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Histochemical Reactions in the Rat Liver After Experimental Application of Vibramycin

Odczyny histochemiczne w wątrobie szczura po doświadczalnym stosowaniu wibramycyny

Vibramycin has a high capacity of penetration from the blood stream to most tissues and sytemic fluids. Its concentration is the highest in the liver, where its level may be several times higher than in the blood. It is even believed that part of the Vibramycin is reabsorbed in the intestinal-hepatic circulation (1, 3).

Tetracyclins are known to be hepatotoxic and when their validity has expired they may, according to literature data (8) cause lesions of the liver. Considering the above mentioned facts it was decided to examine histologically and histochemically the influence of Vibramycin on the morphology and enzymic function of the liver cells of experimental animals after an increased dose of the drug when valid and after expiration.

MATERIAL AND METHODS

White Wistar rats weighing ca 260 g were used in the experiments. They were divided into 3 groups, 2 experimental ones and 1 control. The animals were given intragastrically a preparation of doxycyclin — Vibramycin for consecutive 10 days in the form of suspension in distilled water once daily before feeding in the morning.

Rats of group I received each 8 mg of the valid antibiotic, and group II rats the same amount of the drug with expired validity. The control animals were given 2 ml of distilled water under the same conditions. Over the 10-day period each experimental animal invested 80 mg of Vibramycin.

The next day (24 h) after the last portion of the drug had been given the animals were decapitated and liver segments were taken and fixed in Carnoy and Baker's solutions. The method of staining with haematoxylin and eosin was applied and the activity of acid phosphatase was revealed by the histochemical methods after Gomori, ATP-ase after Wachstein and Meisel, G-6-Phase after Wachstein and Meisel and glycogen after McManus and by the PAS method.

RESULTS

Staining with haematoxylin and eosin

As compared with the control sections the cytoplasm of hepatocytes from the livers of group I rat becomes more acidophilic, the cell nuclei in some cells are enlarged, the capillaries, especially close to the central veins are widened (Fig.1). In the liver of animals of group II structurally changed regions were observed to be filled with cell filtrate. In the neighbourhood of these regions the hepatocytes stained more intensively (Fig. 2).

Acid phosphatase

An intensive reaction in the form of coloured granules was localized in the hepatocytes, especially in the region of the bile ducts in the livers of all the groups (Fig. 3), whereas in both experimental groups the reaction was most pronounced in the Browicz-Kupffer cells. Not only the acid phosphatase activity was enhanced in them, but the cells were enlarged (Fig. 4).

Adenosinetriphosphatase (ATP-ase)

This enzyme exhibited a moderate reaction in the control and group I livers in the bile ducts and walls of the blood vessels (Fig. 5). In the livers of group II animals ATP-ase activity was increased involving also Kupffer's vacuoles in the hepatocytes (Fig. 6).

Glucose-6-phosphatase (G-6-P-ase)

The relation in the livers of the experimental groups was more pronounced as compared with the control picture. The coloured grains were observed above all in the peripheral zones of the hepatic lobules (Fig. 7). A diffusional reaction (apart from the granules) was also noted in the livers of rats of group II.

Glycogen

Glycogen activity was similar in the livers of the controls and those of group I rats (Fig. 8). In sections from the livers of group II rats the reaction was less intensive, but more numerous cells were diffusivelly coloured. The hepatocytes lying close to structurally changed regions showed a particularly strong activity (Fig. 9). The control preparations with diastase gave a negative reaction.

DISCUSSION

After introduction of tetracyclins into therapy (6) described the toxic effect of these compounds on the liver. Among other symptoms jaundice, enlargement of liver and vacuolization of the hepatocytes were noted in the histological picture. After Vibramycin application the side-effects are probably less pronounced (7). It results from other reports that expired tetracyclins are highly toxic (8), however, data concerning Vibramycin are lacking. In the present study it was decided to examine the rat livers after a 10-fold dose of the antibiotic per 1 kg of body weight than that applied to humans, both valid and after expiration of validity.

It was found after staining with haematoxylin and eosin that the acidophilicity of the cytoplasm of the experimental animals was increased, moreover, after the expired drug structurally changed regions appeared to be filled with cell filtrate.

