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FORMULATION OF AZAPROPAZONE SUPPOSITORIES AND EXAMINATION OF ITS EFFECT ON THE GASTROINTESTINAL TRACT OF RABBITS.

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ABSTRACT

The rectal absorption of azapropazone, in rabbits, from different suppository formulations was investigated, and compared with its in-vitro release. A comparison between the rectal absorption of the drug and its absorption from capsule was done. The effect of azapropazone on the gastrointestinal tract of rabbits was studied. It was proved that the rectal absorption of the drug is a function of the nature of suppository bases. Of the tested bases, a base consisting of a mixture of PEG 6000, 1540 and 400 (47:33:20) gave the highest in-vitro release, however Witepsol H15 produced the highest rectal absorption of the medicament. The availability of azapropazone from the capsules was much better than that from the suppositories. The effect of the orally and rectally administered drug on the duodenal mucosa was deleterious with a marked ulceration. No ulcerative changes could be detected on the rectal mucosa in any of the tested groups of rabbits. On the gastric mucosa, there was a pronounced ulcerative changes in case of oral administration.

INTRODUCTION

Azapropazone is one of the most potent non-steroidal antiinflammatory and antipyretic drug which is preferable in the treatment of gout[1] and pre- and post-operative inflammations[2]. The drug is available as capsules, each contains 300 mg[3].

Its oral medication was reported to induce gastrointestinal disorders such as vomiting, ulceration and gastrointestinal haemorrhage[4-6]. Although rectal absorption of several drugs such as analgesics, antispasmodics and local anaesthetics have been reported[7,8] there is a little informations on azapropazone formulations as suppositories.

It was stated that in-vitro tests such as dissolution tests of suppositories are misleading and do not predict the in-vivo absorption[9]. Tentsova et al[10] stated that the evaluation of the effectiveness of a given suppositories by in-vitro tests is insufficient and animal must be used. Concerning the deleterious side effects of the acidic non-steroidal antiinflammatory analgesic drugs on the gastrointestinal tract, it was reported that gastrointestinal ulceration and haemorrhage are the most serious effects induced by their oral administration[11-17].

The aim of this study was to investigate three main points:

1- Formulation of azapropazone in form of suppositories using selected water-soluble and fatty bases,

2- Study of the physicochemical properties, In-vitro release and In-vivo availability of the drug in rabbits, and comparing the results with the capsule form.

3- Investigation of the ulcerogenic effect of the drug in both capsules and suppositories on the gastrointestinal tract of rabbits.

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EXPERIMENTAL

Materials and equipment

Azapropazone (Siegfried, Zofinen, Switzerland); Witepsols H 15, W 35 and E 75 (Dynamit Noble, W. Germany); PEG 6000, 1540 and 400 (prolabo, France); Cellophane membrane, spectrapor M.W. Cutoff: 12,000-14,000 (Fisher Sci. Co. U.S.A.); Formalin 10%, sodium chloride, ethyl alcohol 70 and 90%, eosin 1%, haematoxylin, xylol, potassium monobasic and sodium dibasic phosphate and hard paraffin (Analytical grades). Spectrophotometer (perkin Elmer 505); Erweka hardness tester, model SBT (W. Germany) and Erweka deformation tester, model SSP (W. Germant).

Procedures

Preparation of azapropazone Suppositories

Suppositories were prepared using the fusion method [18].

The suppository bases were selected to cover a wide range of different types of bases. For fatty bases, Witepsol H 15, W 35 and E 75 were used, while a mixture of polyethylene glycols 6000, 1540 and 400 in a ratio of 47:33:20 respectively was chosen to represent the water-soluble type. Azapropazone suppositories, 1 gm, each containing 100 mg medicament were prepared using each time one of the previously mentioned bases. The prepared suppositories were stored at 3-5^{o}C for two days and then for another two days at room temperature before testing.

Evaluation of azapropazone suppositories

Physical properties

suppositories were evaluated for hardness and deformation time according to Erweka* requirements.[19] Weight variation and drug content were determined according to B.P 1980 [19] and B.P.C. 1973[20] respectively.

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hardness tester (2-4 Kg) and deformation time 15 minutes.

Release Study

The method of Krowczinski was adopted[21]. One suppository was placed onto a glass tube (15 x 3.5 cm) covered with cellophane membrane firmly tied. The tube was vertically suspended in a beaker containing 30 ml of phosphate buffer solution (pH. 6.8) to be used as release medium — the temperature of which was maintained at 37±0.5°C using a thermostatically controlled water-bath. One ml aliquot was withdrawn at certain time intervals and replaced by the same volume of buffer solution. The mean of six determinations for each experiment was calculated and the drug concentration was measured spectrophotometrically at 255 nm [22], at which PEGs, Witepsols H 15, W 35 and E 75 showed no interference in the UV. absorbance of the drug.

Bioavailability study

Rabbits weighing 2-2.5 kg were used and should be rectally evacuated before the insertion by using a glass tube (0.5x3 cm) slightly lubricated with petrolatum and inserted in the rabbit rectum. On removing this tube, the rabbit usually defecated[23].

