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Research Article

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Effects of Carbon Tetrachloride on Albumin Synthesis

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ABSTRACT The effects of CCl_4 on albumin synthesis were studied employing the isolated perfused liver. Carbonate-^{14}C was used to measure newly synthesized albumin. 2.5 ml of CCl_4 was administered by stomach tube 2 hr before perfusion. Albumin synthesis decreased from 36 to 5 mg following the ingestion of CCl_4. Preperfusing the livers for 1 hr before measuring albumin synthesis resulted in an increase to 12 mg, and the addition of tryptophan to a final concentration of 10 mM resulted in a further increase to 19 mg. Cortisone did not protect against the toxic effects of CCl_4 when administered to the donor rabbits. Fasting resulted in an increased sensitivity to CCl_4 and an antioxidant was not effective in protecting against the toxic manifestations of CCl_4.

INTRODUCTION

The metabolism of carbon tetrachloride (CCl_4) yields products that are hepatotoxic and cause fragmentation of the endoplasmic reticulum (1–3) and disruption of ribosomes into subunits (4) with subsequent disengagement of the 40S subunit from mRNA. These changes should result in a rapid loss of the ability of the liver to synthesize albumin, since albumin is produced on a polysome bound to the endoplasmic reticulum (5, 6). Other conditions such as fasting and exposure to alcohol also alter the integrity of the endoplasmic membrane-bound polysome (7–11) and both feeding and/or exposure to 10 mM tryptophan have been shown to reverse the acute damage produced in these organelles with restoration of albumin synthesis (11–14).

The present studies were undertaken to determine the effects of CCl_4 on albumin synthesis, and to examine the influence of diet, tryptophan, cortisone, and an antioxidant on the toxic effects of CCl_4. The results indicate that CCl_4 rapidly inhibits albumin production and that the addition of tryptophan does result in some improvement in albumin synthesis.

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METHODS

Protocol. Male rabbits, 1.3–1.5 kg were used as donors and were fed 80–100 g of a standard rabbit chow ad lib. until operation. At 2 hr before surgery, the CCl_4-treated rabbits received 2.5 ml of CCl_4 in mineral oil by stomach tube. The nine experimental groups are indicated in Table I.

These groups were chosen to examine the effects of tryptophan, washout perfusions, cortisone acetate, fasting, and the antioxidant N,N'-diphenyl-*p*-phenylenediamine (DPPD)[1] on albumin production (4). In half of the studies in group 4, the livers were perfused for 60 min before measuring albumin synthesis with carbonate-^{14}C. In the other half, the livers were perfused for 60 min and then transferred to a fresh identical perfusion system where albumin synthesis was measured. The results were the same whether the perfusion for 1 hr before the measurement was in a separate system or not. Thus the data obtained from both methods were combined. Similar results were obtained in group 5. In groups 6–9 the livers were perfused for 1 hr before the introduction of the label.

In groups 6 and 7, cortisone acetate 3 mg/kg body weight was administered intramuscularly 2 hr before the ingestion of CCl_4.

Group 8 was fasted for 48 hr before CCl_4 administration. The two rabbits in group 9 and two control rabbits were injected intraperitoneally with 60 mg of DPPD suspended in 1% gum arabic and Tween (0.5%) at 27 and 3 hr before CCl_4 administration (4).

Perfusion. 2 hr after CCl_4 ingestion, the rabbits were anesthetized with open drop ether and the livers removed and mounted in a humidified chamber for perfusion as previously described (12–15). During surgery, the liver was perfused by an auxilliary perfusion and was deprived of blood for less than 15 sec. The composition of this perfusate was the same as the main perfusate.

The perfusate consisted of 2 parts heparinized rabbit blood and 1 part oxygenated Krebs-Henseleit solution containing 400–500 μmoles of amino acids/100 ml including

[1] *Abbreviation used in this paper:* DPPD, N,N'-diphenyl-*p*-phenylenediamine.