The enhanced acid phosphatase activity, especially in the Browicz-Kupffer cells, of rats of both experimental groups is evidence of increased lytic processes and agrees with the reports of numerous authors (1, 4, 5).

The increased reaction for ATP-ase in the Kupffer vacuoles of liver after expired Vibramycin application is probably connected with an intrahepatic stasis of bile with which the drug is also excreted.

G-6-P-ase activity in the hepatocytes is closely bound with the reaction for glycogen, since with the increase of this enzyme activity, the glycogen activity decreases (2). The enhanced intensiveness of reaction for G-6-P-ase in the livers of the experimental animals and depressed reaction for glycogen point to the influence of Vibramycin hydrocarbon transformations in the liver.

To sum up, Vibramycin, especially when its validity is expired, has an unfavourable influence on the liver in experimental animals, however, not as severe as it might have been expected.

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STRESZCZENIE

Badano wątroby szczurów po podawaniu przez 10 dni zwiększonej dawki wibramycyny (przed upływem terminu ważności i przeterminowanej). Stosowano barwienie histologiczne hematoksyliną i eozyną oraz histochemiczne: wykrywanie aktywności fosfatazy kwaśnej (Fk), adenozynotrójfosfatazy (ATP-azy), glukozo-6-fosfatazy (G-6-P-azy) i glikogenu. U zwierząt doświadczalnych zaobserwowano zmiany w miąższu wątroby (szczególnie po wibramycynie przeterminowanej) wyrażające się pojawieniem obszarów strukturalnie zmienionych. Znacznie zwiększyła się aktywność Fk, ATP-azy i G-6-P-azy, natomiast odczyn na glikogen miał charakter dyfuzyjny. W miejscach strukturalnie zmienionych reakcja na glikogen była znacznie silniejsza niż w wątrobie kontrolnej.

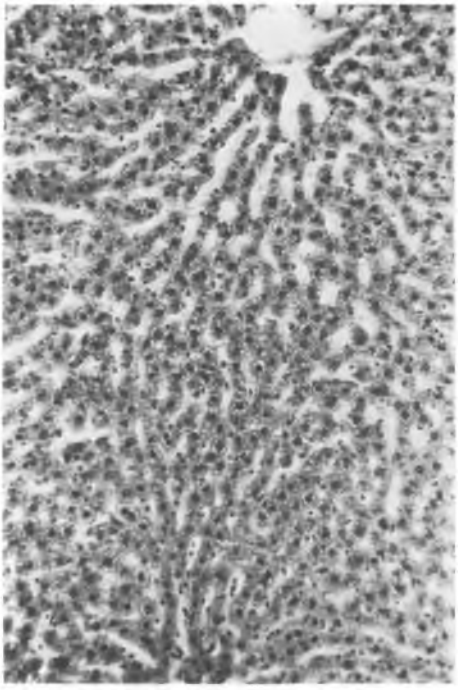

Fig. 1. The liver of the rat, experimental group I. Hematoxylin and eosin. Magn. 200 ×

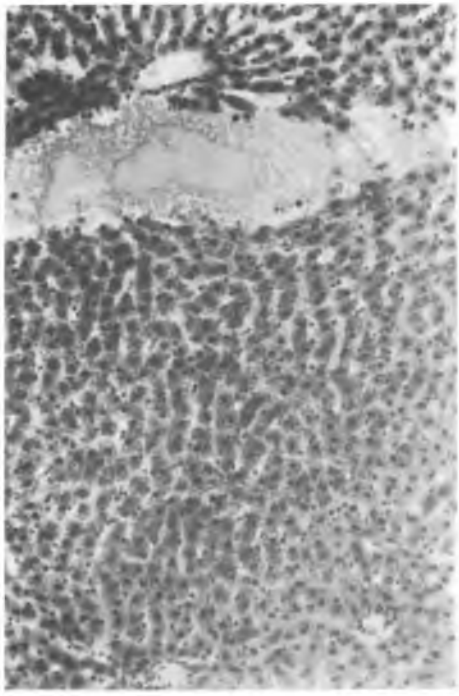

Fig. 2. The liver of the rat, experimental group II. Hematoxylin and eosin. Magn. 200 ×