The animals were divided into three groups, each consisting of six and handling was almost alike. The azapropazone suppositories made from Witepsol H 15 (Formula I) were administered to the first group. The second group was specified for the suppositories prepared using the PEG mixture (Formula II). The third group was given hard gelatin capsules containing the same amount of the drug (Formula III) with the aid of 20 ml water using a certain adaptor for this purpose. Blood samples were withdrawn from the congested aural vein at 1, 2, 3, 6 and 12 hour after insertion of the suppository or administring the capsule. The amount of azapropazone in the serum was determined spectrophotometrically[22], and the data were analyzed according to "t" test[24].

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Gross appearance and histological examinations of the gastro-intestinal tract of rabbits.

Adult rabbits (2-2.5 Kg) were used for this investigation. They were kept under constant diet. The animals were randomly divided into four groups, each comprised four rabbits as follows:

1- First control group: Animals were kept under investigations without medication, (Group 1).

2- Second control group: Animals were given a one gram placebo suppository made from Witepsol H 15, once daily for 30 days, (Group II).

3- Azapropazone suppository group: Each animal of this group was given a one gram suppository containing 100 mg azapropazone and prepared using Witepsol H 15 as a base. The dose was one suppository every day for 30 days, (Group III).

4- Azapropazone capsule group: For a period of 30 days, each animal swallowed a hard gelatin capsule containing 100 mg of the medicament, every day, (Group IV).

The animals were weighed every two weeks and they were noticed for the general activities. At the end of the experiments, the rabbits were sacrificed. Gastrointestianl organs including stomach, duodenum and rectum were dissected, opened out and grossly examined. For histological examinations, the organs were fixed in formaline (10%) and the tissues were processed by the usual paraffin method, sectioned at 6 Mm and stained by haematoxylin and eosin stains[25].

RESULTS AND DISCUSSION

1- Physical properties

The prepared suppositories exhibited a good mechanical properties and were found to be in aggreement with Erweka requirements. Medicated suppositories made from Witepsol H 15

and W 35 exhibited a reasonable deformation time (4 and 4.2 min.) and hardness (3.3 and 3.0 Kg) respectively. Witepsol E 75 showed a higher value for deformation time (7.0 min.), however the hardness value (2.75 Kg) was slightly less than that obtained with Witepsols H 15 and W 35. The hardness and deformation time for the suppositories prepared using PEG mixture were found to be 2.75 Kg and 21.1 min. respectively.

All the prepared suppositories met the acceptable limits of the B.P 1980[19] towards the weight variation (±5%). The content uniformity was found to be within the stated limits according to B.P.C. 1973[20].

2- In-vitro release of azapropazone from the suppositories

Figure 1, shows the release characteristics of azapropazone as a function of suppository bases. The release of medicament from different bases could be arranged as follows: PEG mixture > Witepsol H 15 > Witepsol W 35 > Witepsol E 75. The differences in release from various bases seemed to be greatly affected by the differences in the melting point, deformation time and hardness values of the used bases. It was obvious from Figure 1. that the release from triglycerides member having comparatively low melting point as Witepsol H 15 (m.p. is 33-35°C) was greater than that from triglycerides of high melting range such as Witepsol W 35 (35-37°C) and E 75 (37-39°C) Meanwhile, the release of the medicament from the mixture of PEG which is the water-soluble base was higher than that obtained from the fatty bases. The results were similar to those obtained by other workers [8,26-29] who attributed this increase due to the solubilizing properties of a water soluble excipient.

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3- Effect of suppository base type and route of administration on absorption of azapropazone

Witepsol H 15 (Formula I) and PEG mixture (Formula II) suppository bases proved to be relatively superior regarding to the In-vitro release of the medicament. So, these two formulae were selected for the In-vivo study, compared with the oral hard gelatin capsules containing the same amount of the drug (Formula III) in order to be able to evaluate their relative bioavailability. From the In-vivo data illustrated in Figure 2, a highly significant difference was present (t test). Formula I was characterised by a higher peak serum concecntration than that obtained with formula II, although the peaks of the two rectal formulation were reached after the same time (6 hours). In case of formula I, the curve rose up then fell down rapidly however, in case of formula II the curve rising was slower. This may be attributed to the nature of the PEG base which requires comparatively longer time for dissolution in the rectum.

The peak serum concentration was found to be 890 Ug/ml and 500 ug/ml in case of formula I and II respectively. $AUC_{0-24h.}$ for formula I was about 3798.2 µg/ml hr. and 2900 µg/ml hr. in case of formula II. These results indicated that the serum concentration of azapropazone 0-12 hours intervals was greatly influenced by the type of the base used in the suppository formulations. It was reported that the absorption from the fatty bases is dependent on the rate of partitioning of the drug from the base to the rectal fluid[29], however in case of water-soluble bases such as PEG, the absorption might be affected by the solubility of the drug as well as the base itself [30]. Formula III as shown in Figure 2, was characterised by the highest peak serum concentration which occurred after three hours of administration. The blood concentration-time

curve showed that the drug concentration reached 1056 Ug/ml after three hours then slowly declined up to 220 ug/ml after 12 hours. $AUC_{0-24h.}$ was found to be equal to 7046 ug/ml.hr, and this value is about 2-fold that of formula I (AUC_{0-24h} is 3798.2 Ug/ml hr.) .