25 μmoles arginine, 5 μmoles isoleucine, 69 μmoles L-glutamine. Tryptophan was present at only trace levels (12). Amino acid analyses of the perfusates and the liver were not significantly different from those reported previously (12). Initial glucose levels were between 70–100 mg/100 ml. Rabbit albumin was added to adjust the final albumin level to 3.1–3.4 g/100 ml. The oxygenated perfusate was pumped into the portal vein at flow rates of 1.0–1.4 ml/min per g liver.

To label the intracellular arginine and hence the guanido carbon of albumin, 200 μCi of carbonate-^{14}C (SA 5 mCi/mmole) as carbonate in 0.5 ml of saline was added directly to the portal inflow as a single pulse (12, 13). The perfusion was continued for 2.5 hr after this injection and albumin synthesis calculated from the following expression:

$$\text{Albumin synthesized (mg)} = \text{total perfusate albumin (mg)} \times \frac{\text{Albumin Guanido-}^{14}\text{C SA}}{\text{Synthesized urea-}^{14}\text{C SA}}. \quad (15)$$

Synthesized urea was calculated from the difference between the initial and final urea content of the perfusate and the volume of distribution within the liver and red cell. The space of distribution of urea in the liver and red cell has been determined to average 60%. Urea-^{14}C activity was determined at the end of the perfusion and related to the net urea synthesis (15). The specific activity of this urea carbon was assumed to equal the mean specific activity of the precursor guanido carbon of arginine (16–18). The dose of CO_2-^{14}C varied by as much as 50% and thus the specific activity of the urea carbon and the guanido carbon of albumin varied. For comparison purposes, all the values were normalized to a urea carbon specific activity of 1×10^5.

The concentration of protein in plasma or perfusate was determined with a biuret reagent (19) and protein partition with a Kern microelectrophoresis unit (20).

Albumin was isolated by preparative acrylamide gel electrophoresis, hydrolyzed, and the specific activity of the guanido carbon determined by consecutive treatment with arginase and urease as has been described in detail previously (12–15). Albumin purity was checked by immunoelectrophoresis (21) and the ^{14}C and ammonia N released by enzyme treatment assayed by the technique of Conway and Byrne (22). DNA was determined by the indole method of Ceriotti as modified by Keck (23) and RNA was determined by the method of Fleck and Begg (24). Total hepatic protein was determined by the method of Lowry (25). The description of the techniques for isolation of the bound polysomes has likewise been detailed previously (13). The method described by Blobel and Potter (26) was used. In these isolations and analyses, rabbit liver cell sap, which has been shown to contain a potent and stable RNase inhibitor,[2] was employed.

RESULTS

All rabbits tolerated the mineral oil and the CCl_4 without incident except for slight diarrhea.

The data for the nine groups are given in Table II. The weights of the livers from fasted donors, group 8, were less than controls as expected. However in group 3 and 6 the donor livers were also smaller without obvious explanation.

TABLE I
Protocol

Group	Donor	Perfusate	Remarks
1	Fed	Control	Alb. Syn. measured after 15 min of perf.*
2	Fed CCl_4	Control	Alb. Syn. measured after 15 min of perf.*
3	Fed CCl_4	10 mM Trp.	Alb. Syn. measured after 15 min of perf.*
4	Fed CCl_4	10 mM Trp.	Alb. Syn. measured after 1 hr of perf.*
5	Fed CCl_4	Control	Alb. Syn. measured after 1 hr of perf.*
6	Fed CCl_4-Cort‡	Control	Alb. Syn. measured after 1 hr of perf.*
7	Fed CCl_4-Cort‡	10 mM Trp.	Alb. Syn. measured after 1 hr of perf.*
8	Fasted CCl_4	10 mM Trp.	Alb. Syn. measured after 1 hr of perf.*
9	Fed CCl_4-DPPD§	Control	Alb. Syn. measured after 1 hr of perf.*

* The livers in groups 1–3 were perfused for 15 min before the addition of the carbonate-^{14}C. All other livers were perfused for 1 hr before the addition of the label.
‡ Cortisone acetate 3 mg/kg 2 hr before CCl_4.
§ DPPD = N, N′-diphenyl-*p*-phenylenediamine.

