ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN—POLONIA
VOL. XL, 39 SECTIO D 1985

Katedra i Zakład Histologii i Embriologii. Akademia Medyczna w Lublinie
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Histological and Cytochemical Examination of the Small Intestine in the Rat After Surgical Removal of the Hepatic Lobe and Experimental Administration of Biseptol 480

Histologiczne i cytochemiczne badania jelita cienkiego szczura po chirurgicznym usunięciu płata wątroby i doświadczalnym podawaniu Biseptolu 480

Гистологические и цитохимические исследования тонкой кишки крысы после хирургического удаления доли печени и после опытного применения Бисептола 480

In recent years a new kind of pharmacological procedure has been used, consisting in combining known anti-bacterial substances for joint administration. An example of this use of synergism is provided by Biseptol 480, among the components of which are sulphamethoxyl (SMZ) and trimethaprim (TMP), since tests have shown that the antibacterial effect of each of them when used separately is intensified when the two compounds are combined. It has also been found that TMP, which is a cytostatic, inhibits the decomposition of SMZ, and consequently prolongs the period over which this sulphonamid is active (2, 5, 6).

Biseptol 480 is well tolerated by patients, quickly assimilated from the alimentary tract, reaching a high degree of concentration in organs as early as 2—4 hrs after ingestion. Occasionally, however, during the course of protracted treatment, side-effects involving the alimentary system occur: abdominal pain, vomiting and nausea (5), the causes of which are not fully known. On this account there have been carried out studies which aimed at determining possible contraindications to the application of this drug, and also its toxicity.

MATERIAL AND METHODS

White male rats, from 300—350 g in body weight, were used for the experiments. They were divided into three groups: two experimental and one control. An operation to remove the right lobe of the liver was performed on each of the animals in experimental group I. Two weeks after the operation each rat was given 2 cm^3 of Biseptol 480 water suspension intragastrically for 7 consecutive days after the morning feed, that is, a dose 10 times greater (when converted to kg of body weight) than for humans. In all each rat received 560 mg of the drug. No operation was performed on rats in experimental group II, but they were given identical doses of Biseptol 480 to those used for experimental group I, for 7 consecutive days.

The third group consisted of control animals which were given 2 cm^3 of distilled water in the same way as the doses in the experimental groups.

Twenty-four hours after giving the final dose of Biseptol 480 the animals were decapitated, and after opening the abdominal cavity sections of the small intestine were taken for histological (staining with hematoxylin and eosin) and histochemical tests (detection of the activity of acid phosphatase and alkalic phosphatase by the Gomori method, glucose-6-phosphatase by the Wachstein and Meisel method, lipase by the Gomori and Takamatsu method and polysaccharides by the PAS method after McManus).

RESULTS

Staining with hematoxylin and eosin showed that in comparison with control animals, enterocyte cytoplasm had become more acidophilic in the operated rats, while their intestinal epithelium was observed to be lower (Fig. 1, 2). Preparations from the intestine of animals in experimental group II did not differ from those of control animals (Fig. 3).

Histochemical tests showed that acid phosphatase in control animals exhibited activity primarily in the striated limbus of the enterocytes. A weak reaction was observed in the intestinal glands (Fig. 4). Reduction in enzyme activity was observed in rats in the experimental group (Fig. 5). Reaction was also weaker in the intestines of animals in the experimental group II in comparison with control preparations, but greater than in the group of operated animals.

Alkalic phosphatase in enterocytes in experimental group I exhibited increased activity in comparison with the control, whereas in the intestines of group II of rats it was similar to control preparations (Figs. 7 and 8).

Glucose-6-phosphatase also exhibited a greater reaction in rats from the experimental group I. The enzyme was observed in enterocytes, particularly in the striated limbus (Fig. 6). Pictures of the enzyme's activity in the control and experimental group II were similar.

Lipase, exhibiting a moderate coloured reaction in the intestinal epithelium, the stroma of villi and intestinal glands, did not react by a change in activity to either hepatectomy or to Biseptol 480.