4- Effect of azapropazone suppositories and capsules on the gastro-intestinal tract of rabbits gross appearance.

Slight gross changes were observed in the stomach, duodenum and rectum of animals of groups III and IV. All organs were found to have mild to moderate erythema and friability.

Histological examinations: Gastric mucosa

Rabbits of group II showed a typical mucosa with normal gastric pits and oxyntic cells (Figure 3). The gastric mucosa of group III rabbits showed marked shortening of the gastric pits (Figure 4) and increase in the number of oxyntic cell as compared with that of control groups (I and II). Gastric mucosa of group IV rabbits showed a marked hyperplasia of the oxyntic cells appeared in some areas with subsequent absence of gastric pits and the surface epithelial cells, a normal areas of oxyntic cells and gastric pits were present,(Figure 5).

Duodenum

The duodenum of rabbits after administration of placebo suppositories (group II) only showed normal villi and crypts with mild cellular infiltration (Figure 6). The histological appearance of the duodenal mucosa of group III rabbits showed a marked cellular infiltration in the connective tissue core of the villi extending to the underlying lamina proporia. The epithelial cells covering the villi were lost in the whole surface area and the crypts were rather hypoplastic with shortened epithelial cells. A marked ulceration in some areas was

noticed (Figure 7). On the other hand, the duodenal musosa of rabbits of group IV showed numerous ulceration in some area accompanied with atrophy of both villi and crypts in other areas. A marked cellular infiltration in the connective tissue core of villi was obvious (Figure 8). The damaging changes of duodenal mucosa after oral and rectal medication of azapropazone might be explained by a combined systemic effect and enterohepatic circulation.

Rectum

Animals of group II (Control group) showed no undesirable effects on the mucosal surface of the rectum (Figure 9). However, the rectum of rabbits (group II) after administration of suppositories, the crypts were slighly separated from each other and the surface epithelium was normal (Figure 10). On the other hand group IV showed no effects on the rectal mucosa and a normal histological appearance was found.