Urea synthesis averaged 80±12 mg/100 g wet liver weight during the 2.5 hr of perfusion. In the other groups there was a slight decrease in the absolute amount of urea synthesized. Synthesis of urea was measured at 15–30-min intervals during the perfusion and only those studies which showed a steady urea production were used. A basic assumption in the determination of the synthesis of albumin using carbonate-^{14}C is that the fractional rates of urea and albumin synthesis remain constant during the study period. Within 15 min after the label has been given, 95% of the labeled products, urea and albumin, have been synthesized. Subsequent alterations in unlabeled albumin production would have little effect on the specific activity of the albumin because of the large circulating mass of albumin. This is not so with urea. Since the perfusate is recirculated there is some persistance of the CO_2-^{14}C and labeling of urea continues for a finite period. Thus it is not truly a pulse label. This additional label, coming from a very low specific activity carbonate contributes very little additional activity. However if the continued production of unlabeled urea varies during the study period, then there would be marked alterations of synthesized urea specific activity and calculated values for albumin synthesis would be inaccurate. Setting the total urea production as 100% for the 150 min perfusion period, the fractional rate of urea production in the animals was measured at 15–30-min intervals. The average values were 14±2% at 15

[2] Oratz, M. Unpublished observations.

TABLE II
Albumin Synthesis

A	B	C	D	E	F	G	H	I	J
Group	Liver weight	Urea synthesis	Urea C SA	Perfusate circulating albumin	Albumin Guanido C SA	Albumin synthesis	RNA/DNA	Protein/ DNA	Remarks
	g	*mg/100 g 2.5 hr*	*CTS/mg C* $\times 10^{-5}$	*mg*	*CTS/mg C*	*mg/100 g 2.5 hr*			
1) Fed (10)	61±3	80±12	1	3900± 75	568±61	36±2	2.3±0.2	70±3	Liver preperfused for 15 min
2) CCl_4 (4)	69±4	55±10	1	3700± 80	109±40	5 ±1	2.1±0.2	63±3	Same
3) CCl_4 (4) 10 mM Trp.	43±2	68± 4	1	4100±110	50±10	6±2	1.9±0.2	59±6	Same
4) CCl_4 (7) 10 mM Trp.	62±2	60± 3	1	4400± 90	286±39	19±2	2.3±0.2	66±1	Liver preperfused for 1 hr
5) CCl_4 (5)	60±5	58± 9	1	4400±170	186±26	12±1	2.3±0.3	68±3	Same
6) CCl_4 (4)	49±4	55± 4	1	4560±180	80± 3	8±1	2.4±0.1	72±7	Same: donor rabbit received cortisone acetate 3 mg/kg
7) CCl_4 (5) 10 mM Trp.	67±2	76±12	1	4540±270	297±58	20±4	2.1±0.3	68±5	Same: donor rabbit received cortisone acetate 3 mg/kg
8) CCl_4 (4) 10 mM Trp.	41±3	75± 6	1	3600±140	43± 9	3±1	1.4±0.2	51±5	Same: donor rabbit fasted for 48 hr
9) CCl_4 (2)	65; 51	38; 58	1	4220; 3760	37; 134	3; 9	2.1; 2.5	68; 62	Same: donor rabbit treated with DPPD 27 and 3 hr before CCl_4

The numbers noted in the parentheses represent the number of studies.
±, SEM; Trp., tryptophan.
In groups 6 and 7 cortisone acetate was *not* present in the perfusate albumin synthesis is determined from columns B, D, E, and F.

$$G = F \times E/D \times 100/B.$$

min, 40±2% at 60 min and 70±3% at 120 min of perfusion. Thus, the requirement that urea production remain stable was satisfied.

In livers from fed donors that received CCl_4 (group 2) albumin synthesis decreased to 5 mg/100 g liver per 2.5 hr from a control value of 36 mg. Enrichment of the perfusate with 10 mM tryptophan did not augment albumin synthesis (group 3) unless the livers were exposed to this level of tryptophan for at least 60 min before the measurement of albumin synthesis (group 4). Preperfusion with a control perfusate before measuring albumin synthesis also resulted in some improvement in albumin synthesis but not to the same degree as when excess tryptophan was present (group 5 vs. group 4): P value less than 0.05).