In the test for polysaccharides it was noticed that a strong PAS-positive reaction occurred in mucocytes and in the striated limbus of enterocytes. A coloured reaction was also characteristic of the basement membranes in intestinal glands and in cell membrane. The submucous membrane and muscular membrane also exhibited activity. In the experimental group I of rats the reaction was stronger than in the control and experimental group II (Fig. 9).

It must be emphasized that mucocytes in the control animals exhibited different phases of the secretory process, whereas in rats from the experimental groups the majority of the beaker cells appeared to be in the resting phase.

DISCUSSION

No data have been found in the literature available on enzymatic reactions in different parts of the alimentary tract after experimental administration of Biseptol 480. It was therefore decided to trace the behaviour of some enzymes and polysaccharides in the small intestine of healthy rats given the preparation, and also animals to which the drug was administered two weeks after hepatectomy. These individuals were given ten times larger doses per kg of body weight than given to humans, in order to determine the toxicity of Biseptol 480.

The participation of phosphatases in different stages of cell metabolism is particularly significant if the fundamental importance of phosphorus metabolism in the energetic processes of the cell is taken into account. Acid phosphatase, which is the exponential of intracellular catabolic processes (4, 8, 9) in cells of the intestinal epithelium, is located particularly in lysosomes and the Golgi apparatus. In man and rats the striated limbus, ergastoplasma and lateral cell membranes of enterocytes exhibit intensive activity (4, 8). Reduction of the reaction in the group of operated animals would seem to indicate that the chief reason for this was removal of the hepatic lobe since in animals on which no operation was performed but which were given the drug, acid phosphatase activity was similar to the picture in control rats.

Alkalic phosphatase, primarily responsible for the processes of active transport through the cell membranes and engaged in synthesis of nucleic acids (3, 9) exhibited increased activity in the striated limbus in animals

on which the operation was performed. This phenomenon can be explained after Staszyc (9) by the general biological properties of alkalic phosphatase.

The participation of glucose-6-phosphatase in the specific metabolism of the intestine has not as yet been thoroughly investigated. It has, however, been observed that increase in the amount of smooth membranes in the absorptive cells of the intestinal epithelium is expressed by an increase in the activity of this enzyme (7). Calvert et al. (1) consider that glucose "liberated" by the mucous membrane of the small intestine with the participation of glucose-6-phosphatase may be used to supply the energy necessary for constant differentiation of enterocytes. Beaker cells are devoid of this enzyme. A reagent to glucose-6-phosphatase in the intestine of the control group was manifested in the form of granular matter in the striated limbus and supranuclear and parabasal cells. It if is accepted that the increased reaction observed in operated animals forms evidence of increase in the amount of smooth membrane in enterocytes, this would mean that metabolism was accelerated in the latter.

Lipase did not alter its activity in the small intestine of animals in the two experimental groups, whereas polysaccharides exhibited increased reaction in operated animals. As is well-known, the process of mucinogenesis is connected with certain stimuli (9) and it would therefore appear that in this case greater reaction was caused by the operation than by the administration of the drug.

To sum up this discussion it must be added that standard histological examination revealed only slight changes in the two experimental groups, in respect of: height of epithelium, acidophilism, of cytoplasm or compact arrangement of cells. These changes may, however, be considered as physiological.

REFERENCES

1. Calvert R. et. al: Development Pattern of Glucose-6-Phosphatase Activity in the Small Intestine of the Mouse Fetus. Histochemistry **63**, 209, 1979.
2. Garrod L. P. et al.: Antybiotyko- i chemioterapia. PZWL, Warszawa 1983.
3. Gutschmidt S. et al.: Kinetic Characterization on Unspecific Alkaline Phosphatase at Different Villus Sites of Rat Jejunum. Histochemistry **69**, 189, 1980.
4. Hugon J., Borges M.: Fine Structural Localization of Acid and Alkaline Phosphatase Activities in the Absorbing Cells of the Duodenum of Rodents. Histochemie **12**, 42, 1968.
5. Kędra M. et al.: Zastosowanie Biseptolu w leczeniu zakażeń przewodu pokarmowego. Pol. Tyg. Lek. **9**, 371, 1974.
6. Klimowicz A.: Farmakologia Kliniczna Kotrimoksazolu (Biseptolu). Post. Hig. Med. Dośw. **37**, 537, 1983.

