components in the same way as described earlier[5]. The cadmium content determined in the high-molecular weight fraction I (V_e/V_o at 'void volume') was compared with that of cadmium found in the low-molecular weight fraction II (V_e/V_o 2.8–3.4). The results were expressed as a percentage of the total quantity of cadmium found in both fractions.

Results and discussion. The figure shows a typical result of column chromatography on Sephadex G-100 of the bile samples collected during the 1st 3 h after i.v. administration of 0.6 mg of Cd/kg b.wt[5]. Cd^{2+} cations were eluated both at 'void volume' (fraction I) and in the fraction with V_e/V_o around 3 (fraction II). The quantity of cadmium in both fractions was approximately the same.

In the table the amounts of Cd found in both fractions I and II in relation to the dose administered and to Cd pretreatment are shown. Results are expressed in percentage of the total quantity of cadmium found in both fractions after chromatographic fractionation. The table clearly shows that the cadmium content of the low-molecular weight fraction II increases when higher doses of Cd are administered. On the contrary Cd pretreatment increases Cd content in the high-molecular weight fraction I. These results are in a good conformity with cumulative biliary excretion of Cd: with increasing dose of Cd administered, cumulative biliary excretion of Cd increases. It may be therefore said that cumulative biliary excretion of Cd correlates with Cd content in the low-molecular weight fraction II. In case of Cd pretreatment the Cd content in the fraction II decreases, and similarly there is a decrease in the biliary excretion of Cd. After i.v. injection of the dose of 2 mg of Cd/kg b.wt, more than 98% of the cadmium in bile samples was associated with a low-molecular weight compound, with molecular weight less than 4000 (probably Cd-glutathione complex)[1]. After administration the dose of 2.625 mg Cd/kg b.wt we found 96.9% of Cd in fraction II (table). According to Cherian and Vostal[1] the low cumulative biliary excretion of Cd and the higher percentage of Cd accumulation in the liver in rats given low doses suggest that liver initially has a high affinity for cadmium and therefore only a small percentage of Cd is transported to the bile. With higher doses of Cd the cadmium binding sites in the liver may be saturated and more Cd is available for biliary excretion. In this case Cd might form a complex with low-molecular weight compounds in the bile.

Cadmium pretreatment induces formation of metallothionein in the liver resulting in higher retention of Cd in the liver since the Cd-metallothionein complex is only poorly excreted in the bile[7]. In rats pretreated with Cd we found the higher portion of Cd in bile in the high-molecular weight fraction I. The nature of the macromolecules binding Cd in the bile has not yet been identified. It seems that there exists a correlation between the binding capacity of liver for cadmium, the cumulative biliary excretion and the binding of Cd in the bile.

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Does an excess in liver proline increase the accumulation of collagen induced by carbon tetrachloride?

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Summary. A 20-fold, diet-induced increase in liver proline does not result in an increased accumulation of hydroxyproline following chronic carbon tetrachloride administration.

Collagen accumulation is the hallmark of cirrhosis resulting from a variety of different etiologies. It has been shown that collagen accumulation induced by carbon tetrachloride (CCl_4), ethanol and schistosomiasis, both in animals and in humans, is correlated with the levels of free proline in the liver[2–4]. Two possible explanations are conceivable for this correlation: a) that the amount of proline in the liver controls the amount of collagen synthesized or b) that the correlation between the increase in collagen and proline is an epiphenomenon in which proline levels are not causally related to actual collagen synthesis. This distinction, besides its interest in relation to the mechanisms that control collagen synthesis, may have clinical implications in cirrhosis since proline levels in the liver, and thus fibrogenesis, could be modified through manipulations in dietary proline.

In the present experiments we have increased the amount of proline in the diet of animals administered carbon tetrachloride and we have determined its effect on liver proline levels and on collagen levels, measured chemically as hydroxyproline.

Materials and methods. Male Wistar rats weighing 150 g (Canadian Breeding Laboratories, Ottawa, Canada) were divided into 6 groups with 4–5 animals per group: I. CCl_4 – high proline diet; II. CCl_4 – normal chow diet; III. Control (corn oil instead of CCl_4) – high proline diet; IV. Control – normal chow; V. CCl_4 – high alanine diet; VI. Control – high alanine diet.

The high proline diet consisted of a standard rat Purina Chow containing an added 30% l-proline. Animals consumed this diet ad libitum. The high alanine diet served as a control for a non-specific effect of ureogenesis. An excess of aminoacids, through an increased urea synthesis, has been shown to sensitize the liver to hypoxia[5], a condition that might accentuate the CCl_4-induced hepatotoxicity. Therefore, we carried the 2 extra groups of animals in which proline was replaced equimolarly by alanine, an aminoacid known to induce a very high degree of ureogenesis and of necrosis after hypoxia[5].