Because cortisone acetate has been shown to result in reaggregation of the endoplasmic reticulum-bound polysome in the absence of new RNA synthesis, its effect on CCl_4 exposed livers was studied. Pretreating the donor with cortisone acetate, 3 mg/kg body weight 2 hr before CCl_4 administration failed to result in any improvement in albumin synthesis (group 6) unless tryptophan was present in the perfusate (group 7).

Starvation has been reported to increase the sensitivity of an animal to the toxic effects of CCl_4 (27) and as is seen in the fasted animals (group 8) that despite the presence of tryptophan albumin synthesis was near zero.

Antioxidants have been suggested as preventing the toxic manifestations of the metabolites of CCl_4. However, the pretreatment of two donor rabbits with DPPD 27 and 3 hr before CCl_4 ingestion failed to prevent the decrease in albumin synthesis (4). There was no decrease in albumin synthesis in control studies with DPPD or with mineral oil and these studies were included in group 1.

Endoplasmic reticulum-bound polysome profiles (Fig. 1) were obtained in the groups studied and were either aggregated as seen in the fed control groups or disaggregated after CCl_4. Minimal reaggregation was noted with perfusion for 1 hr.

Hepatic RNA/DNA and protein/DNA ratios were 2.0–2.6 and 59–72 in control groups and were not altered by CCl_4, tryptophan, or cortisone. In the fasted studies (group 8) there was a significant decrease in both hepatic protein and RNA as was noted previously (13).

Lactate-pyruvate ratios were unaltered by either CCl_4, tryptophan, DPPD, or by cortisone. The ratios ranged between 8–20 at the start of perfusion and decreased to values betwen 2–6 at the end of the study. Pyruvate levels were 0.4–0.7 μmoles/ml in control perfusions and were not altered in the experimental groups. Oxygen extraction was steady and bile flow ranged between 2–3 ml/hr in all studies.

DISCUSSION

The isolated perfused rabbit liver is an effective model for the study of hepatotoxic agents. The donor and the perfusate can be subjected to specific alterations and the effects of varying times of perfusion examined conveniently.

Employing this model system, the administration of CCl_4 2 hr before the measurement of albumin synthesis has been shown to result in a decrease in albumin synthesis of 86%. This loss of hepatic albumin-producing capacity was associated with a marked disaggregation of the endoplasmic membrane-bound polysome (Fig. 1). Smuckler et al. have shown that CCl_4 administration results in an alteration in the endoplasmic reticulum and the ribosomes are separated from this structure as well as from mRNA (1–3). No loss of total RNA was noted. These workers also described a decreased incorporation of glycine-^{14}C into total hepatic and plasma proteins in vivo. Our results are in accord with these findings including the observation that hepatic RNA/DNA and protein/DNA ratios were unaltered by CCl_4 ingestion.

Smuckler et al. have demonstrated that the effect of CCl_4 or its metabolites altered the interaction between ribosome and mRNA (1–3). Gravela and Dianzani (4) concluded that CCl_4 acted by preventing the binding of ribosomes to mRNA after a normal polysomal translation, since CCl_4 had no effect when the ribosome was kept bound by the use of cycloheximide. Since both tryptophan (12–14) and cortisone (28) have been shown to favor ribosome mRNA interaction resulting in some degree of reaggregation of the disaggregated-bound polysome, these agents were studied in an attempt to find a means of reducing the toxicity of CCl_4. Tryptophan failed to prevent the inhibition of albumin production by CCl_4 when the amino acid was given to the donor and when the donated liver was exposed to high concentrations of tryptophan for 15 min. In order to determine if longer perfusions might result in some improvement, perhaps by permitting exchange or removal of some of the toxic metabolites of CCl_4, the livers were perfused for 1 hr before the introduction of the label. Some improvement was noted and albumin synthesis rose to about one-third the normal rate. Longer exposure, with 10 mM tryptophan in the perfusate, did result in more improvement in albumin synthesis indicating that endogenous mRNA and ribosomes can still interact to form albumin. This increased albumin production lends support to the concept that metabolites of CCl_4 act either to alter the attachment of ribosomes with mRNA or polysomes with the endoplasmic reticulum; since these interactions are necessary for albumin production.

