COPPER CONTENT IN THE LIVER OF MAN AND EXPERIMENTAL ANIMALS WITH ACUTE TOXIC HEPATITIS

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The copper content in the liver was lowered in persons dying from toxic degeneration of the liver and in rabbits and rats poisoned with carbon tetrachloride. Administration of microdoses of copper to the animals restored the copper concentration in the liver and had a normalizing effect on the structure of the organ.

The biological role of copper is the subject of an extensive lierature [1, 4, 5]. Disturbances of the copper concentration in the blood and internal organs and of copper metabolism have been demonstrated in various diseases, some of them infectious [2, 7, 8]. The authors' previous investigations, using emission spectral analysis, demonstrated an increase in the blood copper concentration at the height of infectious hepatitis [6].

In the present investigation the method of quantitative emission spectral analysis, using the ISP-30 apparatus and the Okamoto–Usman histochemical method, was used to study the concentration and localization of copper particles in the liver of ten healthy persons dying accidentally, and 7 patients with infectious hepatitis dying with manifestations of toxic degeneration of the liver. The copper concentration was also studied in the liver of 12 healthy chinchilla rabbits and of rabbits with acute hepatitis produced by a course of three parenteral injections of 40% CCl_4 solution in peach oil, in a dose of 0.5 ml/kg per injection, at intervals of 24 h (18 animals).

In order to study whether copper can be used for therapeutic purposes in acute liver lesions, the effect of enteral administration of a single microdose (53 rabbits) and of three parenteral injections (96 rats) of microdoses of copper (0.6 and 0.3 mg/kg per injection) on the concentration of copper in the liver was investigated.

EXPERIMENTAL METHOD

Before spectral analysis, the dried organs were incinerated in a muffle furnace at 400-420° to obtain a whitish-gray powder. The mean ash index was 1:80.

Pieces of liver measuring 0.3 × 0.2 cm for histochemical investigation were fixed in Carnoy's fluid and in a 0.1% alcoholic solution of rubeanic acid, passed through alcohols of increasing concentration, and embedded in paraffin wax. Dewaxed sections were stained with hematoxylin-eosin and by the Feulgen and Brachet reactions for DNA and RNA, with the appropriate controls; acid mucopolysaccharides were determined by Hale's reaction and neutral mucopolysaccharides by the McManus method.

EXPERIMENTAL RESULTS

The results of spectral investigation of copper in the human liver were as follows: in the healthy liver its concentration was 0.61±0.03 mg%, and in the liver of patients dying from toxic degeneration the copper concentration was sharply reduced to 0.24±0.01 mg%.

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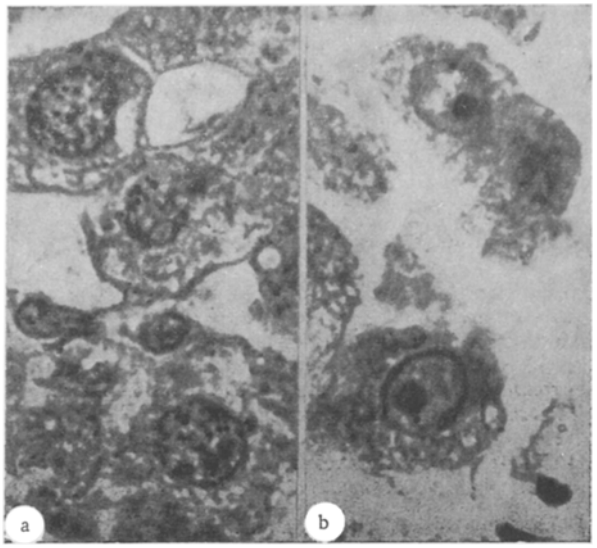

Fig. 1. Photomicrograph of human liver. a) Healthy person dying accidentally; b) patient dying from toxic degeneration of the liver: marked decrease in number of copper granules in nuclei of hepatocytes. Okamoto – Usman method, 900 ×.

