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ORIGINAL PAPERS

IN SITU MODIFICATIONS OF $NADH_2$-CYTOCHROME-C-REDUCTASE AND LACTATE DEHYDROGENASE IN LIVER, IN THE EXPERIMENTAL CARBON TETRACHLORIDE POISONING

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Abstract. The present study tried to determine the variations in the enzymatic, oxidoreductive and aerobic activity of the $NADH_2$-cytochrome-C-reductase (diaphoresis) by histochemical methods, in comparison to that of the glycolic anaerobic enzyme (lactate dehydrogenase), using an experimental model of poisoning with carbon tetrachloride (CCl_4) in rats. The liver was investigated at 24 hours after poisoning. Two standardized histochemical techniques were used. Intensity of reaction, the parameter used, was determined by qualitative evaluation of color, as visualized with optical microscope. The diaphorase presented an intense and very intense decrease of reaction in vascular endothelia and in hepatocytes .In hepatocytes, the decrease of the enzymatic, oxidative and aerobic activity appeared especially in the pericentrolobular area. The intensity of reaction was unequal in middle area of the lobular cells and maintained its intensity in the periportal biliary hepatocytes. The lactate dehydrogenase presented different changes from a liver lobule to another, either by highly diminishing of reaction in the hepatocytes of the entire lobule, or by diminishing of reaction unequal from a hepatocyte to another in the same lobule. In some liver lobules appeared modifications in the metabolism of the hepatic lobule for both of the studied oxidative enzymes. It was more obvious for the lactate dehydrogenase. There were also noticed some structural modifications, with cellular necrosis and/or numerous lipid drops in the cytoplasm. The observations and the results of the experimental intoxication emphasize the noxious consequences which some pesticides will have on the organism, expressed on the liver in the present study.
Keywords: carbon tetrachloride poisoning, liver histochemistry, experimental intoxication

Introduction

The multiple activities of hepatocytes are realized through rich enzymatic complexes. Nowadays their study is the subject of many investigations in the modern hepatology.

These new analytical methods, based on previous studies of electronic microscopy (1,2), differential centrifugations (3,4), cytology, biochemistry and histochemistry (5,6) cytometric and histophotometric studies, etc., lead to the possibility of sketching a new structural-enzyme profile at cellular or at lobular (morphologic liver unit) levels.

The clinical or experimental studies made on homogenate and sections, biopsy punctures and fragments drawn intraoperative leaded to determine the pattern of an enzymatic profile of the normal and the pathological liver. Its modification makes one of the basic elements in the biological syndrome of acute and chronic hepatic diseases and will be developed in the present paper. An experimental model of the changes of some oxidoreductive enzymes at the rat liver in the carbon tetrachloride poisoning is presented.

Materials and methods

Two groups of male Wistar rats, weighing 150-170 grams have been investigated as follows: a

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	Hepatocyte	Kupffer Cell	Blood Vessels	Connective cells
Control group				-,+
				+
			++	++
	+++	+++	+++	+++
Carbon tetrachloride poisoning	-,+		-,+	-,+
	+		+	+
	++	++		++
	+++			

Table I. The intensity of reaction for $NADH_2$-cytochrome-c-reductase

	Hepatocyte	Kupffer Cell	Blood Vessels	Connective Cells
Control Group	-,+			
	+	+	+	+
	++			
	+++			
Carbon tetrachloride poisoning	-,+		-,+	-,+
	+	+	+	+
	++	++		
	+++	+++		

Table II. The intensity of reaction for lactate dehydrogenase

Legend
- \- Negative Reaction;
- -,+ Low Intensity Reaction
- \+ Medium Intensity Reaction
- ++ Intense Reaction
- +++ Very Intense Reaction

control group that was not treated and one group of rats injected subcutaneously with low-dose (0, 25 ml/100g body weight) carbon tetrachloride. The animals have been killed by exsanguination, 24 hours post injection. The liver fragments were sectioned at cryotome at - 20˚C, to be used for the following histochemical techniques:

- $NADH_2$ –cytochrome C-reductase (diaphorase)- Diculescu et al method 1964 (7)
- Lactate dehydrogenase- Pearse method 1972 (8)

Intensity of reaction, the parameter used, was determined by qualitative evaluation of color, as visualized with optical microscope.

Results

The liver in the control group

$NADH_2$-cytochrome-C-reductase

The enzyme activity appears as an intense and very intense reaction in the cytoplasm of hepatocytes. Their nuclei remain without reaction (figure 1).

