STUDIES ON CARBON TETRACHLORIDE INTOXICATION†

I. The Effect of Carbon Tetrachloride on Incorporation of Labelled Amino Acids Into Plasma Proteins.

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The defect leading to the panorama of morphological changes in the rat liver in carbon tetrachloride intoxication was said by Christie and Judah (G. S. Christie and J. D. Judah, 1954) to be due to injury to mitochondria and an alteration of the activities of the mitochondrial oxidative enzyme systems. These investigators reported the changes as occurring 12 to 16 hours following administration of the noxious agent. In further investigation of this problem, our efforts were directed toward finding the earliest discernible changes in liver cells and their enzymes. From this study emerged the fact that mitochondrial structure by light and electron microscopy and associated enzyme activities appear unaltered in the first 4 hours following treatment. The electron micrographs of rat livers examined 3 hours after treatment showed easily recognizable changes in the endoplasmic reticulum. These changes consist of marked dilation of the cisternae of the coarse endoplasmic reticulum and apparent dislocation of the granules from the membranes. Retrospectively, the beginning of this change was apparent at 60 minutes and possible at 20 minutes following treatment. The dilation of cisternae is similar to that observed by Oberling and Rouiller (Ch. Oberling and Rouiller, 1956) and Bassi (M. Bassi, 1960). The coarse endoplasmic reticulum has been associated with protein synthesis in cells. Therefore on

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the basis of the electron microscopic evidence, it seemed reasonable to look for a defect in protein synthesis by the liver soon after carbon tetrachloride administration.

Fibrinogen and albumin are produced primarily, if not solely, by the liver. The rate of incorporation of radioactive amino acid into fibrinogen and albumin, assuming constant exposure of the protein synthetic mechanism to the amino acid pool, should provide a first order test of this hypothesis. Therefore, the following experiments were designed to examine this.

METHODS AND MATERIALS

Male albino rats (Sprague-Dawley) weighing initially 250 to 350 grams were fasted for 18 hours prior to the experiments. Carbon tetrachloride was given in mineral oil, 1:1 by volume, via stomach tube without anesthesia. The dose was 1.0 ml. of the mixture per 100 gm. of body weight; controls received 0.5 ml. of mineral oil per 100 gm. of body weight. Following intubation the animals were permitted tap water ad libitum but no food.

Two hours following intubation the rats were given 25 μC/100 grams body weight of glycine-1-C^{14} in saline intravenously under light ether anesthesia. The rats were subsequently bled by cardiac puncture without anesthesia.

All cardiac bleedings were performed with siliconized syringes and needles (1 inch #22 gauge) and in each instance 1.0-1.5 cc. of blood was obtained and coagulation prevented with 1 volume 1.0 M sodium citrate to 10 volumes of blood. Plasma was separated by centrifugation. Fibrinogen was precipitated with 10.5% sodium sulfate (50 volumes of sulfate solution to 1 volume plasma) overnight at room temperature, collected by centrifugation, resuspended in 10.5% sodium sulfate, recentrifuged and finally dissolved in a phosphate-citrate buffer, pH 7.3. From this solution the purified fibrinogen was precipitated by mixing with an equal volume of 10% trichloroacetic acid in water. The precipitate was collected on #42 Whatman filter paper 47 mm. in diameter utilizing a Millipore filter chimney, washed with 5% trichloroacetic acid, absolute methanol and absolute ether, dried, and counted in a front window-gas flow Geiger Müller

tube. The fibrinogen was subsequently recovered from the filter paper and analyzed by Blombäck's method (B. Blombäck and M. Blombäck, 1956).

The albumin was separated as a methanol extract of the trichloroacetic acid precipitated total plasma protein, reprecipitated with 10% trichloroacetic acid, collected in the same manner as the fibrinogen, washed with 5% trichloroacetic acid and absolute ether, and counted. Total nitrogen was determined by the micro-Kjeldahl method.

Purity of the fibrinogen and albumin was checked by zone electrophoresis in paper, agar and starch, by paper and column chromatography, and by ultracentrifugation. Self absorption calculations were made for both proteins and all recordings converted to infinite thinness.

Hematocrits were determined by the micro hematocrit method, plasma protein concentrations by biuret and micro-Kjeldahl methods, and plasma protein patterns by paper and starch block electrophoresis. Blood volumes were determined by the method of Benditt et al. (E. P. Benditt, R. L. Straube, and E. M. Humphreys, 1946).