Employing the isolated liver perfused with 200 mg/100 ml ethanol, tryptophan has also been shown to exert a protective effect (13). In both of these studies with alcohol and CCl_4, significant disaggregation of the endoplasmic membrane-bound polysome occurred and tryptophan resulted in some reaggregation. However the degree of reaggregation could not be related to the increment in albumin synthesis (13), a result not unexpected since

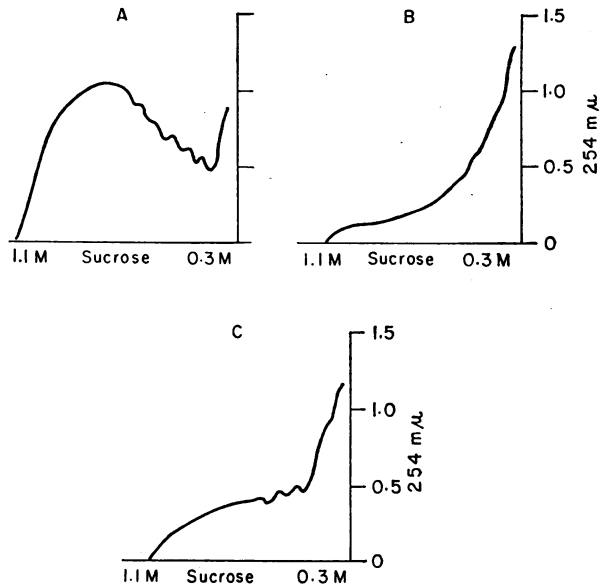

FIGURE 1 Endoplasmic-bound polysome profiles: (*A*) from fed and fed DPPD-treated donors; (*B*) CCl_4 fed, fasted, or cortisone treated; (*C*) CCl_4 fed donors where livers were preperfused for 1 hr before the addition of CO_2-^{14}C. Marked disaggregation occurs with CCl_4 and only minimal reaggregation is seen when the liver is perfused for an additional 1 hr. The addition of tryptophan does not result in any further increase in aggregation.

the specific polysome for albumin synthesis can not be isolated as yet (Fig. 1).

Cortisone acetate, at a dose of 3 mg/kg body weight administered 2 hr before the ingestion of CCl_4 failed to inhibit the loss of albumin production. To the contrary, there was even a lower value found than with preperfusion alone (groups 5 and 6, P less than 0.05). This result suggests that this short-term exposure to cortisone may have actually stimulated the microsomal enzyme system responsible for CCl_4 metabolism. Thus the toxicity of CCl_4 would be enhanced. Cortisone administered to the donor rabbit however failed to alter the response to tryptophan added in excess to the perfusate.

Other factors which have been considered to alter the toxicity of CCl_4 are the removal of protein from the diet of rats and the administration of antioxidants. Starvation has been shown to enhance the toxicity of CCl_4 (4, 27). In four livers from donors, fasted for 48 hr, and then treated with CCl_4, albumin synthesis was essentially nil and practically no radioactivity was incorporated into albumin. While it is possible that CCl_4 was absorbed faster in these animals and the liver exposed to a larger dose, protein synthesis has been shown to be inhibited by a dose as small as 0.5 ml/kg (1). Tryptophan did not reverse these findings and the total RNA and protein within the hepatic cell remained low (group 8). In two studies the antioxidant, DPPD, failed to augment

albumin production though no toxic effects of this agent were observed.

Albumin synthesis is extremely sensitive to CCl_4 probably secondary to the alterations in the cytoplasmic protein-synthesizing system. Tryptophan has been shown to restimulate albumin synthesis if given shortly after CCl_4 ingestion and if present in the perfusate at high levels. While the mechanism of this action of tryptophan is not known it is interesting to speculate that tryptophan aids in the combination of available functionable particles that are still capable of protein synthesis.

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