Influence of a Liver Preparation on Liver of Cats Receiving Carbon Tetrachloride. (26429)

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While lipotropic substances and proteins, especially those providing certain essential amino acids, exert a protective action on the liver, the participation of other unknown factors is suggested by clinical observations on patients with hepatic cirrhosis. That the liver may be the source of such unknown factors is implied in several studies. Beneficial effects of crude liver preparations have been reported in patients with decompensated cirrhosis of the liver who were maintained on either a moderate or high protein diet(1,2,3). Analyses of the available data of the various liver preparations indicate that their content of known lipotropic agents (*e.g.*, methionine and choline) was too small to account for the beneficial effects obtained over and above those obtained from the diet alone. A few experimental animal studies also suggest that liver extracts partially protect against experimental liver injury produced by dietary means or hepatotoxic agents(4,5,6,7). The results from animal studies, however, have been equivocal. In the present study an attempt has been made to evaluate by cytological quantitative determinations the influence of a lipotropic liver extract on the pattern and degree of fatty degeneration resulting from carbon tetrachloride.

Procedure. This study was conducted on 32 healthy adult male cats acclimatized by previous residence for 4 weeks in individual cages in a constant temperature room. The animals were maintained on a diet of fresh horse meat *ad lib* supplemented with commercial preparations of cat food (fish and chicken). Daily records of animal weight and food and water intake were kept. Daily food consumption was in each case far in excess of the calculated minimum requirements for both caloric and protein adequacy.

The 32 cats were divided into 4 groups: Group I, 9 cats: carbon tetrachloride* was given intramuscularly every second day, 0.8 g per kilo body weight, for 5 periods extending over 10 days. Group II, 7 cats: a liver preparation was given daily, 0.3 ml per kilo body weight, by deep intramuscular injection for a period of 20 days. Group III, 9 cats: the liver extract was given daily as in Group II but in addition carbon tetrachloride was given as in Group I from 11th to 20th days. Group IV, 8 cats: basal diet alone.

The liver preparation† (Ripason) used was a sterile protein-free water-soluble fraction processed from fresh untreated liver of cattle. It is represented by the manufacturer as containing the following per ml: cystine 105 μg, methionine 100 μg, histamine 2.0 μg, and vit. B_{12} less than 0.01 μg. In terms of per cent of dry residue, total N was 6.2, amino N 2.3, total P 2.1, and amino acids 0.6.

The animals were killed rapidly by overdosage with pentobarbital sodium by intracardiac puncture. Sections of 5μ thickness were made from each of 12 uniformly distributed areas of the liver. Degree of liver damage was determined by a fat droplet count procedure similar to that described by Tanyol and Rehfuss(8). Microscope slides of liver sections were examined under power of 5 $\times$ ocular and 4mm objective. The size of the examination field was reduced uniformly to a circle 6.5mm in diameter. A count in 20 consecutive fields was made of the fat droplets seen in each slide and 3 consecutive slides obtained from each area by serial section were used. A total of 60 fields for each area of liver was thus examined. Areas which were within the field but which did not contain liver cells (*e.g.*, periportal spaces, larger vessels, parts severed from main section, etc.) were not included in the count.

The data on fat droplet counts from the 4

* Tetrachlormethane reagent, sp.gr. 1.59.

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TABLE I. Influence of Administration of a Liver Preparation on Hepatotoxic Effect of Carbon Tetrachloride.

Treatment	Animal No.	Fat droplets, mean count per 20 fields
Group I:		
Carbon tetrachloride, alternate days for 10 days	1	16
	2	64
	3	461
	4	198
	5	802
	6	532
	42-0	60
	42-1	16
	42-2	0
	N = 9	
Group II:		
Liver preparation daily for 20 days	14	0
	15	0
	16	16
	17	16
	42-6	0
	42-7	0
	42-8	0
	N = 7	
Group III:		
Carbon tetrachloride, alternate days for 10 days plus liver preparation daily for 20 days	7	0
	8	0
	9	0
	10	20
	11	16
	12	168
	42-3	13
	42-4	0
	42-5	0
	N = 9	
Group IV:		
Diet alone, untreated	C 1	0
	C 2	0
	C 3	0
	C 4	0
	D 5	10
	D 6	0
	D 7	0
	D 8	0
	N = 8	

series of experiments were tabulated and significance of differences between them tested by the ranking method described by Wilcoxon(9).

