Sera of rats fed zein possessed significantly lower concentrations than sera from rats in any other group. Fraction II of rats fed lactalbumin and gluten was significantly higher than that of rats fed casein or zein. A significantly smaller percentage of fraction II was found in sera of zein-fed rats than in the sera of any other group.

Discussion. The importance of amount and proportion of serum proteins and lipoproteins in sera of animals is only partially understood. Diets were formulated similar to those fed by James and El Gindi(6) who reported that protein quality markedly influenced carotene utilization in the rat. Since Erwin, *et al.*(3), and others have found that albumin binds Vit. A and carotene and probably forms a transportation mechanism for these nutrients, it appeared likely that the effect of dietary protein quality was mediated through alteration in albumin content of the sera. However, the effect of dietary protein quality on alteration of albumin proportion in total serum protein could not account for differences observed in carotene utilization by the rat. Unfortunately, total serum albumin per cent was not measured.

Several investigators(7) observed that quantity of dietary protein altered serum cholesterol content. More recently, others(8) found that quality of dietary protein also influenced the level of serum cholesterol. Serum cholesterol has been found to be largely associated with certain lipoprotein fractions and, in rats, with alpha lipoprotein. The results of this study suggest that the influence of dietary protein quality on serum cholesterol is mediated through alteration of quantity and type of serum lipoproteins. That is, dietary protein affects the transportation mechanism for cholesterol and probably does not contribute to its synthesis.

Summary. Serum proteins and lipoproteins were fractionated in sera from male and female rats fed 4 pure protein sources respectively. Almost all lipo- and serum protein fractions were affected by sex. Serum protein fractions did not vary greatly in magnitude but statistically significant changes were attributed to dietary proteins. Type of dietary protein markedly altered the fractions of lipoproteins in the rats.

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Effect of Carbon Tetrachloride on Release of Free Fatty Acids by Rat Adipose Tissue.* (25535)

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Calvert and Brody(1) presented evidence that the mechanism of carbon tetrachloride hepatotoxicity involves a primary action on the central nervous system. According to their hypothesis carbon tetrachloride administra-

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TABLE I. Effect of Subcutaneously Injected Epinephrine on Release of Free Fatty Acids from Rat Epididymal Adipose Tissue.

		No. of animals	Min.*	FFA released†	Significance‡
Exp. I	Controls	6		3.22 ± .51	
	Epinephrine, 1 mg/kg	6	30	5.54 ± .75	$P < .05$
	Idem	6	60	3.52 ± .52	$P > .50$
Exp. II	Controls	6		6.72 ± .76	
	Epinephrine, .1 mg/kg	5	30	9.74 ±1.15	$.1 > P > .05$
	" 1.0 "	7	"	10.50 ± .67	$P < .005$

* Time elapsed from epinephrine inj. to sacrifice of rats.
† Free fatty acids released, μeq/g of adipose tissue in 3 hr, mean ± stand. error.
‡ Statistical significance of data was determined by comparing mean values for experimental groups with mean values of corresponding control group, in Student's t test.

tion evokes a sympathoadrenal discharge. The released catechol amines are believed to cause liver necrosis due to hepatic vasoconstriction and a fatty liver resulting from accelerated release of lipid from adipose tissue. One means by which lipid of adipose tissue is mobilized is as free fatty acids‡ bound to plasma albumin (2). Release of these fatty acids from adipose tissue is influenced by catechol amines. Administration of epinephrine and norepinephrine to human subjects and to dogs results in an elevated level of plasma free fatty acids (3-6). Further, addition of both these amines *in vitro* to the excised epididymal fat pad stimulates release of free fatty acids(7,8). If the epididymal fat pad from epinephrine treated rats exhibited an increased rate of release of free fatty acids *in vitro* this tissue would provide a method for testing whether administration of carbon tetrachloride causes a sympathoadrenal discharge.

