

COLLAGEN AND ELASTIN IN THE LIVER OF RATS INTOXICATED WITH
MERCURIC CHLORIDE

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Intoxication of rats with mercuric chloride (0.5 mg Hg/kg of body weight, daily for 10 weeks) increased the hepatic contents of soluble and insoluble collagen and elastin. The increase was associated with elevated serum aminotransferase and alkaline phosphatase activities, and decreased total protein level in serum. Inflammatory changes were found in the liver. An increase in the fibrous protein content suggests that inflammatory reaction to mercuric chloride can result in hepatic fibrosis.

Inorganic mercurials accumulate mainly in the kidney and liver (1-5). Hepatotoxicity of mercury has been reported in experimental animals and workers occupationally exposed to mercurial compounds (6-11). Various mechanisms have been suggested to contribute in the pathogenesis of the hepatic damage. In general, the signs and symptoms of hepatitis have been observed. Chronic hepatitis in a majority of cases leads to fibrotic response of the liver characterized by over-accumulation of collagen (12-17). Collagen and elastic fibres distort the liver cytoarchitecture and produce the hepatic insufficiency (18, 19).

In the present paper, we report increased collagen and elastin contents in the liver of rats intoxicated for 10 weeks with mercuric chloride.

MATERIAL AND METHODS

Male Wistar rats, aged nine weeks (190 ± 10 body wt.) were used in the experiment. Animals were placed in two groups, 15 rats each. The experimental group was given aqueous solution of mercuric chloride, by gavage, in a daily dose of 0.5 mg Hg/kg of body weight, for 10 weeks. Control animals were given an equal amount of water. Rats were fed on commercial pellet diet. Both food and water were freely available. At the

end of the treatment period rats were killed by decapitation. Blood was collected from the cervical veins and liver was excised at autopsy. Collagen fractions were extracted from the liver tissue with a procedure described by *Grasedyck and co-workers* (20). The amount of collagen in the extracts was measured as hydroxyproline. Hydroxyproline was determined with the method of *Stegemann* (21). Elastin content in the liver tissue was measured as described by *Robert and co-workers* (22). In blood serum samples alanine and aspartate aminotransferase and alkaline phosphatase activities were assayed with kits from Lachema (Czechoslovakia); total bilirubin level was determined according to the method of *Jandrassik and Grof* (23); total protein concentration was assayed with the method of *Lowry and co-workers* (24). Tissue samples used for histological examination were fixed in neutral formaldehyde solution and stained with haematoxylin and eosine. Statistical significance of the differences was calculated with Student's t-test.

RESULTS

Table 1 shows that mercury treatment increased the collagen and elastin contents in the liver. Both the soluble and insoluble fractions of collagen were increased.

Table 1.

Collagen and elastin content in the liver of rats intoxicated with mercuric chloride (mean \pm SD)

Animal group	Collagen (μmol of hydroxyproline per g of wet tissue)				Elastin (mg of protein per g of wet tissue)
	Total	Neutral-salt soluble	Acid soluble	Insoluble	
Control n = 15	0.396 \pm 0.042	0.134 \pm 0.015	0.115 \pm 0.021	0.147 \pm 0.025	0.96 \pm 0.08
Mercury intoxicated n = 15	0.632 \pm 0.052*	0.198 \pm 0.023*	0.154 \pm 0.044**	0.280 \pm 0.034*	1.4 \pm 0.12*

Statistical significance of the differences from the corresponding controls: * $P < 0.001$, ** $P < 0.01$

Biochemical indices of the liver functions are shown in Table 2. Alanine and aspartate aminotransferase activities in serum were significantly elevated. Alkaline phosphatase activity and total bilirubin level were also slightly increased and total serum protein concentration decreased. These biochemical changes appeared to reflect the hepatocellular damage after mercury poisoning. Morphological studies performed in the rats treated with mercuric chloride showed the presence of inflammatory alterations

Table 2.

Indices of the liver function in serum of rats poisoned with mercuric chloride (mean \pm SD)

Animal group	Alanine aminotransferase ($\mu\text{mol}/\text{dm}^3$)	Aspartate aminotransferase ($\mu\text{mol}/\text{dm}^3$)	Alkaline phosphatase (IU/ dm^3)	Total bilirubin ($\mu\text{mol}/\text{dm}^3$)	Total protein (g/ dm^3)
Control n = 15	1.40 \pm 0.23	1.57 \pm 0.22	91.0 \pm 8.0	9.4 \pm 0.2	68.2 \pm 5.1
Mercury intoxicated n = 15	2.20 \pm 0.31*	2.30 \pm 0.28*	104.0 \pm 9.8**	13.5 \pm 1.5*	48.08 \pm 3.3*

Statistical significance of the differences from the corresponding controls: * $P < 0.001$, ** $P < 0.01$