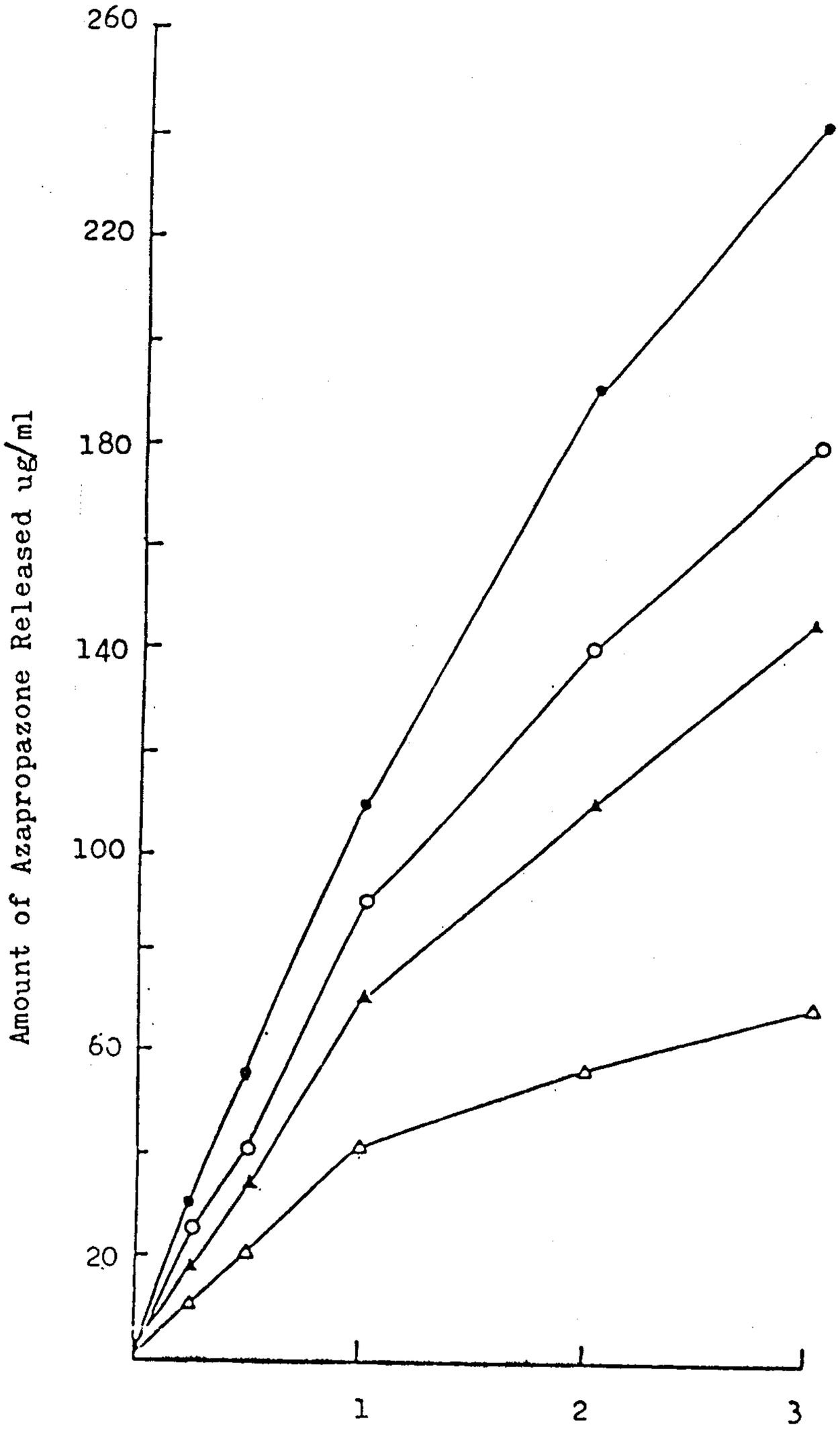

Figure 1. Release Characteristics of Azapropazone from Different Suppository Bases.

● PEG mixture; ○ Witepsol H 15; ▲ Witepsol W 35 and △ Witepsol E 75.

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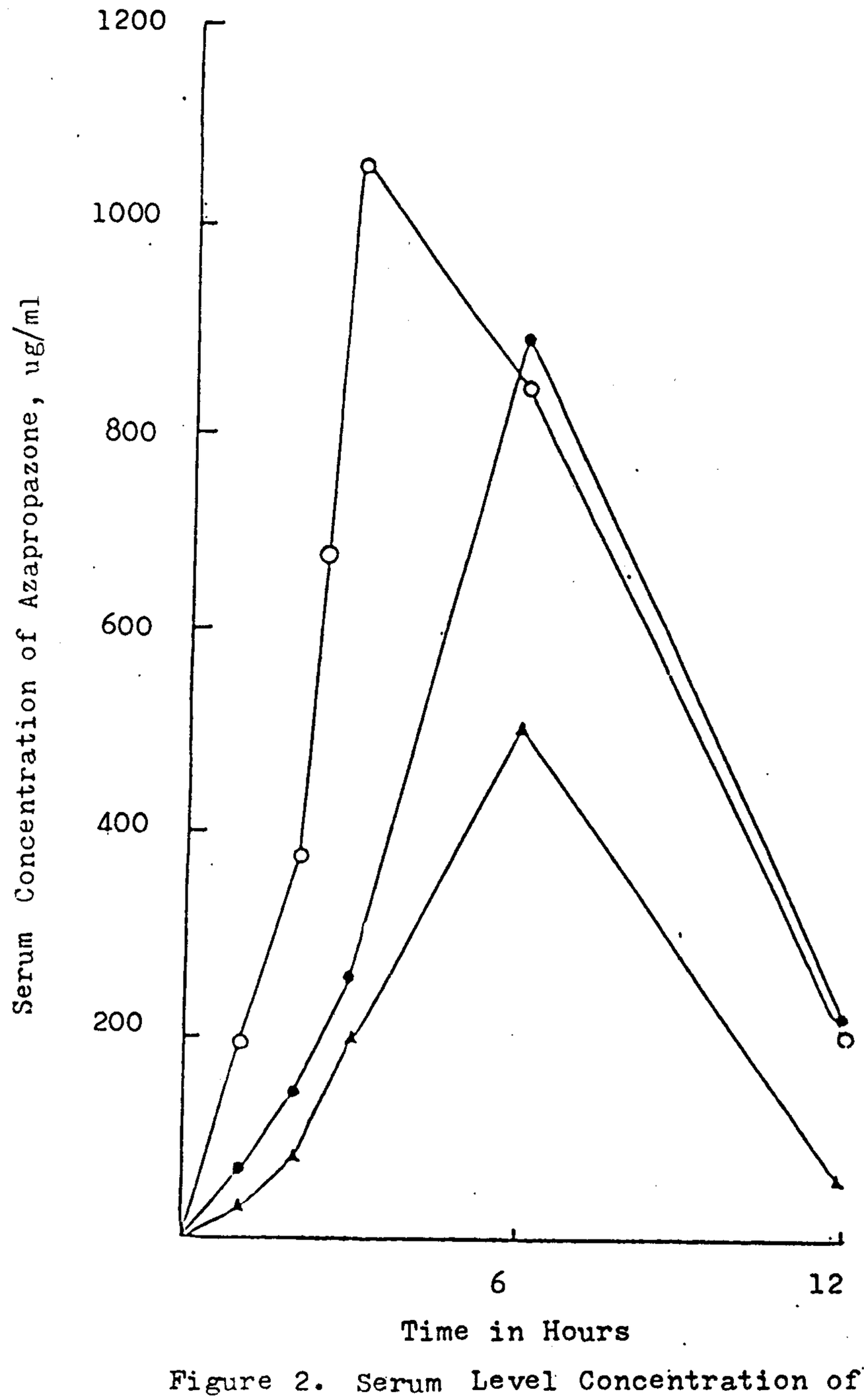

Figure 2. Serum Level Concentration of Azapropazone administered orally and rectally.

