. Oxygen uptake was unaffected by adding glucose to the incubation medium.

$^{14}CO_2$ and lactic acid production. Table III shows data on the evolution of $^{14}CO_2$ derived from the metabolism of uniformly labeled D-glucose-^{14}C by liver slices. In rats dosed with CCl_4, $^{14}CO_2$ production was approximately double that of the control value in all cases; it was not significantly affected by vitamin E status or dietary casein level. It is important to note that exogenous glucose was metabolized to a very small extent, calculated to be less than 0.1% according to these $^{14}CO_2$ studies, which is in agreement with the finding that added glucose did not increase oxygen uptake. For example, in the 18% casein, vitamin E-sufficient, glucose control, 3.66 mμmoles of glucose–carbon/mg of dry weight was recovered as CO_2, compared to 119 mμmoles of oxygen taken up/mg of dry weight.

Table III also shows a sizable net production of lactic acid in the presence of exogenous glucose by liver slices from all CCl_4-dosed rats. This is not seen for liver

TABLE III. Effect of CCl_4 on Production of $^{14}CO_2$ and Lactic Acid from Glucose by Rat Liver Slices.[a]

Dietary vitamin E[b]	CCl_4 dosage	$^{14}CO_2$ (cpm/mg of dry wt. tissue/50 min)		Lactic acid[c] (μg/mg of dry wt. tissue/50 min)
		Dietary casein (18%)	Dietary casein (10%)	
+	—	5.30 ± 0.71 (7)[d]	5.93 ± 0.46 (7)	−0.49 ± 0.22 (4)
+	+	10.06 ± 1.21[f,e] (6)	11.09 ± 1.37[f] (7)	2.97 ± 0.62[f] (4)
—	—	5.54 ± 0.57 (6)	5.35 ± 0.68 (8)	−1.69 ± 0.34[e] (5)
—	+	9.54 ± 0.93[f] (6)	11.47 ± 0.94[f] (8)	2.69 ± 0.60[f] (5)

[a,b] See Table I. Incubation medium was a modified Krebs–Ringer phosphate solution containing D-glucose (0.011 *M*) and uniformly-labeled D-glucose-^{14}C (1 μCi).

[c] Values are for net production which is the difference between values in the absence and presence of D-glucose. Data from both 18% and 10% casein rats were combined (see text).

[d] Mean ± SE; number of rats in parentheses.

[e] Superscripts refer to significant effects ($p < 0.05$) related to CCl_4 dosage (^{f}) or vitamin E deficiency (^{e}).

slices from control rats, nor were there significant differences due to vitamin E status. Values represent the combined data from rats receiving either 10% or 18% casein, since all rats from both these groups responded the same to CCl_4 intoxication as far as the production of lactic acid by liver slices was concerned.

Discussion. Christie and Judah (6) observed that, in rats fed stock diets, oxygen uptake by liver slices decreased 44% at 20 hr after administration of CCl_4 by intubation. Our findings of altered oxygen uptake due to oral dosage with CCl_4 agree in direction and degree with these early observations. However, Hove and Hardin (9) had previously observed a onefold increase in oxygen uptake by liver slices 24 hr after the injection of small amounts of CCl_4 into rats fed a 10% casein diet deficient in vitamin E. As confirmed in our study, Hove (1, 2) had noted that higher casein levels or vitamin E supplementation gave protection against CCl_4 lethality. The only known differences in experimental design between our study and that of Hove and Hardin (9) are that 10% dietary fat was used instead of 20% and that CCl_4 was intubated orally rather than injected subcutaneously.

Hove (15) suggested that the increased oxygen uptake due to CCl_4 dosage which he had previously observed may have been caused by catalytic peroxidation of additional unsaturated fat in the tissue, and not by an increased rate of glucose oxidation. Although the data reported here clearly show an increased liver-fat content and a decrease in oxygen uptake by liver slices from rats which received CCl_4, they do not necessarily refute Hove's hypothesis. One possible explanation for the discrepancy in findings might be related to differences in the quantity or types of fat produced in the livers in response to administered CCl_4 in the two studies. Hove and Hardin (9) observed a larger liver-fat differential due to CCl_4 than that reported in this study, and if this differential is constituted primarily by lipids containing unsaturated fatty acids, an accelerated oxidation of unsaturated fatty acids might conceivably more than compensate for the oxygen uptake depression due to an impaired tricarboxylic acid cycle (6, 7).

The increased $^{14}CO_2$ production from uniformly labeled D-glucose-^{14}C which we observed in liver slices from CCl_4-dosed rats may be due to (i) an increased receptivity of the liver cell to glucose entry; or (ii) a greater rate of metabolism of intracellular glucose through the pentose phosphate pathway; or (iii) a combination of (i) and (ii). Morrison *et al.* (7) detected elevated specific activities of cell sap enzymes involved in glycolysis and the pentose phosphate pathway in degenerating liver cells from CCl_4-dosed rats. The CCl_4-induced modification in our animals must have been more than sufficient to offset a loss of $^{14}CO_2$ pro-

duction by the impaired TCA cycle. Weldon and co-workers (8) did not find that CCl_4 had an appreciable effect upon CO_2 production from uniformly labeled glucose-^{14}C. However, they investigated the effects of exposing liver slices to CCl_4 *in vitro*; this would bypass a possible *in vivo* "activation" of CCl_4 (4, 5).