The pericentral and the periportal biliary lobular

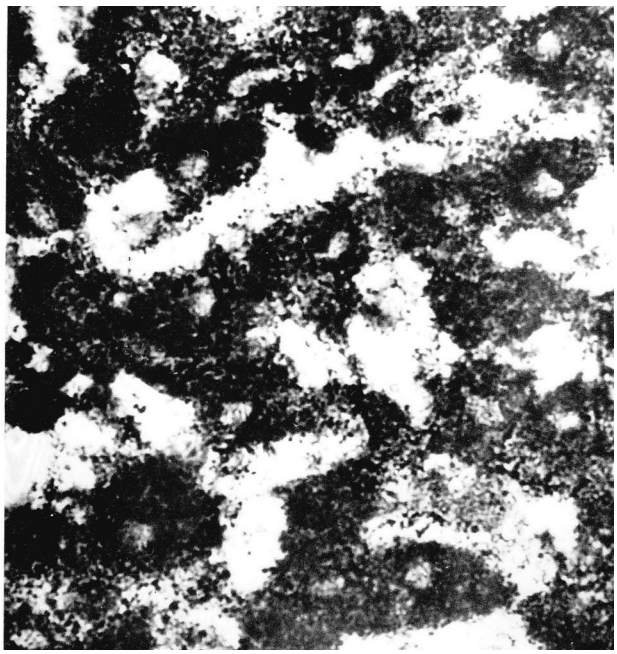

Figure 1. Rat liver in control group.NADH2-cytochrom-C-reductase appears very intense , like dark formazanic granules wich fill the entire cytoplasma of the hepatic cell. The nucelus is non-reactive.

Figure 2. Rat liver in control group.NADH2-cytochrom-C-reductase.Very intense reaction in the periportal and pericentrolobulare heptocytes.;Intense reactions in the middle area of the liver lobule.

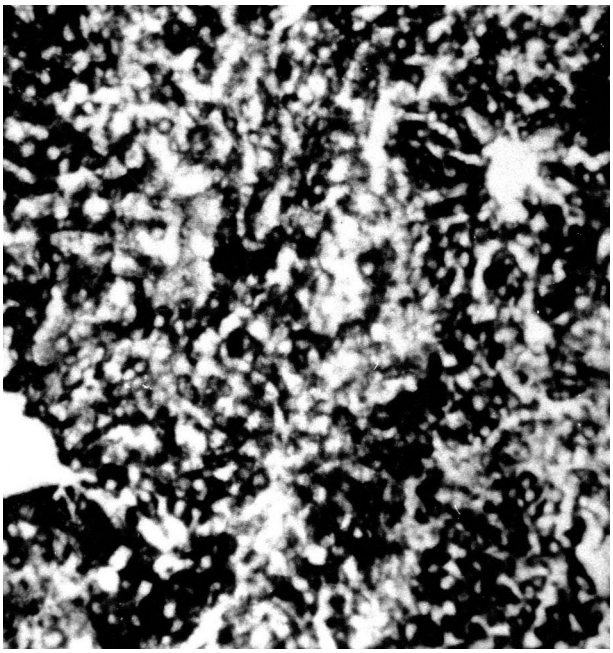

Figure 3. Rat liver in control group.Lactate-dehydrogenase presents different degrees in reactions in the hepatocytes, the most intense ones are in the cells of the pericentrolobular areas. MO x132

areas present the most intense enzymatic reactions in the liver cells (figure 2).

The Kupffer cells also appear very intensely positive. In the connective tissue from the Kiernan spaces can be observed the connective cells of fibroblasts type, with an enzymatic reaction of different intensities (medium, intense and very intense).

Lactate Dehydrogenase

The activity of the enzyme presents unequal intensity in the cells from the same liver lobule, or from one lobule to another, sometimes creating a mosaic or mottled appearance (figure 3).

The Kupffer cells have a medium intensity reaction. In the portobiliar spaces can be generally observed moderate reactions in vascular endothelial and connective perivascular cells.

The liver in the experimental group

$NADH_2$-cytochrome-C-reductase

Enzymatic reaction intensity is nonuniform within the same liver lobule or different from one lobule to another (figure 4).

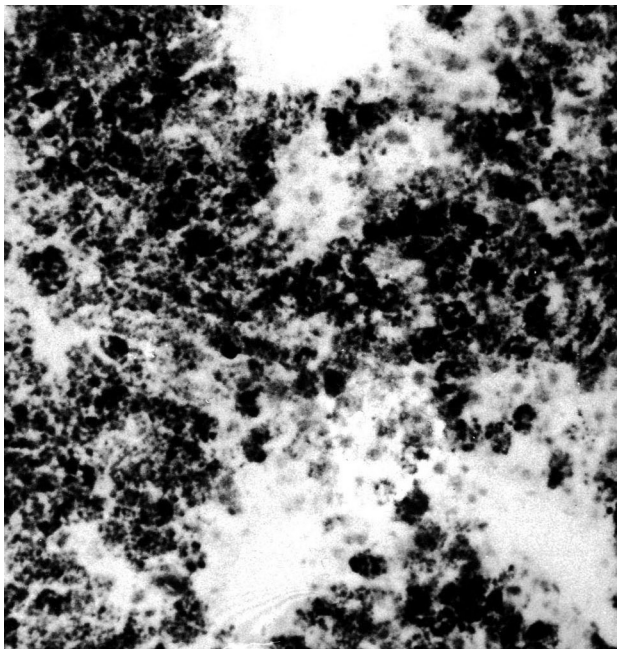

Figure 4. Rat liver with carbone tetrachloride poisoning. The enzyme has an uneven reaction in the hepatocytes. M.O.x132