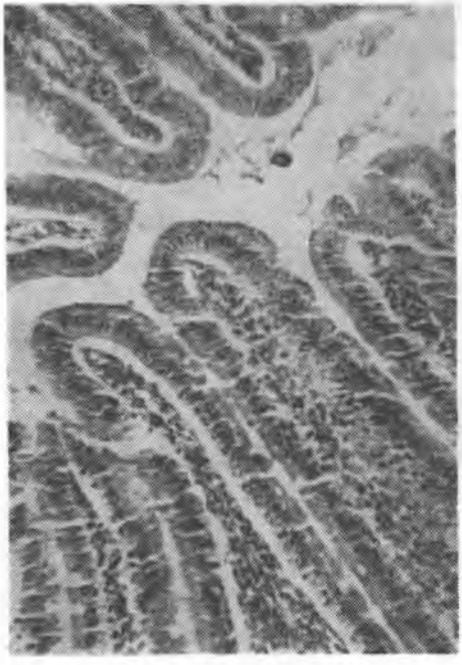


Fig. 1

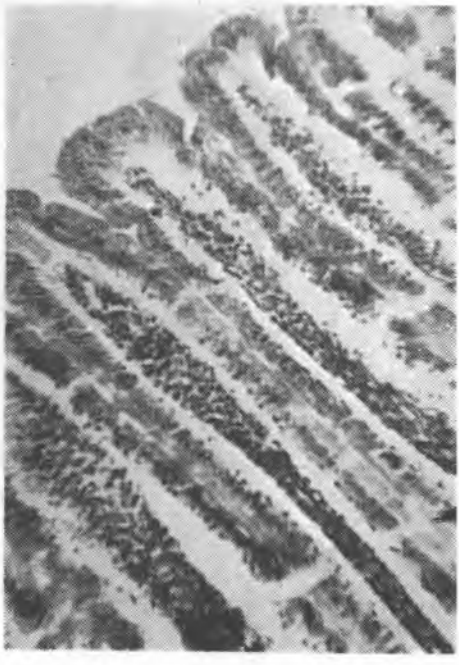


Fig. 2

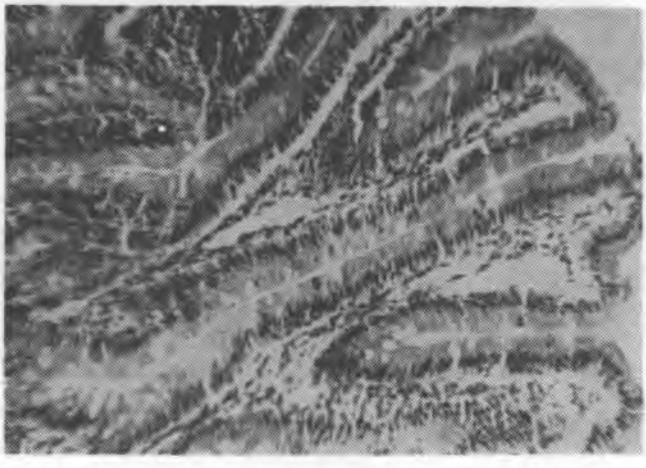
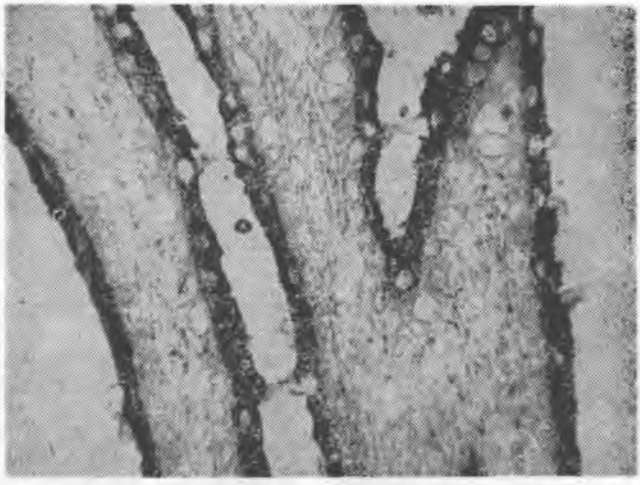