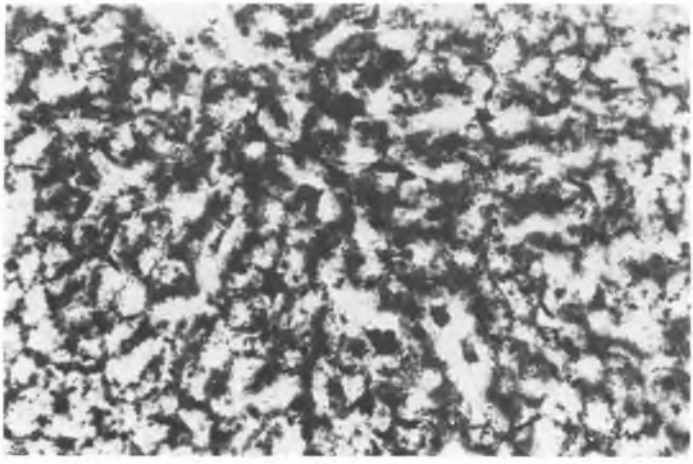

Fig. 3. The liver of the rat, experimental group I. Acid phosphatase by Gomori method. Magn. 200 ×

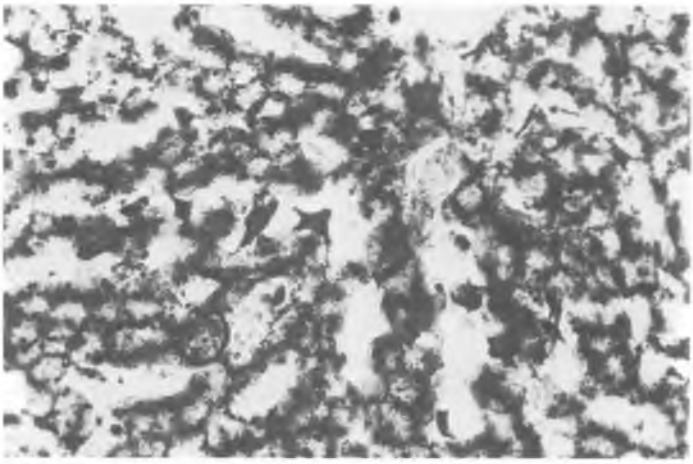

Fig. 4. The liver of the rat, experimental group II. Acid phosphatase by Gomori method. Magn. 200×

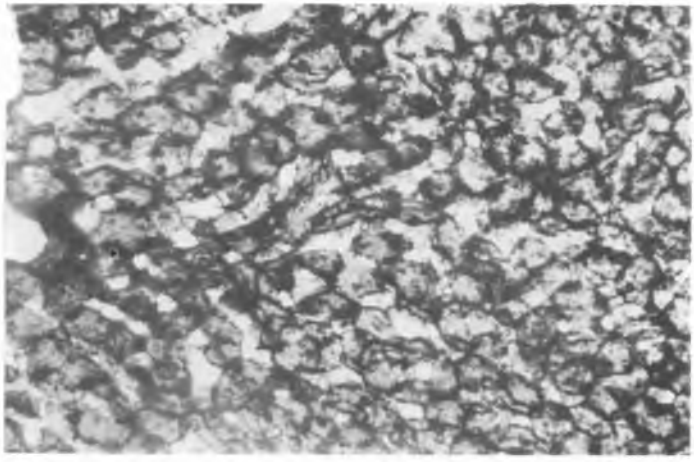

Fig. 5. The liver of the rat, control group. Adenosinetriphosphatase by Wachstein and Meisel method. Magn. 200×