○ Capsules; ● Witepsol H 15 Suppository and ▲ PEG Suppository.

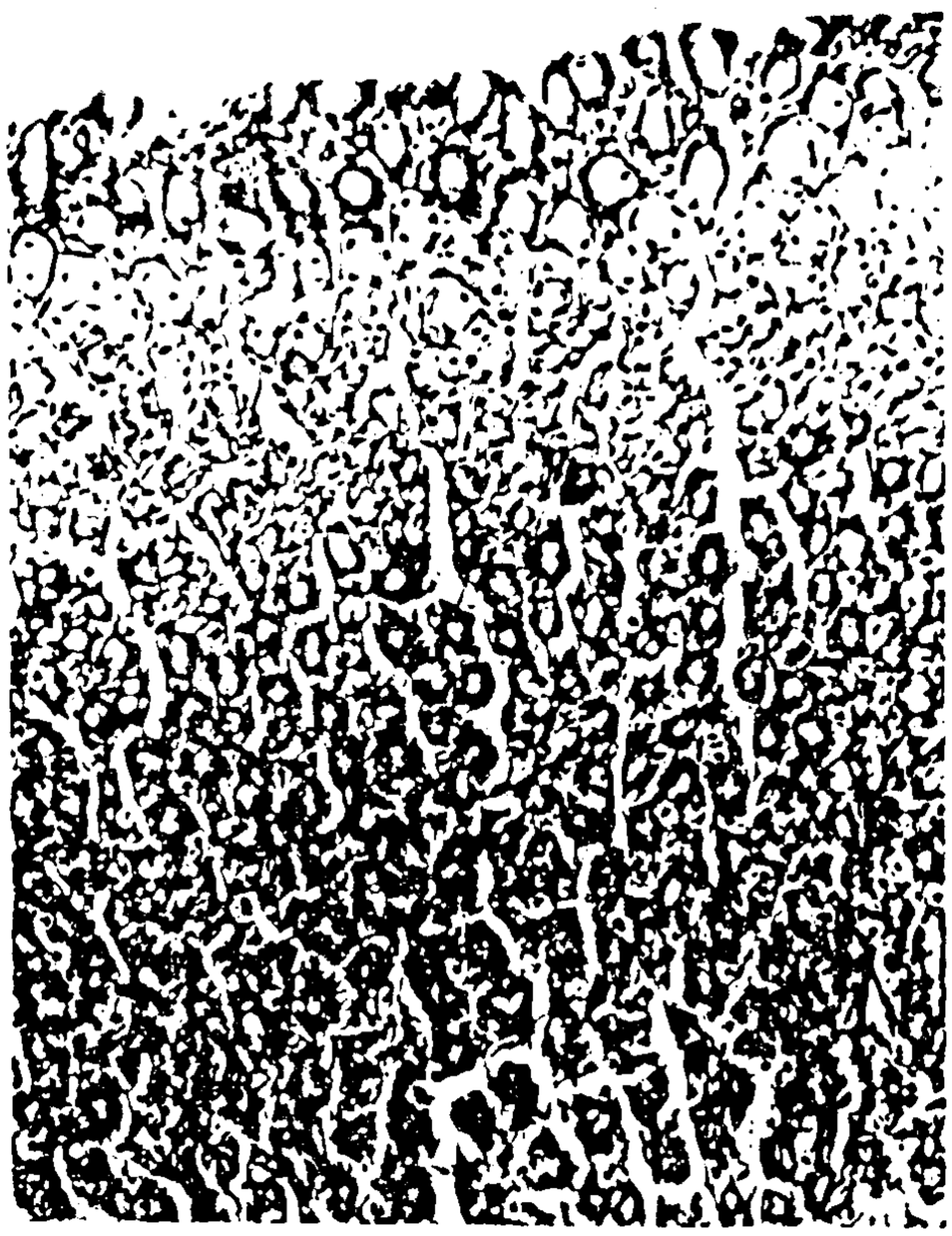

Figure 3. Gastric mucosa of a rabbit (Group II) after administration of plain suppository (Hx & E X100).

Figure 4. Gastric mucosa of a rabbit (Group III) after administration of 100 mg azapropazone suppository (Hx & E X 100).