In the pericentrolobular region, enzyme activity decreases strongly and unevenly in hepatocytes, in some of them up to negativity (the lack of activity). In the lobular area, hepatocytes appear structurally altered; deleting cellular contour, pyknotic nuclei, cytoplasm disorganization, or lipid loading.

The Kupffer cells are intensely reactive. Cellular and vascular structures in portobiliar space present diaphasic low and sometimes medium reactions. The fibroblasts are low, medium and intensely positive (figure 5).

Lactate Dehydrogenase

In some liver lobules, enzyme activity decreases sharply with a uniform response of hepatocytes. Pericentrolobular and periportobiliar hepatocytes remain intensely reactive (figure 6). In other liver lobules, the mosaic appearance is maintained, due to different degrees of cellular reactivity to lactate dehydrogenase.

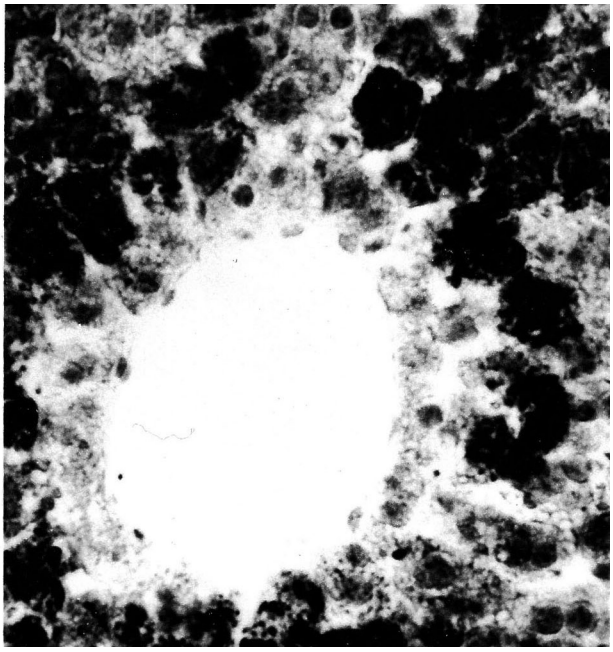

Figure 5. Rat liver with carbone tetrachloride poisoning. The pericentrolobular area presents an uneven enzymatic activity in the hepatocyte , accompanied by structural alterations of the hepatic cells. M.O. x 540

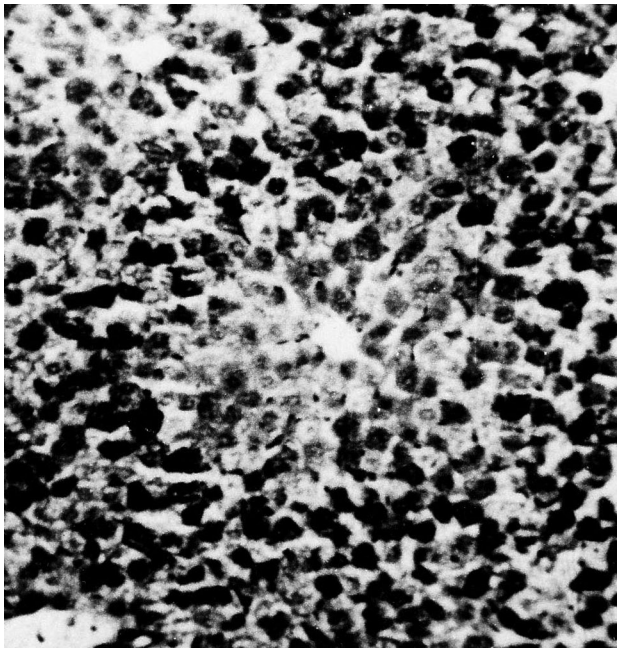

Figure 6. Rat liver with carbone tetrachloride poisoning. Powerful uneven enzymatic activity from a hepatocyte to another, from a cellular group to another, and from one lobule to another. M.O.x 132

The Kupffer cells present medium, intense and very intense reactions.

A small number of lobules show intense activity of lactate dehydrogenase concentrated only in hepatocytes around the centrolobular vein. Cells in the external and middle areas of those lobules have no reaction. In Kiernan space reaction is generally weak in the vascular endothelia, in the epithelium of the biliary duct and in fibroblasts.