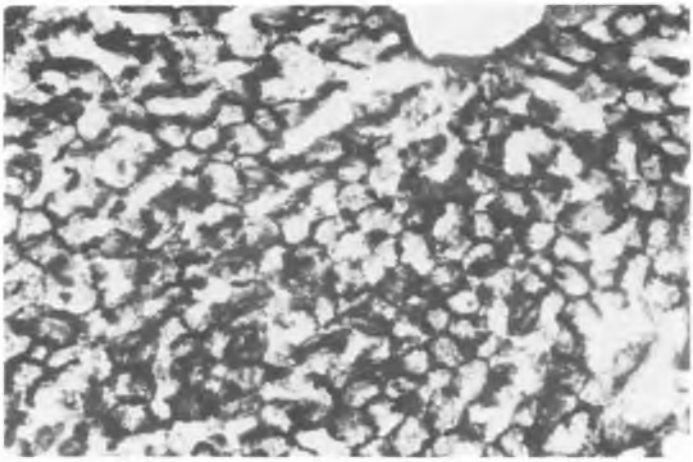

Fig. 6. The liver of the rat, experimental group II. Adenotriphosphatase by Wachstein and Meisel method. Mag. 200×

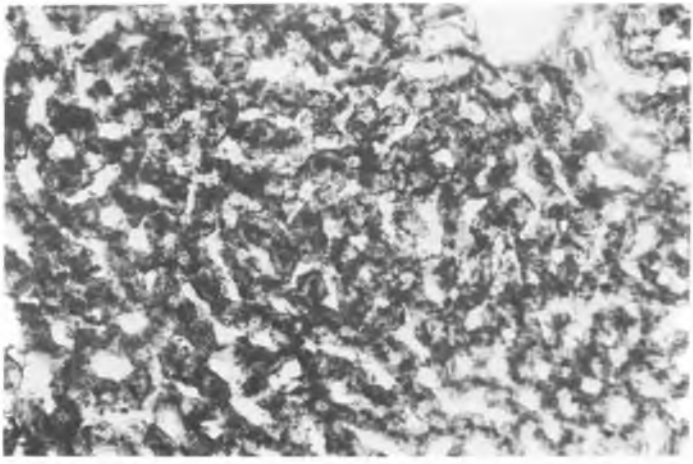

Fig. 7. The liver of the rat, experimental group I. Glucose-6-phosphatase by Wachstein and Meisel method. Magn. 200×

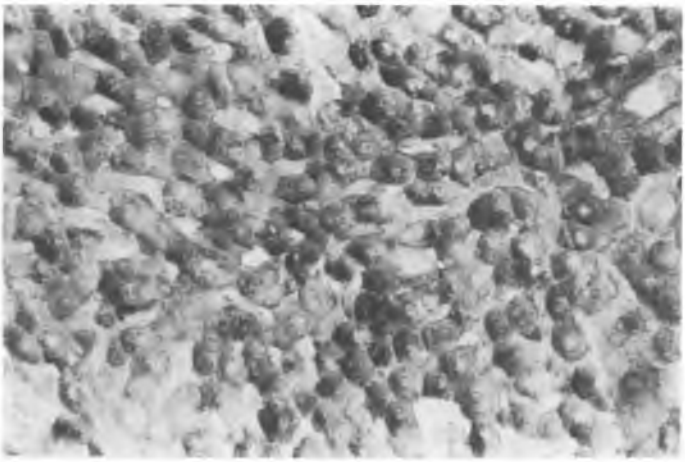

Fig. 8. The liver of the rat, experimental group I. PAS method by McManus. Magn. 200×

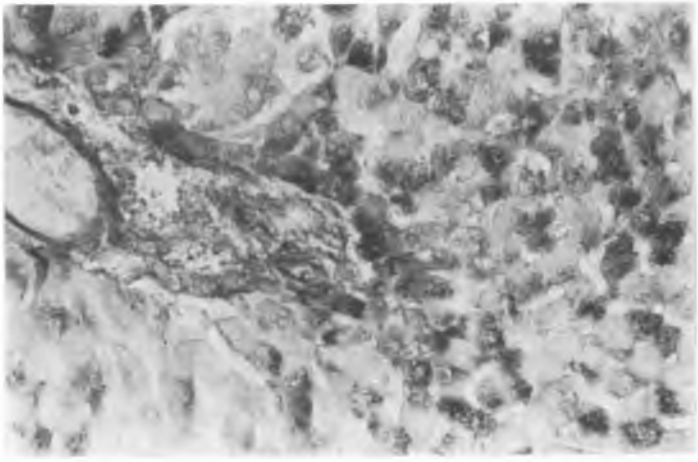

Fig. 9. The liver of the rat, experimental group II. PAS method by McManus. Magn. 200×