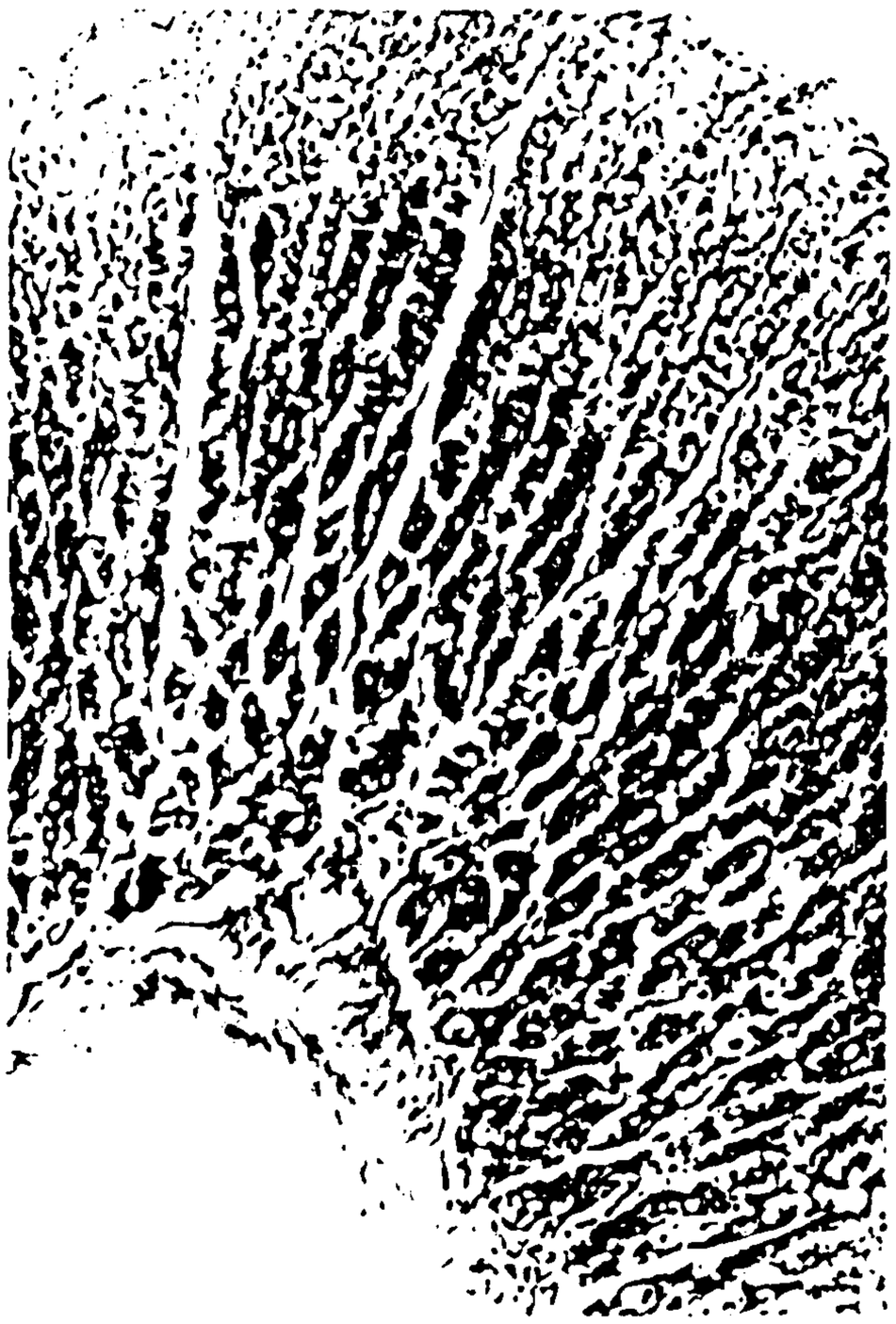

Figure 5. Gastric mucosa of a rabbit (Group IV) after administration of 100 mg azapropazone capsule (Hx & E X 100).

Figure 6. Duodenum of a rabbit (Group II) after administration of plain suppository (Hx & E X 100).