It might appear that the CCl_4 effects of decreased oxygen uptake and increased $^{14}CO_2$ production are contradictory. The effect on oxygen uptake is primarily concerned with endogenous respiration, for the addition of glucose to the medium did not significantly alter either oxygen uptake or the CCl_4 effect. However, the effect on $^{14}CO_2$ production involves exogenous glucose, which was previously noted as proceeding at an extremely slow rate and not contributing to the overall respiration as measured by oxygen uptake.

The net production of lactic acid in the presence of exogenous glucose seen only in our CCl_4-dosed rats may be explained by increased glycolysis (7) coupled with decreased TCA cycle function (6), allowing the accumulation of lactic acid as a metabolic product of exogenous glucose. Preliminary experiments in our laboratory did indeed show a depressed oxidation of exogenous lactic acid by liver slices from CCl_4-dosed rats, confirming the reports of others that this toxin does alter TCA cycle oxidation.

Although an air atmosphere was used in these studies, the absence of a net production of lactic acid by the control liver slices indicated that they were probably not rendered anaerobic. Another indication that oxygen was not a limiting factor is evident from the fact that the depression of oxygen uptake by CCl_4 in these studies was approximately the same magnitude as reported by Christie and Judah (6) where an oxygen atmosphere was employed. Furthermore, it is unlikely that the glycolytic stimulation due to CCl_4 is dependent upon a reduced oxygen atmosphere since Morrison *et al.* (7) showed an increase in the specific activity of enzymes involved in glycolysis in livers rapidly removed from rats treated with CCl_4.

The protection by dietary vitamin E or adequate protein levels against CCl_4-induced lethality cannot be correlated with its effects on CCl_4-altered carbohydrate metabolism. Carbon tetrachloride-induced decreases in oxygen uptake and increases in $^{14}CO_2$ production by liver slices in the presence of exogenous glucose were mostly unaffected by the vitamin E status or dietary casein levels of rats studied; neither was the accumulation of lactic acid in the incubation media of liver slices from CCl_4-dosed rats significantly altered by these dietary treatments. The increases in liver size and liver fat, which are symptoms typical of CCl_4 intoxication, were also not modified by altered dietary protein or vitamin E. Our studies suggest that the lesion(s) resulting in fatality to rats ingesting low levels of CCl_4 must be either of a more sophisticated nature in carbohydrate metabolism than that which we have measured, not solely dependent upon effects therein, or not related to carbohydrate metabolism at all.

Summary. Vitamin E deficiency and low protein intake, factors reported to accentuate the overt toxicity symptoms of CCl_4 in rats, were investigated for their effects on CCl_4-induced alterations of carbohydrate metabolism by rat liver slices. Rats fed for 5 weeks on purified diets containing 18 or 10% casein with or without added vitamin E were fasted 24 hr, intubated orally with 1 ml of CCl_4 in olive oil per kg of body weight, and then fasted during the subsequent 24 hr. Protection against CCl_4-induced lethality by adequate dietary protein and/or vitamin E was observed. However, the following CCl_4 effects were unaffected by lowering dietary casein or vitamin E levels: (i) increased liver size and fat content; (ii) lowered oxygen uptake and increased production of $^{14}CO_2$ from uniformly labeled glucose-^{14}C by liver slices; and (iii) a net production of lactic acid (seen only with liver slices from CCl_4-dosed rats). Thus it is concluded that in rats, the protection against CCl_4 toxicity by dietary vitamin E or adequate protein levels is not related to any effects on these parameters of CCl_4-altered carbohydrate metabolism.

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Pharmacologic Dissociation of Immunologic Release of Histamine and Slow Reacting Substance of Anaphylaxis in Rats* (33437)

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The immunologic pathways leading to the release of histamine and slow reacting substance of anaphylaxis (SRS-A^{rat}) in the rat can be dissociated in terms of the responsible homolgous immunoglobulins (1,2) and the participating target cells (3,4). Histamine release from rat mast cells is mediated by a heat labile (5), "mast cell sensitizing" (6) or homocytotropic (7) antibody; the immunoglobulin class of the homocytotropic antibody has not yet been established, but it appears to be distinct from the recognized classes, IgG, IgA, and IgM (8, 9). The formation and release of SRS-A^{rat} is mediated by a heat stable antibody of the IgGa class (2) and requires the presence of the polymorphonuclear (PMN) leukocyte (3) but not the peritoneal mast cell.

The present report reveals that these two distinct pathways can be selectively blocked *in vivo* by pharmacologic agents which act after antigen–antibody interaction but prior to the formation and release of the mediators. Diethylcarbamazine (3, 10) and certain structural analogs prevent the immunologic elaboration of SRS-A^{rat} but not of histamine and serotonin, whereas disodium cromoglycate (11) suppresses the antigen-induced release of the amines but does not inhibit the release of SRS-A^{rat}.

Materials. Diethylcarbamazine citrate (1-diethylcarbamyl-4-methylpiperazine) (Hetrazan, Lederle Laboratories, Pearl River, New York) was kindly supplied by Dr. H. G. Lockhard (Lederle Laboratories). Disodium cromoglycate (1, 3-bis[2-carboxychromon-5-yloxy]-2-hydroxypropane) (FPL-670, Fison's Pharmaceuticals, Loughborough, Leicestershire, England) was donated by Dr. J. S. G. Cox. Isonicotinic acid hydrazide and nicotinamide were obtained from Mann Research Laboratories, Inc. (New York). Isopropylisoniazid (Iproniazid) was donated by Hoffman-LaRoche, Inc. (Nutley, New Jersey), and monoethanolamine was acquired from Fisher Scientific Co. (Fairlawn, New Jersey). Cho-

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