Results. The appetites of the animals of Group II (liver extract) and Group IV (basal diet controls) were well maintained. Group I (carbon tetrachloride) and Group III (carbon tetrachloride plus liver extract) cats showed about a 10% decrease in food intake only during the last 2 days. In all animals of the 4 groups body weights showed no net decrease throughout the experiment. Other than an increasing resistance to needle injections, the animals showed no behaviour peculiarities during the observation period. No evidence of inflammatory reaction was found at sites of liver extract injections.

In general, the livers of the cats that received carbon tetrachloride (Group I) appeared on gross inspection to be slightly paler and more granular than those of non-intoxicated animals (Groups II and IV) but the differences could not be regarded as marked. Microscopic examination of the liver revealed fatty infiltration of all liver lobes in 8 of the 9 animals. Fat droplets were distributed diffusely rather than concentrated in any one region. Data on fat droplet count are summarized in Table I.

On the other hand, the microscopic picture of the livers of cats treated with both carbon tetrachloride and the liver extract (Group III) was different from the above. Fatty changes were marked in one cat, slight in 3, and not demonstrable in the remaining 5 (Table I). The cats treated with the liver preparation had significantly smaller numbers of fat droplets than the untreated animals, with a probability of rank T less than 0.05%(9). Fig. 1 and 2 show sections typical for livers of cats receiving carbon tetrachloride alone, and of cats receiving carbon tetrachloride plus liver extract.

While the usual signs of nuclear degeneration (karyolsis, karyorrhexis and pyknosis) and cytoplasmic degeneration (vacuolization and loss of cell contour) were much more marked in livers of intoxicated cats, their absence in the liver extract treated series can not be regarded as specific to the treatment.

Discussion. All evidence of hepatic damage due to carbon tetrachloride may disappear spontaneously if the toxic agent is withdrawn (and animal is maintained on a stock diet)(10,11,12). In the present experiments, a low incidence of hepatic damage during carbon tetrachloride intoxication was found only when associated with administration of the liver preparation. Whether this represented stages in regression of established lesions or reflected prophylactic properties of the liver extract we do not know.

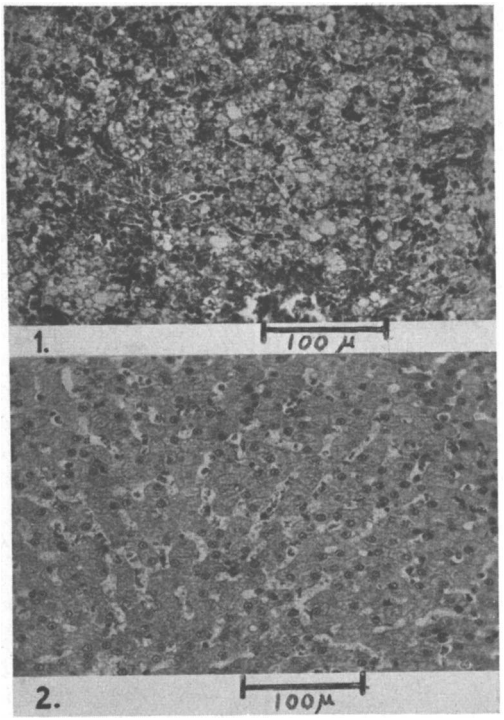

FIG. 1. Section of liver of cat which received intramuscularly 5 evenly spaced doses of carbon tetrachloride over a 10-day period.

FIG. 2. Section of liver of cat which received a protein-free water soluble liver extract daily in addition to carbon tetrachloride over a 10-day period.