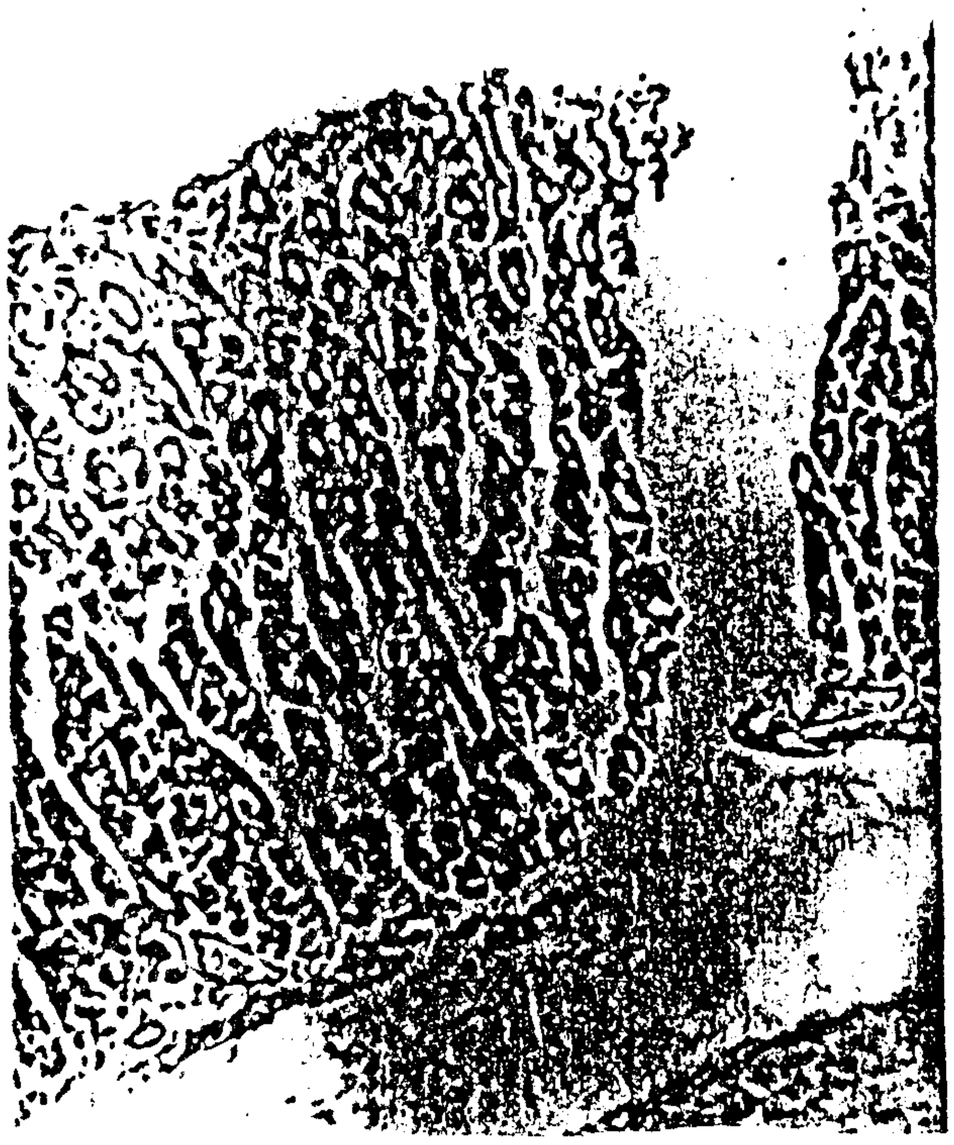

Figure 7. Duodenum of a rabbit (Group III) after administration of 100 mg suppository (Hx & E X 100).

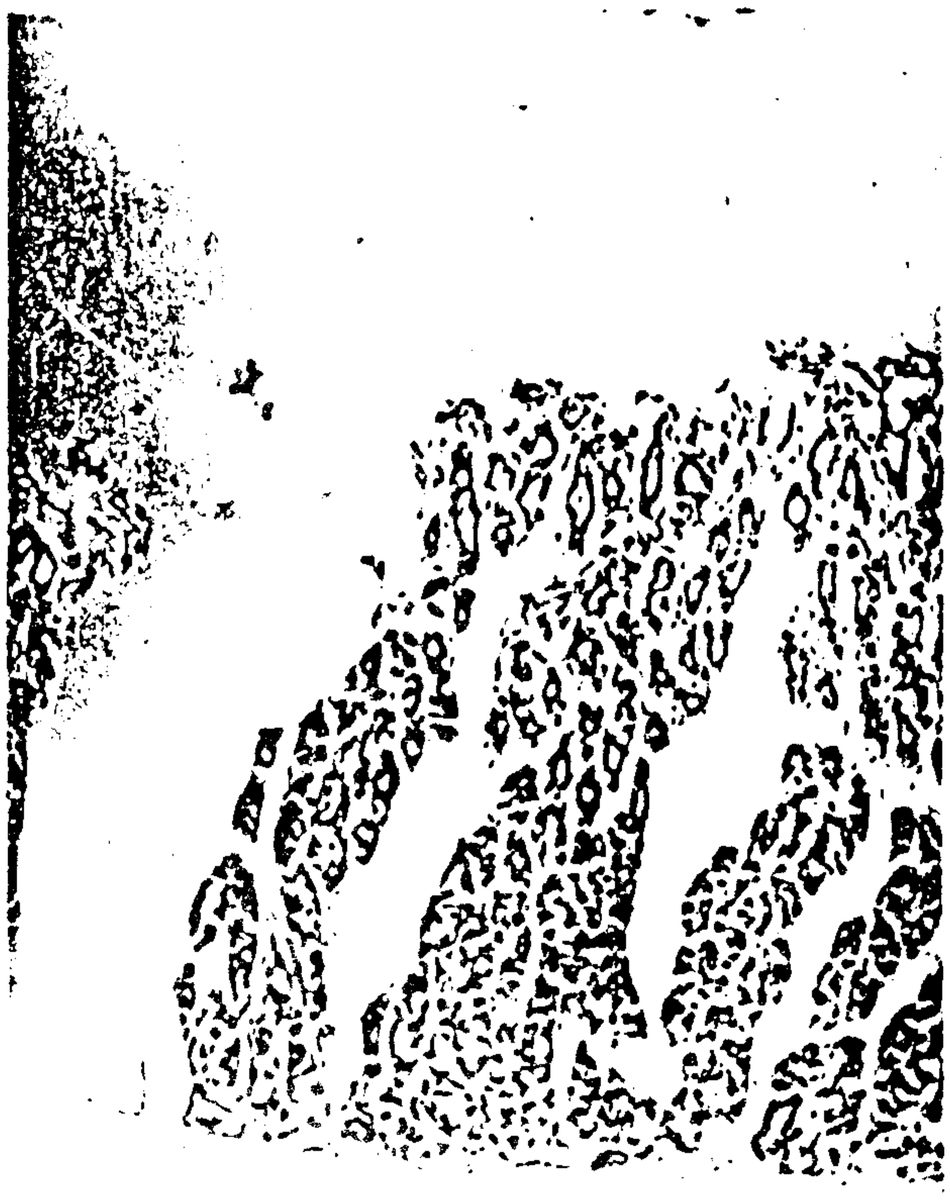

Figure 8. Duodenum of a rabbit (Group IV) after administration of 100 mg azapropazone capsule (Hx & E X 100).