Methods. Male albino rats of Sprague-Dawley strain, 113-190 g, previously fasted for 16 hours, were used. The animals were selected so that in each experiment maximum weight range was no more than 35 g. Carbon tetrachloride was administered *via* stomach tube under light ether anaesthesia as a 1:1 mixture with mineral oil, at dose of 0.5 ml of mixture/100 g body weight. Two control groups were employed, except where noted. One group was untreated, except for starvation; the other received 0.25 ml of mineral oil orally under light ether anaesthesia. Epinephrine (adrenalin in oil, Parke, Davis and Co., 1:500) was given subcutaneously at dose of 1 mg or 0.1 mg/kg of body weight. Control animals received 0.1 ml of peanut oil. Animals were decapitated, epididymal fat bodies removed, weighed, and incubated for 3 hours at 36°C in Krebs-Ringer phosphate solution containing 5% albumin, at pH 7.4. Free fatty acids released were determined as previously described(9).

Results. Administration of epinephrine to rats at a dose of 1 mg/kg 30 minutes prior to removal of epididymal fat bodies elicited a significant increase in release of free fatty acids into the medium (Table I). No significant change was observed in free fatty acids released when animals were given the same dose of epinephrine, but killed 60 minutes after injection. When epinephrine was given at a dose of 0.1 mg/kg 30 minutes before sacrifice an increased release of free fatty acids was observed, but the significance statistically was low.

Release of free fatty acids from adipose tissue excised from rats at various times after carbon tetrachloride administration showed no significant changes in comparison with untreated controls or to the control group which received mineral oil alone (Table II).

These time periods were chosen for the following reasons. Increased release of free fatty acids *in vitro* is evident 30 minutes after epinephrine administration. A significant increase in chemically determined hepatic fat is not evident until 3 to 4 hours(10), and the time at which liver fat is increasing maximally is about 9 hours following carbon tetrachloride feeding.

Discussion. Dole(3) and Gordon and

‡ The term free fatty acids is intended to have the same meaning as unesterified or nonesterified fatty acids.

TABLE II. Effect of CCl_4 Poisoning on Release of Free Fatty Acids from Rat Epididymal Adipose Tissue.

Exp.	Treatment of animals	No. of animals	Hr*	FFA released†
1	Control	6		5.12 ± .53
	Mineral oil	6	.5	4.70 ± .43
	CCl_4	6	.5	5.48 ± .64
2	Mineral oil	6	4	7.72 ± .61
	CCl_4	6	4	6.01 ± .76
3	Control	7	9	5.39 ± .58
	CCl_4	8	9	5.29 ± .60

* Time elapsed from CCl_4 administration to sacrifice of rats.

† Free fatty acids released, μeq. g of adipose tissue in 3 hr, mean and stand. error. All experimental mean values were not significantly different from corresponding control values in Student's t test.

Cherkes(4) showed that administration of epinephrine to human subjects was followed by marked, prompt, and short-lived rise in plasma levels of free fatty acids. Gordon and Cherkes(7) subsequently showed that when epinephrine was added to rat epididymal adipose tissue *in vitro* there was an accelerated release of free fatty acids into the incubation medium. The data of Table I extend these observations to show that epididymal adipose tissue excised from rats given epinephrine subcutaneously exhibits an increased rate of release of free fatty acids. These data establish that the change in adipose tissue metabolism induced by epinephrine *in vivo* persists in the excised tissue *in vitro*.

With regard to the suggestion of Calvert and Brody(1) that carbon tetrachloride elicits a discharge of the sympathoadrenal system, it seems reasonable to expect that epididymal adipose tissue of rats poisoned with carbon tetrachloride should exhibit an increased rate of release of free fatty acids *in vitro*. This expectation was not realized (Table II); however, these data cannot be considered as sufficient to disprove the hypothesis. It is possible that in carbon tetrachloride poisoned rats, a small continuous discharge of epinephrine is producing a continuous accelerated release of non-esterified fatty acids from adipose tissue, and that the alteration in metabolism of the adipose tissue does not persist *in vitro*. However, that this is unlikely is shown by the observation of Spitzer and Miller(11) that plasma free fatty acids dropped sharply and remained depressed for 48 hours after subcutaneous administration of carbon tetrachloride to dogs. Thus the interesting and provocative data reported by Calvert and Brody(1) may involve mechanisms other than those suggested by these authors.

Conclusions. 1. Administration of epinephrine to rats increased release of free fatty acid from adipose tissue *in vitro*. 2. Administration of carbon tetrachloride had no effect on release of free fatty acids from adipose tissue *in vitro*. 3. These results are not consistent with the hypothesis that carbon tetrachloride administration elicits a supporting adrenal discharge.

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