Changes in serum proteins and in major liver constituents following carbon tetrachloride and a high-fat choline-free diet in rats

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Summary. Both carbon tetrachloride and a high-fat, low-protein choline-free diet cause hepatomegaly in rats due to an accumulation of both lipids and proteins with a concomitant increase in deoxyribonucleic acid content. CCl_4 causes a decline in hepatic glycogen. Serum albumin level is decreased in CCl_4-treated rats and increased in those fed the experimental diet.

Hepatomegaly is a common manifestation of liver steatosis which is generally attributed to fat accumulation. However, recently it has been shown that the hepatomegaly occurring after long-term feeding of ethanol results in an accumulation of protein which is quantitatively as important as the increase in lipid[1]. Both an enhanced protein synthesis and defective export contribute to this accumulation[2]. These data led us to test whether 2 other steatogenic agents, i.e. CCl_4 and a high-fat, low-protein and choline-free diet (hF-Cf), also induce hepatomegaly with concomitant changes in protein and lipid contents. These 2 treatments were chosen since both cause a defect in protein export[3-5], while acting differently on total protein synthesis. It has been found that these syntheses decrease after CCl_4 administration[6], remain unchanged with choline deficiency[4] and increase after a low-protein diet[7,8]. The investigation was expanded to other main constituents of the liver i.e. water, glycogen and deoxyribonucleic acid (DNA). Results on serum protein content are also reported.

Materials and methods. Nonfasted female Wistar albino rats, bred in our colony, weighing 150–200 g, were used. A group of rats was s.c. injected for 6 days with 0.25 ml/100 g b.wt/day of a 20% solution of CCl_4 in seed-oil and killed 24 h after the last injection. Another group was fed for 7 days the hF-Cf. A third group, the control, was fed a normal pelletted chow. The main constituents of both diets have been reported elsewhere[9]. The diets and water were given ad libitum. The rats were stunned by a blow on the head and bled at the neck. Total protein and albumin of serum were determined, respectively, with the biuret reagent and the bromcresol green colorimetric method, as purchased from Sclavo S.p.A., Siena, Italy. The livers were excised and their weights (wet and dry), total lipid[10], protein[11], glycogen[12] and DNA[13] contents measured. The statistical significance was evaluated with Student's t-test[14]. No statistical significance was attached to differences with a probability value $p > 0.05$.

Results. The results are summarized in the table. CCl_4 causes a decrease in rat b.wt of about 9.9% as compared with the values found at the start of the treatment, while normal- and hF-Cf fed-rats show no significant differences. The serum total protein concentration decreases in CCl_4-treated rats by 731 mg/100 ml, the reduced albumin level (428 mg/100 ml) accounts for about 59% of this decrease. Also in rats fed the hF-Cf diet, the serum total protein content appears to decrease (637 mg/100 ml), but, in this case, the albumin level is above the normal value (522 mg/100 ml). It appears, therefore, that the increase in serum albumin in hF-Cf fed rats is concomitant with a decrease in other serum proteins.

Livers from CCl_4-treated rats increase in wet weight by about 24%. In these rats, both total lipids and proteins show a strong increase that, has been shown on the whole, to be 573 mg/100 g b.wt, 191 of which are proteins. The augmentation in lipids plus proteins appears to be greater than the increase found for liver dry weight (382 mg/100 g b.wt). This may be explained by the concomitant decrease in liver glycogen (174 mg/100 g b.wt). DNA and protein content increase, respectively, by about 26% and 30% in livers of CCl_4-treated rats as compared with controls. Liver wet weight/dry weight ratios do not differ statistically between normal and CCl_4-treated rats.

Livers from hF-Cf fed rats increase in wet weight by about 12% and this is associated with a concomitant increase in

Summary of the effects of CCl_4 and of a steatogenous diet on serum proteins and on liver constituents of rats

	Control	CCl_4	Diet
Body weight (g)	187 ± 7.8^a–188 ± 4.4^b	172 ± 4.3^a–$155 \pm 4.2^{b,c}$	180 ± 6.9^a–184 ± 6.6^b
	(20) (20)	(20) (20)	(20) (18)
Serum total protein	7.842 ± 0.1895	7.111 ± 0.1605^d	7.205 ± 0.1863^d
(g/100 ml)	(19)	(14)	(15)
Serum albumin	3.615 ± 0.1168	3.187 ± 0.1220^e	4.137 ± 0.1210^e
(g/100 ml)	(19)	(14)	(15)
Liver wet weight	3.895 ± 0.0753	4.831 ± 0.111^d	4.367 ± 0.144^e
(g/100 g b.w)	(25)	(19)	(18)
Liver dry weight	1.156 ± 0.014	1.538 ± 0.080^d	1.569 ± 0.1160^e
(g/100 g b.wt)	(23)	(20)	(15)
Liver wet weight	3.419 ± 0.059	3.291 ± 0.125	2.852 ± 0.108^d
Liver dry weight	(25)	(20)	(15)
Hepatic protein content	643 ± 19.2	834 ± 61.2^e	738 ± 59.9^d
(mg/100 g b.wt)	(24)	(20)	(15)
Hepatic lipid content	48.0 ± 5.21	430 ± 52.2^d	290 ± 64.8^e
(mg/100 g b.wt)	(19)	(19)	(13)
Hepatic glycogen content	349.5 ± 68.113	175.4 ± 20.756^d	275.9 ± 28.657
(mg/100 g b.wt)	(5)	(5)	(5)
Hepatic DNA content	10.4 ± 0.52	13.1 ± 0.63^d	11.9 ± 0.60
(mg/100 g b.wt)	(10)	(9)	(8)