Carbon tetrachloride was prepared in a 1:1 solution in corn oil and injected s.c. at a dose of 1 ml CCl_4 kg/b.wt, 3 times weekly. Diet manipulation and CCl_4 administration were

started at the same time and were maintained for 30 days. Animals were then killed and their livers were analyzed for hydroxyproline and free proline. Proline was determined by the method of Troll and Lindsley[6] as modified by Rojkind and Gonzalez[7]. Hydroxyproline was determined as described by Prokop and Udenfriend[8].

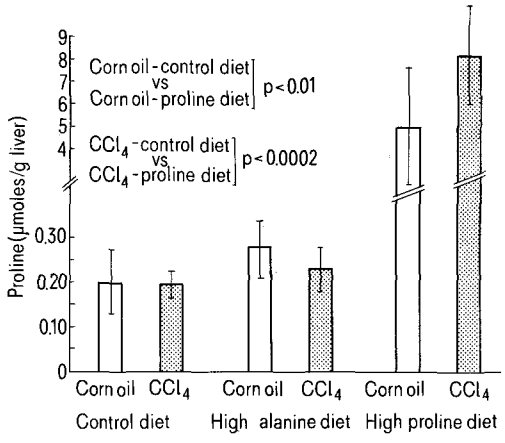

Fig. 1. Proline content of the liver in animals fed either a normal chow diet or chow diets enriched with proline or alanine. Animals were given 1 ml/kg CCl_4 3 times weekly for 30 days, or an equivalent volume of corn oil (controls).

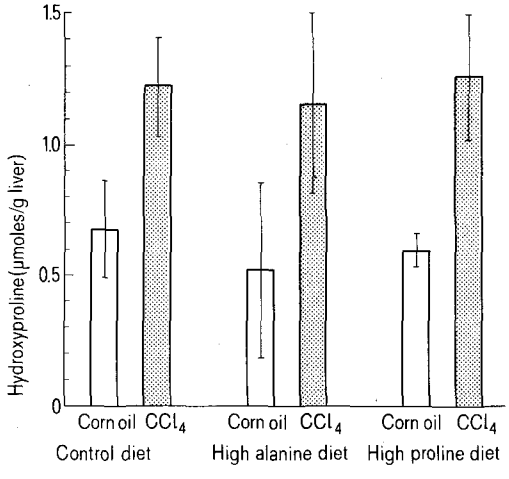

Fig. 2. Hydroxyproline content of the liver in animals fed either a normal chow diet or diets enriched with proline or alanine. Carbon tetrachloride and corn oil were administered as described in figure 1.

Results. Figure 1 shows that supplementation of chow diet with proline, as used in our experiments, resulted in a 20-fold increase in free proline in the liver. Concomitant administration of CCl_4 for 30 days did not result in an increase in free proline expressed per g of liver, as compared to that in the corn oil control group. However, when data are expressed per total liver, rats receiving the high proline diet and CCl_4 showed a significant increase in free liver proline, as compared to corn oil controls (CCl_4: 106 ± 22; Corn oil: 60 ± 26 μmoles/liver, $p < 0.03$). Figure 2 shows that hydroxyproline accumulation in the liver following CCl_4 is not influenced by the amount of proline in the diet. The high proline diet which resulted in a 20-fold increase in the levels of proline in the liver did not induce an increase in hydroxyproline over the levels in controls fed normal chow diet. Similarly, when expressed as hydroxyproline per total liver, CCl_4-treated animals fed the high-proline diet did not show higher hydroxyproline levels when compared to animals fed normal chow diet. Actually, a small but significant reduction was observed: (CCl_4-high-proline diet, 16.6 ± 2.3 μmoles/liver; CCl_4-chow diet, 20.9 ± 1.5 μmoles/liver; $p < 0.02$). For all the parameters studied in this work, animals fed the high-alanine diet did not differ from chow-fed rats.

Discussion. While a number of studies have shown a correlation between liver contents of proline and collagen, measured as hydroxyproline, in several conditions leading to cirrhosis[2-4] these do not necessarily imply a cause-effect relationship in which the regulatory mechanism of collagen synthesis is the availability of proline.

Our experiments show that vastly different proline levels in the liver do not influence the accumulation of collagen in the liver following the administration of a cirrhogenic agent such as CCl_4. We therefore suggest that the correlation that exists between proline levels and hydroxyproline accumulation in the liver in several conditions is an epiphenomenon and not the mechanism for increase in collagen formation.

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Effect of centrally active drugs on dopamine oxidation by rat brain catecholamine oxidase[1]

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Summary. Centrally active drugs of the phenothiazine-, butyrophenone- and iminodibenzyl class markedly decreased the rate of dopamine oxidation in the presence of rat brain catecholamine oxidase.

Vander Wende and Spoerlein[2] reported the presence of an enzyme in rat brain, which catalyzes the oxidation of dopamine, noradrenaline and adrenaline in vitro. Although the enzyme appears to be widely distributed in the central nervous tissue, the role of the enzyme is unknown. It has been suggested that catecholamine oxidase activity may play a role in the maintenance of normal mental function by controlling the relative levels of catecholamines[3,4], or by