TABLE 1. Copper Concentration in Liver of Rabbits with Acute Experimental Hepatitis (M±m)

Group of rabbits	Copper concentration (in mg %)
Intact	0.49±0.006
With hepatitis	0.27±0.004
Receiving copper	0.57±0.007
With hepatitis and receiving copper	0.54±0.009

Histochemical tests of the healthy human liver revealed very many small dark granules of copper on the surface of the nuclear membranes of the hepatocytes. The copper granules did not extend outside the nuclei, and the Kupffer cells were free from them. Sections of the liver from persons dying in hepatic coma showed a sharp decrease in the number of unchanged hepatocytes (Fig. 1). Most liver cells showed degenerative changes. The number of copper granules on the surface of the karyolemma was considerably reduced. Hepatocytes whose nuclei were completely without copper granules were frequently seen. Copper granules appeared, sometimes in large numbers, in the cytoplasm of the reticular cells from the liver of some patients dying from toxic degeneration of the liver.

The experiments on animals also revealed a decrease in the copper concentration in the liver in toxic hepatitis (Table 1).

Histochemical investigation showed a marked decrease in the number of copper granules in the hepatocytes (Fig. 2), an increase in the cytoplasmic RNA content, a decrease in the glycogen content, and an irregular distribution of neutral mucopolysaccharides in the cytoplasm in acute experimental hepatitis. A small quantity of Hale-positive substances, which were absent in the liver cells of healthy animals, appeared in hepatocytes with a deformed, disintegrating nucleus.

Administration of microdoses of copper to the healthy animals and to animals with hepatitis, both when a single enteral dose was given (rabbits) and, to a greater degree, after three parenteral injections of doses of 0.6 and 0.3 mg/kg (albino rats), led to a marked increase in the concentration of the trace element in the liver. After administration of copper in doses of 0.3 mg/kg parenterally for 3 days, a sharp increase in the number of Kupffer cells in the liver tissue was found 24 h after the last injection, which may account for reports in the literature that copper stimulates antibody formation [3, 4].

Copper was also found to have a definite normalizing effect on liver structure. Although the edema and cloudy swelling of the liver parenchyma, characteristic of acute hepatitis, still remained, they were not diffuse but localized in character. The cytoplasm of the hepatocytes stained clearly with pyronine, and the cytoplasmic RNA was uniformly distributed. The number of RNA granules remained increased, as during hepatitis. The cell nuclei were less deeply stained than normally. The number of Kupffer cells was clearly increased.

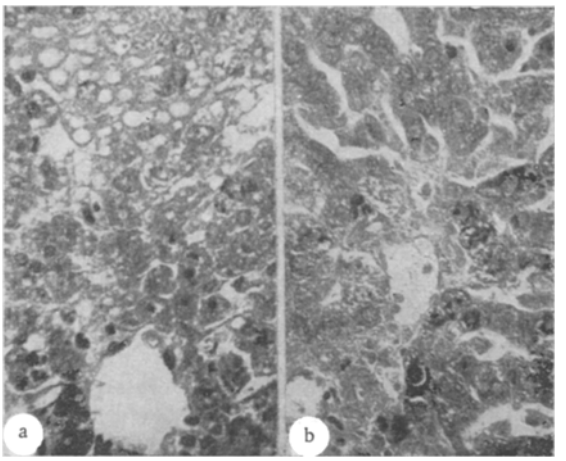

Fig. 2. Photomicrograph of rat liver. a) Acute CCl_4 hepatitis; b) acute CCl_4 hepatitis + copper, 0.3 μg/kg parenterally daily for 3 days. Hematoxylin-eosin, 400 ×.

Consequently, both under clinical conditions (in patients with infectious hepatitis who developed toxic degeneration of the liver) and in experiments on animals, acute liver damage is accompanied by loss of the trace element copper. In experiments on animals, enteral and parenteral administration of microdoses of copper helps to restore the copper concentration in the liver and the normal morphological structure of the liver parenchyma.

Parenteral injection of copper daily for 3 days produces a marked increase in the number of Kupffer cells. In the authors' opinion, this explains the ability of copper to stimulate immunogenesis.

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