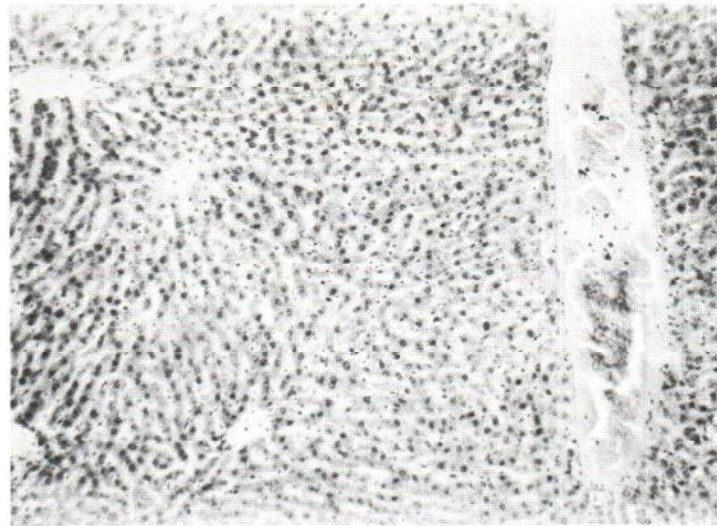


Figure 1. *Liver congestion with widening of the sinusoids. (Magn. 80x)*

within the liver parenchyma. Inflammatory infiltrations were seen particularly around the biliary ducts (Figures 1 and 2). Livers were congested and widened sinusoids were seen (Figure 3).

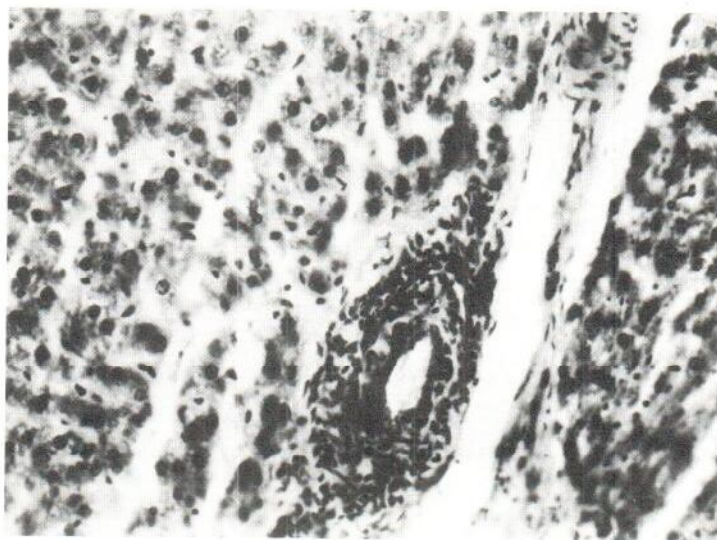


Figure 2. *Inflammatory infiltration around the biliary ducts. (Magn. 200x)*

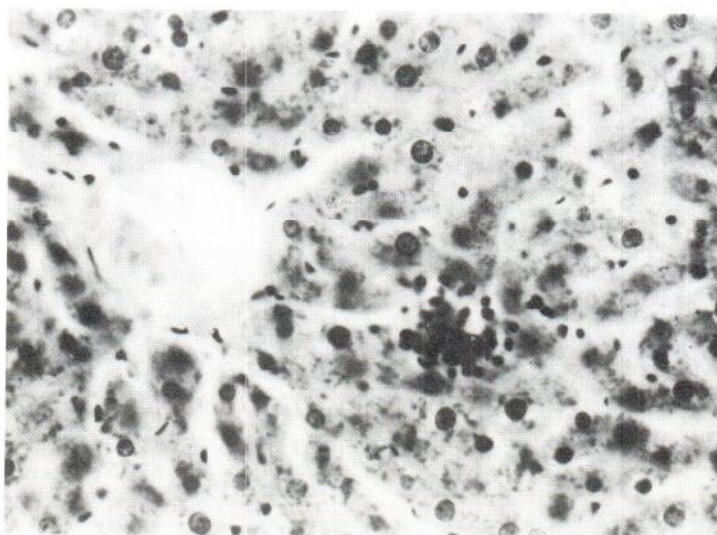


Figure 3. *Inflammatory infiltration around the necrotic hepatocytes. (Magn. 400x)*

DISCUSSION

Intoxication of rats with mercuric chloride under conditions of the presented experiment caused severe impairment of the liver function. Inflammatory changes, located mostly in the surrounding of the biliary tract, and impaired hepatic function with an elevated accumulation of collagen in the liver suggest the initial stage of hepatic fibrosis. The mechanism of this phenomenon remains unclear. It is possible that fibrosis is a non-specific response of the liver parenchyma to noxious stimuli. The fibrotic response is preceded by inflammation as it has been reported in the kidneys (1, 3, 5). On the other hand, as heavy metals have been shown to influence collagen synthesis and degradation, (25, 26) a direct effect on collagen metabolism cannot be discarded.

Further studies to elucidate molecular and cellular mechanisms of mercury-induced hepatic damage as well as the kinetics of development of hepatitis in relation to collagen accumulation are needed.

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Sažetak

KOLAGEN I ELASTIN U JETRI ŠTAKORA OTROVANIH ŽIVINIM KLORIDOM

Trovanje štakora živinim kloridom (0,5 mg Hg/kg tjelesne težine na dan tijekom deset tjedana) imalo je za rezultat povećan sadržaj topljivog i netopljivog kolagena i elastina u jetri. Povećanje je dovedeno u vezu s povišenim aktivnostima aminotransferaze i alkalne fosfataze u serumu, i sa smanjenim nivoom ukupnog proteina u serumu. U jetri su zamijećene upalne promjene. Povišen sadržaj vlaknastog proteina upućuje na to da upalna reakcija na živin klorid može dovesti do fibroze jetre.

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