Acta vet. scand. 1969, *10*, 288—291.

Brief communication

HISTOCHEMICAL DETECTION OF COPPER IN THE LIVER FROM SHEEP CHRONICALLY POISONED BY COPPER

Continued ingestion of copper in excess of the nutritional requirement leads in all animals to its passive accumulation within the tissues, especially the liver. Up to certain levels varying greatly with the species high concentrations of copper in the liver appear to impose no physiological hardship on the animal. Above these levels there may occur a catastrophic liberation of a high proportion of the copper into the blood stream resulting in extensive hemolysis and jaundice usually followed by death. Sheep are more prone to the hemolytic crisis of chronic copper poisoning than other animal species. The hemolytic crisis of chronic copper poisoning is associated with centrolobular necrosis of the liver. The copper concentration in the liver is usually 300 p.p.m. or more (wet tissue) compared with about 50 p.p.m. in normal sheep (*Simesen & Møller* 1969).

It has been the aim of this investigation to examine if a specific histochemical method of copper demonstration in the liver tissues from sheep could be a help in establishing the diagnosis of chronic copper poisoning.

Copper has been visualized histochemically in liver tissue from calves (*Weiss et al.* 1967).

MATERIAL AND METHOD

The rubeanic acid method was used for the histochemical demonstration of copper. This method can be used for both fresh and formalin fixed tissue. The rubeanic acid (dithio-oxamide) binds with copper to form a black precipitate (copper rubeanate). Regarding the detailed technical procedure, reference is given to *Uzman* (1956) and *Thompson* (1966).

The method was applied to liver tissues originating from six chronically copper poisoned sheep (three dead, one killed and liver biopsies from two), and from two normal sheep after slaughter (controls). The diagnosis of a copper poisoning was

quantitatively chemically verified on liver tissue from all sheep. Duplicate sections from all livers were stained with Harris-hematoxylin-Eosin.

RESULTS

In the conventionally stained sections from the six poisoned sheep toxic damage of the parenchyma with periportal cirrhosis and heavy intrahepatic cholestasis were found. A normal histological picture was found in the sections of the two controls.

In the rubeanic acid-treated sections the following observations were made.

The three dead sheep: The black precipitate appeared equally distributed in the hepatic lobules, preferably localized periportally and around the central veins. It was present in the cytoplasm of the liver cells as small and large amorphous granules, mainly in a peribiliary localization. In several intrahepatic bile plugs copper precipitations were noted. In the nuclei of the liver cells no copper was detected, nor was it found in Kupffer cells.

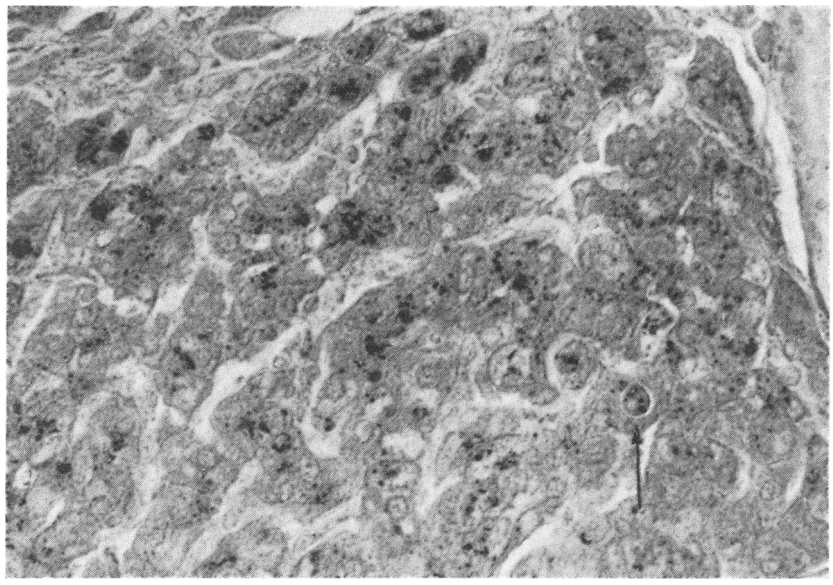

Figure 1. Formalin-fixed liver tissues originating from a sheep suffering from chronic copper poisoning. The copper precipitations are localized intracytoplasmic and periportally. The arrow points to an intrahepatic bile plug containing copper. (Rubeanic acid with Eosin as contrast × 300).

Two types of copper-containing liver cells could be distinguished. One type were intact cells where the protein-bound copper was found as distinct granules sharply separated from the surroundings. The other type were cells with regressive changes where the cytoplasm was diffusely grey-coloured, though very often containing some darker indistinct clumps.

The killed sheep, and biopsies from two sheep: An unequal distribution of the copper among the liver lobules was found. In the affected lobules the copper was predominantly located periportally and in broad "strings" running from the triads towards the central veins. Only intact cells with distinct granules were found. Here, too, copper-containing bile plugs could be detected.

The two control sheep: The copper was observed as fine grey dusty precipitations diffusely distributed in the liver cell cytoplasm, predominantly periportally.

DISCUSSION

A certain difference in the copper distribution in the livers of the dead sheep and the others was found. In the sheep dead during the hemolytic crisis the copper was present in all liver lobules, predominantly periportally and around the central vein, whereas in sheep in the accumulating phase it was located in fewer functional units.

In the dead sheep the histochemical picture of the degenerated liver cells could be interpreted as a release of copper bound to protein, decomposed by the necrobiosis of the cells during the hemolytic crisis.

Copper precipitations in the bile plugs establish that an excretion of copper occurs also during the accumulation phase. This states the supposition put forward by *McCosker* (1968).

In the control livers the copper precipitations were very fine, grey and dusty, in contrast to the accumulation phase, where the precipitations were found as both small and large black granules.

Sheep chronically poisoned by copper develop toxic damage of the liver cells, periportal cirrhosis, as well as intrahepatic cholestasis (*Nillson* 1968, *Simesen & Møller* 1969), but the same changes also occur after ingestion of hepatotoxins, e.g. pyrrolizidin alkaloids.

Using a histochemical copper reaction on liver biopsies from sheep suspected of suffering from chronic copper poisoning it

seems possible to add evidence to the establishment of the diagnosis. The method might also contribute to the further elucidation of the pathogenesis of chronic copper poisoning in sheep.

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(Received July 9, 1969).