Discussions

The administered poison produces metabolical and structural changes in the liver, especially in the hepatocytes. The metabolism is intensely altered for the studied oxidative enzymes, pointing out that the disruption of their activity has its target on both mitochondria by diaphorase as well as on cytosol by lactate-dehydrogenase.

Decreased activity of the $NADH_2$-cytochrome-C-reductase (diaphorase) produces a disruption in the mitochondrial function. This settlement was mentioned as a very important, fundamental element in the dysfunction of liver cells, under the toxic action of carbon tetrachloride (9), since the early studies.

Changes, expressed by the diminishing of the coenzymes content and of the substances' power of oxidation, can appear. The increase of the permeability of mitochondrial membranes, becoming very vulnerable to the activity of carbon tetrachloride as a lipid solvent can occur(10).The mitochondrial membranes , which have lipids in their structure, present a high level of alteration after inhaling the poison.

The enzymatic activities are also lowered because of the modification in some cations. Thus, under the toxic activity, the concentration of calcium in hepatic mitochondria is increased, while the concentration of potassium is decreased. These observations were obtained experimentally by repeated and prolonged inhalation of carbon tetrachloride in rats (11,12).

Altered membranes can also be observed in the rough endoplasmic reticulum, with the detaching of numerous ribosomes that had spread in the cytoplasm. An intense reduction in glucose 6-phosphatase activity and protein synthesis also appears.

The lysosome membranes are destroyed and the acid hydrolases functions fall partially or completely. Structural and ultra structural changes of cell organelles also produce disturbances of the cytosolic enzymes.

Lactate-dehydrogenase has a very nonuniform activity in hepatocytes. It presents negative reactions up to very intense reactions, sometimes exceeding the aerobic enzyme activity, the $NADH_2$-cytochrome-C-reductase. In consequence, in terms of toxic action, metabolism of carbohydrates is achieved especially by the anaerobic oxidative way.

Accumulation of lipids in hepatocytes, suggested by some authors (13) is achieved by complex mechanisms that include:

- mobilization of fatty acids from peripheral fat stores;
- the blockage of triglyceride secretion from liver cells;
- decreased mobilization of large percentages of triglycerides from the liver into the blood;
- modifying of surface tension of the membrane and cytoplasm lipids that lead to their fragmentation.

Therefore carbon tetrachloride hepatic toxicity is explained through the decrease of enzymatic activities, located on cell organelles' membranes. In this case the enzyme studied, the diaphorase, which is characteristic to the mitochondrial oxidative activity, shows a pronounced decrease of its activity.

Both oxidative enzyme changes, aerobic and anaerobic, appear not only in the hepatic cells of the same lobule but also in the ones of the neighboring lobules, thereby creating metabolic conversions in different regions .In the pericentrolobular region appear active hepatocytes, especially for lactate-dehydrogenase. Enzymatic activities are simultaneously developed in the same lobule, in the centrolobular and periportal areas. An active section appears in the center of the liver lobule, flanked by low oxidative pericentrolobular and periportal activities zones.

Certainly, these pathological issues are strongly related to the functional moments in which the liver cells are found, the local conditions during the changes with the adjacent blood vessels and the percentage of oxygen in various parts of the liver lobule. The internal lobular areas are also the headquarter of some pronounced structural alterations such as deleting the contour of the hepatocytes, cells with pyknotic nuclei, cytoplasm loaded with lipid drops, fragments of the membrane of the cells' organelles (mitochondria, rough endoplasmic reticulum, lysosomes). Kupffer cells and macrophages of the Kiernan space have a very intense enzymatic reaction , trying to remove damaged cells and this way to build a protection for complexes of the hepatic cells activities.

The observations given, sustains without any doubt, the idea that carbon tetrachloride produces serious alteration in the hepatocytes metabolism and the liver function. It is recommended to be attentive with all products that might contain this substance. Some examples are: pesticides (14), industrial emissions in fluorocarbon and semiconductors production. It should be noted that CCl_4 presents a real carcinogenic risk and is an active fibrillogenetic factor, leading to fibrosis or hepatic cirrhosis.

Conclusions

This study evaluated on rats, the toxicity of carbon tetrachloride on oxidative, aerobic ($NADH_2$-cytochrome-C-reductase) and anaerobic (lactate dehydrogenase) enzymes. Their activity, observed *in situ*, is profoundly changed, presenting: functional decline of the diaphorase in most of the hepatocytes, nonuniform reactions of both studied enzymes, lobular metabolic aerobic-anaerobic conversions in the pericentrolobular and periportobiliar liver cells. The hepatocytes, mostly the pericentrolobular ones, present structural modifications with the deletion of the cellular contour, pyknotic nuclei and lipidic accumulation.

The study draws the attention towards the prevention of use of this highly toxic substance in the industry.

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