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Figure 9. Rectal mucosa of a rabbit (Group II) after administration of plain suppository (Hx & E X 100).

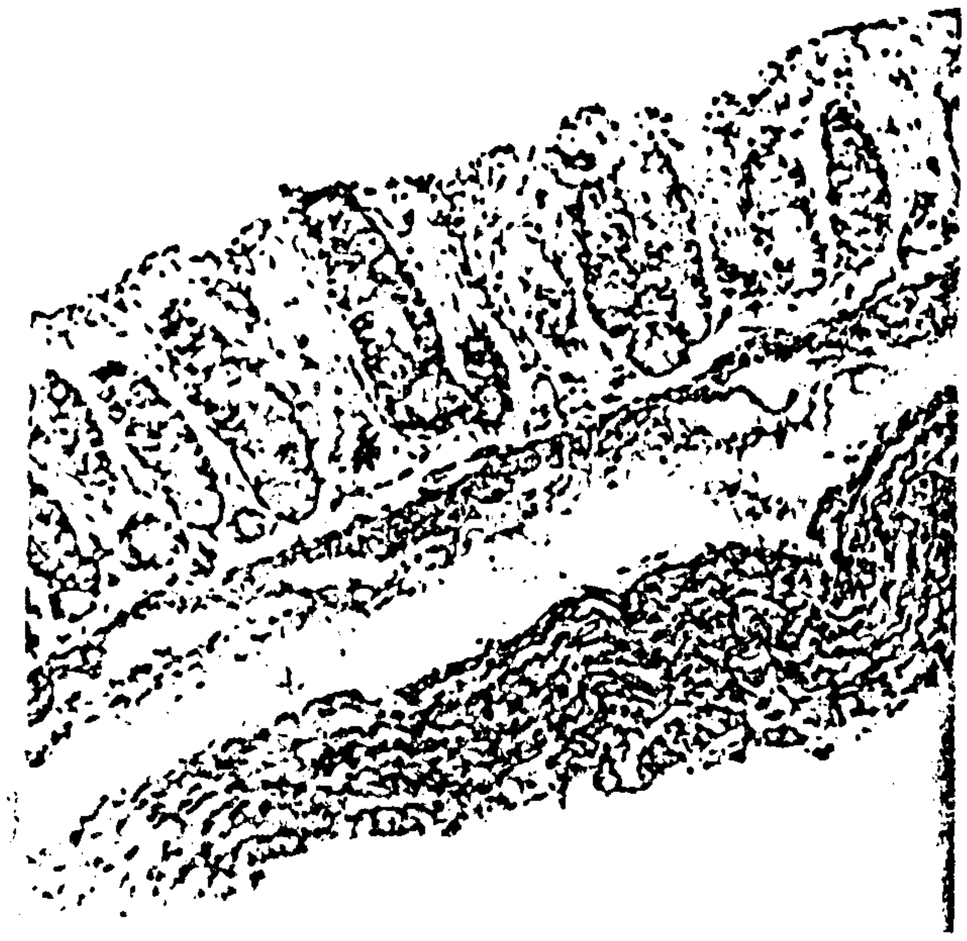

Figure 10. Rectal mucosa of a rabbit (Group III) after administration of 100 mg azapropazone suppository (Hx&E X 100).

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صياغة اقماع الاذابروبازون واختبار تأثيرها على القناة الهضمية للارانب

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قسم الصيدلانيات ـ كلية الصيدلة ـ جامعة المنصورة

تم دراسة امتصاص الاذابروبازون من الاقماع الشرجية وقورن ذلك بالاتاحة المعملية للدواء من قواعد الاقماع المختلفة . كذا دراسة امتصاص الدواء من الاقماع ومقارنته مع امتصاص الدواء من الكبسولات . وقد تناول البحث دراسة تأثير الدواء من الكبسولات والاقماع على القناة الهضمية للارانب .

وقد وجد ان خليط عديد الايثيلين جليكول المكون من عديد اثيلين جليكول ٦٠٠٠ ، ١٥٤٠ ، ٤٠٠ بنسبة ٤٧ : ٣٣ : ٢٠ على الترتيب يعطى اعلى اتاحة معملية بينما كانت الاتاحة الحيوية للدواء عالية من قاعدة ويتبسول هـ ١٥ . وقد وجد ان للدواء تأثيرا تآكليا بدرجات متفاوته على امعاء الارانب من الكبسولات والاقماع .

ولم يلاحظ وجود تغيرات هستولوجية على انسجة المستقيم للارانب المعطاه الدواء فى صورة اقماع شرجية بينما كان ذلك واضحا لتلك التى اعطيت الكبسولات .

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