In hepatic injury removal of lipid from the liver cells can be brought about only when the diet provides lipotropic factors, principally supplementary methionine, choline, or a choline precursor. In addition the supply of dietary protein must be adequate to provide the essential amino acids necessary as structural materials for reparative processes in the liver cells(13). The effectiveness of the liver preparation in these experiments probably was not due to simple provision of a lipotropic agent since the amounts of choline and methionine were far below the minimum requirements. Furthermore, prevention by methionine or choline supplements of hepatoxic effects of either carbon tetrachloride(14,15) or chloroform(16) is not observed in the rat, cat, or dog maintained on a high protein intake. The ameliorating effects of the liver preparation would also appear not to have been due to its content of Vit. B_{12} since the amount of Vit. B_{12} so administered was less than .003 μg/kilo above that provided by the stock diet.

Protection of the rat liver against hepatatoxic agents by suspensions of tissue(17), xanthine compounds(18,19), and even colloidal carbon(20) have been reported. This effect has been interpreted as being due to increased body tissue catabolism incident to inflammatory reactions at injection site(20). In the present experiments, however, there were no inflammatory reactions noted. Moreover, in another study(16) we have repeatedly failed to find in carbon-tetrachloride intoxicated cats any protection against liver damage from similarly administered protein-free water-soluble preparations of heart muscle or stomach tissue. The evidence supports the view that the beneficial effects of the liver extract were not the result of a non-specific reaction but rather due to a lipotropic factor. More definite chemical characterization of the lipotropic factor(s) must await further purification of active liver fractions.

The absence of an injury pattern would appear not to support the view that different degrees of fatty and other degenerative changes reflect stages in a progressive invasion from the central vein outward until the whole lobule is involved. Finally it should be noted that none of the liver sections from our relatively short-term experiments demonstrated the presence of fibrous tissue or reticulum seen in liver biopsy specimens from patients with hepatic cirrhosis. The report that certain liver extracts inhibit growth and migration of fibroblasts(21) suggests the study of the effectiveness of the liver preparation used by us in chronic hepatic injury by toxic agents.

Summary. Diffuse fatty infiltration of all liver lobes was found in cats given carbon tetrachloride intramuscularly. A statistically significant reduction in degree of fatty infiltration was found when the cats were also given injections of a protein-free aqueous fraction of liver. The nature of the lipotropic action is unknown.

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Distinctive Cytopathology of ECHO Viruses Types 22 and 23.*† (26430)

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The cytopathology of the enteroviruses in rhesus monkey kidney tissue culture has been described(1). All of the enteroviruses studied (poliovirus 1-3, Coxsackie B1-B5, A9, ECHO 1-14) manifested a characteristic cytopathic effect (CPE) in fixed and stained preparations. Only ECHO virus type 10 (Reovirus type 1) was found to have features which differed from the other enteroviruses. In the present paper, the study was extended to ECHO serotypes 15-24. Types 22 and 23 were found to have distinctive CPE. The cytopathology of the remainder was similar to that previously described for the members of the enterovirus group.

Materials and methods. Tissue cultures. Rhesus monkey kidney coverslip cultures were prepared as described previously(1). Cultures were maintained in 1.8 ml of a medium consisting of 0.5% lactalbumin hydrolysate, 0.1% yeast extract (Difco), 0.2% bovine albumin, Fraction V (Armour and Co.) in a base of Earle's saline with 200 units penicillin, 200 μg streptomycin per ml and inoculated with 0.2 ml undiluted virus§ suspension. Titers of ECHO 22 and 23 were 5.1 and 5.3 log TCD_{50}/0.1 ml respectively. At selected intervals, coverslips were removed and fixed for staining. HeLa cell cultures grown in a medium containing calf serum were also used(3).

Histologic technic. Hematoxylin and eosin stain as modified by Reissig was used routinely(4). Feulgen stain was also used(5). Cultures were fluorochromed with acridine orange at pH 6.0(6) and examined under the fluorescence microscope. The fluorescent equipment consisted of a Reichert 'Lux X' high intensity mercury vapor arc lamp, 2 BG12 ultraviolet filters and a modified model

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