Gross autopsy and microscopical examination were performed on all animals.

EXPERIMENTAL OBSERVATION

Three and four hours following carbon tetrachloride treatment there is a marked depression of incorporation of labelled amino acid into fibrinogen and albumin. There is not during this time a significant alteration in the plasma protein concentration or relative concentrations of the various protein species. There is an initial small decrease in plasma volume, reflected in an increase in hematocrits, and also manifested by a decrease in the plasma protein space, determined using homologous glycine-C^{14} labelled plasma protein.

The results of this series of experiments are shown in Table #1. The mean activity of fibrinogen at 60 minutes after glycine-1-C^{14} administration is 552 c/m/m, whereas the treated animals' fibrinogen shows a mean activity of only 274 c/m/m. The difference between these values at 60 minutes is statistically significant. The divergence in the activity is even greater at 120 minutes,

TABLE #1

		Protein Specific Activity		
Fibrinogen		Mean c/m/m*	S.D.**	$\bar{p}$***
TIME†				
60 minutes	control	552.6 (8)	±122.5	<0.05
	experimental	274.9 (7)	±167.5	
120 minutes	control	1305.6 (8)	±263.5	<0.01
	experimental	391.5 (6)	± 93.6	
Albumin				
60 minutes	control	522.76(9)	±181	<0.01
	experimental	63.12(5)	± 40.2	
120 minutes	control	1131.5 (5)	±132	<0.01
	experimental	99.16(5)	± 68.9	

† Time following glycine-1-C^{14} administration, CCl_4 treatment given two hours prior to the glycine administration.
* Counts/minute/mgm at infinite thinness.
** Calculated by formula (9) page 6, Mathematical Statistics by C. E. Weatherburn, Cambridge University Press, 1957.
*** Calculated using method in Chapter X, Weatherburn.
( ) Following c/m/m refer to number of animals used.

$\bar{p}$ <0.05 is considered significant.

at which time the treated animals show roughly 30% the incorporation compared to the controls.

The divergence of the albumin values for 60 and 120 minutes is even more striking. Other experiments indicate that this trend continues to 300 minutes. In experiments in which glycine and carbon tetrachloride were administered simultaneously there is seen at 60 and 120 minutes a lesser but discernible depression of the incorporation of glycine into fibrinogen and albumin when compared with control animals.

Other experiments now in progress indicate the uptake of labelled amino acid by liver is similar in CCl_4 treated and in control animals, indicating similar exposure of the synthetic mechanism of the liver cells to the labelled amino acid.

DISCUSSION

Present evidence indicates that protein synthesis occurs in or on the ribonucleoprotein particles of the microsomal structure of liver cells (J. Brachet, 1955). These particles correspond to the "granules" of the rough endoplasmic reticulum observed in the electron microscope. The present studies show that the synthesis of specific proteins by the liver is substantially depressed by CCl_4 treatment. Furthermore, this appears to be due to an alteration in the intracellular protein synthetic mechanism consistent with the observation in the electron microscope of an early alteration in the form of the endoplasmic reticulum.

It seems reasonable to suppose that the fatty metamorphosis observed in livers of CCl_4 treated animals is due to lack of protein with which triglyceride may be coupled to be transported from the cell. Consistent with this formulation is the evidence that the rate of triglyceride formation remains unimpaired within the time limits used in our experiments (R. Recknagel and D. Anthony, 1959) (B. Lombardi and R. Recknagel, 1961) (R. Recknagel and B. Lombardi, 1961).

The swelling of the liver cells ("cloudy swelling" or "hydropic change") appears to be due primarily to a dilation of the cisternae of the endoplasmic reticulum without mitochondrial change. The later oxidative enzyme changes may also be secondary to altered protein synthesis, and cell death may be the result of these several changes.

SUMMARY AND CONCLUSIONS

The earliest discernible change in liver cells of carbon tetrachloride poisoned rats is in the endoplasmic reticulum. Associated with this is demonstrated a severe depression in synthesis of the proteins fibrinogen and

albumin by the liver. Lipid deposition, mitochondrial changes, and necrosis, which follow later, may all be dependent upon the defect in protein metabolism.

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