Fig. 3



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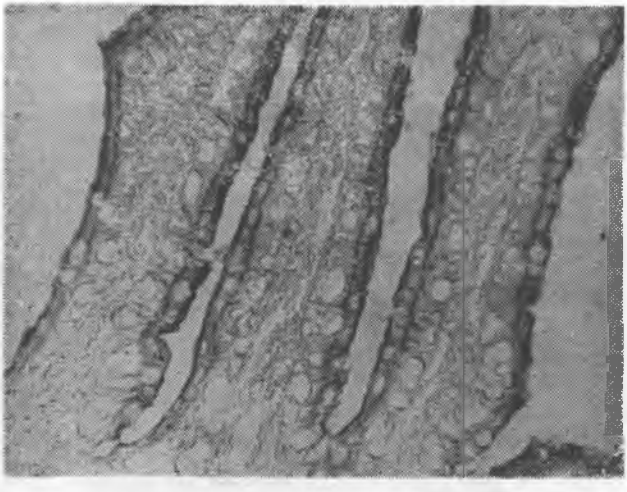

Fig. 5

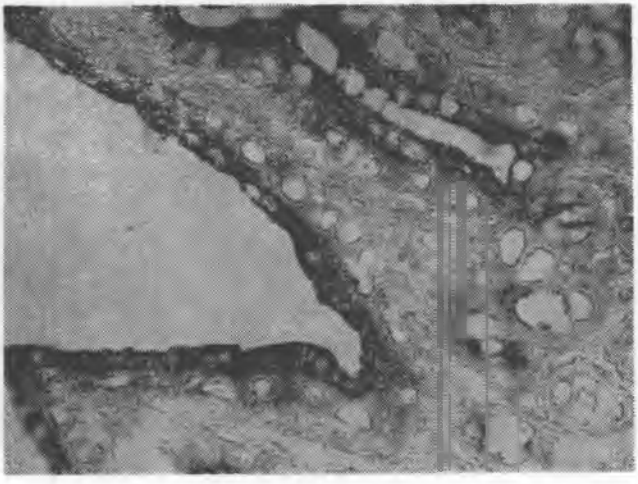

Fig. 6

Fig. 7

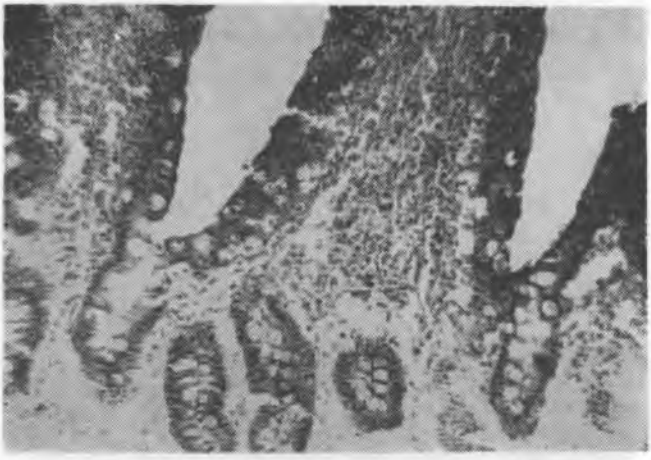

Fig. 8

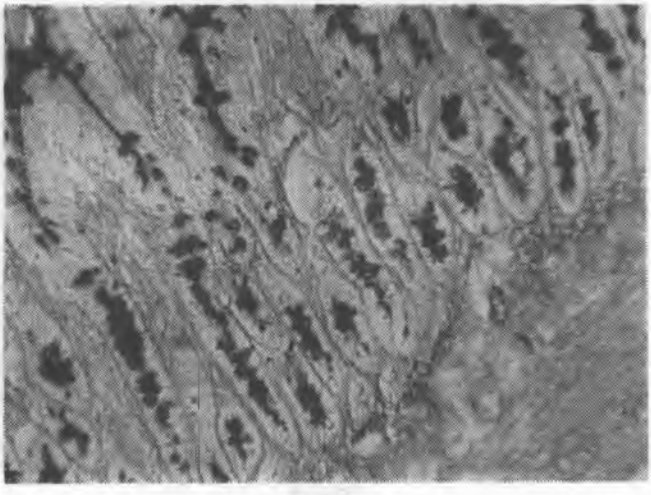

Fig. 9

7. Ménard D.: Ultrastructural Localization of Intestinal Glucose-6-Phosphatase Activity During the Postnatal Development of the Mouse. Histochemistry 67, 53, 1980.
8. Ono K.: Ultrastructural Localization of Acid Phosphatase Activity in the Small Intestinal Absorptive Cells of Adult Rats. Histochemistry 62, 113, 1979.
9. Staszyc J.: Wpływ doświadczalnej wagotomii na cytofizjologię komórek enterochromafinowych. Ann. Univ. M. Curie-Skłodowska, Lublin, Sectio D 26, 191, 1971.

Otrzymano 12 IV 1985.

EXPLANATION TO FIGURES

Fig. 1. The small intestine of the rat, control group. Hematoxylin and eosin. Magn. 200×.

Fig. 2. The small intestine of the rat, Ist experimental group. Hematoxylin and eosin. Magn. 200×.

Fig. 3. The small intestine of the rat, IInd experimental group. Hematoxylin and eosin. Magn. 200×.

Fig. 4. The small intestine of the rat, control group. Acid phosphatase by Gomori method. Magn. 200×.

Fig. 5. The small intestine of the rat, Ist experimental group. Acid phosphatase by Gomori method. Magn. 200×.

Fig. 6. The small intestine of the rat, Ist experimental group. Glucose-6-phosphatase by Wachstein and Meisel method. Magn. 200×.