Values are the mean ± SE of the number of determinations given in parentheses. [a] Start weight; [b] final weight; [c] $p < 0.01$ as compared with the start b.wt of the same group; [d] $p < 0.01$; [e] $p < 0.05$ from controls.

dry weight of 413 mg/100 g, b.wt, 242 mg of which are lipids and 95 mg are proteins. Although not statistically significant, the increase in DNA (14%) parallels that of proteins. The glycogen content does not appear to be statistically modified. In hF-Cf diet fed rats, 76 mg of constituents other than lipids and proteins contribute to the increase in liver dry weight. Liver wet weight/dry weight ratios are decreased in this group of animals by about 16% which indicates that the increase in some constituents is not concomitant with an addition of water.

Discussion. We cannot at present advance hypotheses about the mechanism leading to the changes found in serum proteins under the present experimental conditions. The modifications in DNA, glycogen and protein content of the liver caused by CCl_4 are in agreement with previous findings[15,16]. From these results one may conclude that the accumulation of proteins has an important role in determining hepatomegaly both in CCl_4-treated and in hF-Cf fed-rats. Since DNA changes run parallel to those of proteins, one may think that the 2 modifications are causally connected. The DNA increase may be due to various causes, such as the presence of infiltrating cells, an augmentation in the number of binucleated cells or hepatic regeneration. CCl_4 could affect the hepatic glycogen level through an increased release of catecholamines by the adrenal medulla[17], since these substances reduce the hepatic glycogen level[18]. Another possibility is that, since CCl_4-treated rats probably suffer from malnutrition, as shown by their loss in b.wt, the reduction in liver glycogen may be due to a sort of starvation also capable of strongly decreasing the hepatic glycogen level[19]. The hypotheses on the pathogenesis of fatty liver have been discussed elsewhere[20].

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Collagenolytic activity of eosinophilic granuloma in vitro

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Summary. Lytic activity of eosinophilic granuloma and other tumours was studied in vitro on collagen substrate. Collagen degradation was measured through the release of hydroxyproline-rich peptides into the medium. The in vitro lytic action was at a maximum in the case of EG and was correlated with the presence of histiocytic cells.

Eosinophilic granuloma (EG), Hand-Schüller-Christian and Letterer-Siwe diseases are the 3 clinical forms of histiocytosis X[2]. Whatever the clinical form, histiocytosis X is related to a tumoral cellular proliferation probably of histiocytic origin and showing an ultra-structural marker called X body[3]. Biochemical[4] and membrane-receptor studies[5] were used to identify this tumoral histiocytic cell. Tissue culture is an useful tool in the study of histiocytosis X since the histiocytic cells (with their X body) can be maintained for several days and weeks in vitro, while neither neutrophil nor eosinophil polymorphonuclear cells are ever identified after 1 week in the same conditions[6].

As the destructive effect of EG upon bone tissue suggests a cellular release of active proteolytic substances, we intended to demonstrate and to quantify in vitro the lytic activity of the EG cellular population using a collagen film as the substrate for culture. Tumour fragments of various type and origin were used as controls of the lytic activity.

Material and methods. Tumoral material. Fragments of 5 clinically and histologically demonstrated EG originating from bone lesions of children were taken and put on the collagen film in Leighton tubes. Explants of various tumours from both children and adults (table 1) were placed under the same conditions.

Collagen substrate. The collagen substrate was prepared according to the recommendations of Ehrman and Gey[7] and Bornstein[8]. The rat tail tendons were cut free and immersed in a solution of 0.1 M acetic acid. After a gauze filtration, the acetic acid collagen solutions was dialysed against distilled water until the desired viscosity was obtained. The dialysed collagen solution was spread on the surfaces of coverslips. Exposure to ammonia vapour gelled the solution. The coverslips were washed with distilled water and immersed in the nutritive medium (MEM + 10% calf serum) with antibiotics (penicillin, 200 units/ml and streptomycin 0.1 mg/ml). These coverslips can be kept for at least 1 month under refrigeration.

Culture. 6–8 explants (0.1 mm^3) were distributed on each coverslip in a sterile Leighton tube. 4–12 collagen coverslips were used for each tumour. The explants were put on the collagen film in a minimum of medium (0.2 ml) to assure their adhesion on the coverslip. Medium (1.8 ml) was added the day after and its renewal occurred once a week. When the cultures were terminated, the coverslips were fixed with Bouin solution and stained with hemalum eosin.

Enzymology. 1. Acid phosphatase[9] and leucyl aminopeptidase[10] activities were tested on the coverslips following the growth of the culture. 2. Collagenase activity. Collagen degradation was determined by the release of hydroxyproline-rich peptides into the medium. After acid hydrolysis of the peptides, hydroxyproline was separated by chromatography[11] and determined by Stegemann's method[12]. The