Fig. 7. The small intestine of the rat, experimental group. Alcaline phosphatase by Gomori method. Magn. 200×.

Fig. 8. The small intestine of the rat, IInd experimental group. Alcaline phosphatase by Gomori method. Magn. 200×.

Fig. 9. The small intestine of the rat, Ist experimental group. PAS method by McManus. Magn. 200×.

STRESZCZENIE

Jelito cienkie szczura po hepatektomii badano histologicznie (barwienie hematoksyliną i eozyną) oraz histochemicznie (wykrywanie: fosfatazy kwaśnej i fosfatazy zasadowej według metody Gomoriego, glukozo-6-fosfatazy według metody Wachsteina i Meisel, lipazy według metody Gomoriego i Takamatsu oraz wielocukrów metodą PAS według McManusa). Wykazano, że Biseptol 480 powodował niewielkie zaburzenia w aktywności badanych enzymów, wyrażające się zwiększeniem (fosfataza zasadowa, glukozo-6-fosfataza, wielocukry) bądź zmniejszeniem (fosfataza kwaśna) odczynu.

РЕЗЮМЕ

Тонкую кишку крысы после гепатоктомии исследовано гистологически (окрашивание гематоксилином и эозином) и гистохимически (выявление: кислой фосфатазы и щелочной фосфатазы методом Gomori, глюкозо-6-фосфатазы методом Wachstein и Meisel, липазы методом Gomori и Takamatsu и полисахариды методом PAS по McManus). Показано, что Бисептол 480 вызывал небольшие растройства в активности исследованных энзимов, проявляющиеся увеличением (щелочной фосфатазы, глюкозо-6-фосфатазы) или понижением (кислой фосфатазы) реакции.