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ASSESSMENT OF LOCAL AND SYSTEMIC AVAILABILITY OF TRIBENOSIDE AFTER RECTAL ADMINISTRATION IN RATS

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SUMMARY

The local and systemic availability of tribenoside administered rectally as a suppository was studied in comparison with those in the oral administration in rats, using [^{14}C]-labelled tribenoside. In the rectal administration, the systemic bioavailability calculated with the orally administered solution as the reference standard was almost 60%. On the other hand, high concentration of tribenoside was retained for a prolonged period of time in the tissues of the rectum and anus, particularly in the lamina propria without being metabolized after rectal administration. The average concentration of the drug in those tissues for 1–24 h was almost 50 times higher than that in the oral administration. These findings suggested that the rectal application of tribenoside suppositories for treatment of hemorrhoids could offer a greater advantage with regard to direct delivery of the drug close to the actual site of the action before diluted in the systemic circulation and metabolized by the liver passage, or with regard to the duration of the drug action.

INTRODUCTION

Tribenoside (ethyl-3,5,6-tri-O-benzyl-D-glucofuranoside) was synthesized as an anti-inflammatory and anti-allergic agent by Huber and Rossi (1968). Various pharmacological activities of tribenoside such as anti-inflammatory, anti-arthrotic, amine-release-inhibitory, membrane-stabilizing and venotropic properties have been documented (Jaques, 1977).

Tribenoside has been most often administered as an oral dosage form for the treatment of chronic venous insufficiency and hemorrhoids. The rectal dosage form such as suppository has also been developed.

The absorption of tribenoside has been studied both in animals and humans (Keberle and Schmid, 1971; Tanayama et al., 1974). The bioavailability of tribenoside from the rectal suppository in humans was reported to be from one-fourth to one-fifth as much as

that from an oral dosage form (Keberle and Schmid, 1971). On the other hand, tribenoside applied topically together with an irritant solution has been known to suppress the aedema caused by such an irritant in the mouse ear (Riesterer and Jaques, 1968). Later, Jaques et al. (1969) found that the drug applied topically to the mouse ear was absorbed percutaneously, and retained in that organ for a longer period of time (Jaques et al., 1969). These findings suggest that in spite of the lower systemic bioavailability, if the rectal suppository is intended for the treatment of hemorrhoids, it is expected to be more favourable than the oral administration, since the drug is absorbed close to the site of the action which allows the drug to distribute itself there before being diluted and metabolized in the systemic circulation.

The aim of the present work is to estimate the tissue distribution of tribenoside as well as the bioavailability in the systemic circulation when the drug is administered rectally as a suppository to rats.

MATERIALS AND METHODS

Labelled compound

Tribenoside (ethyl-3,5,6-tri-O-benzyl-D-glucofuranoside) labelled at 3,5,6-benzyl moieties with carbon-fourteen (see Fig. 1) was obtained from Ciba-Geigy. The specific activity was 16.0 μCi/mg and the radiochemical purity was ascertained to be more than 98% by thin layer chromatography. Other chemicals were of reagent grade.

According to the formula shown in Table 1, an exact amount of [^{14}C]tribenoside diluted with non-labelled tribenoside (the resulting specific activity was accounted to be 6.3 μCi/mg) as suppository base was added to it. The drops of molten mixture of the ingredients weighing 50 mg as administration dose were taken on a mold using micropipettes, and immediately cooled to a low temperature (about 5°C). The subsequent solid pellets were kept in a refrigerator as rectal suppositories.

Experimental animals

Male SD-JCL rats weighing 200 g were fasted for 16 h prior to the experiments but water was allowed ad libitum.

In vivo experiments on absorption, excretion and distribution

For the oral administration, doses of 10 mg/kg of [^{14}C]tribenoside diluted with non-labelled tribenoside (63.0 μCi/kg) were dissolved in 2 ml of polyethylene glycol 400 and

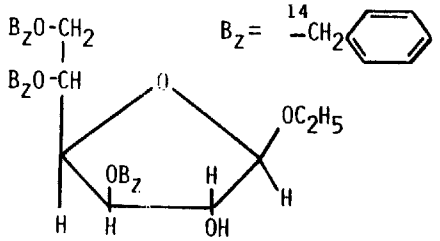

Fig. 1. Chemical structure of tribenoside.

TABLE 1

FORMULA OF RECTAL SUPPOSITORY OF TRIBENOSIDE

Tribenoside ([^{14}C]-tribenoside)	40 mg (252 μCi)
Witepsol W-35	960 mg
Total	1000 mg

administered to the rats without anesthetization. For the rectal administration, the rats were anesthetized with intraperitoneal injections of the mixture of pentobarbitone, 50 mg/kg and sodium phenobarbitone, 100 mg/kg. A suppository was inserted into the rectum about 1 cm from the anus, and then the anus was closed with an adhesive agent for 6 h to avoid any leakage of the drug.

The blood specimens were withdrawn from the tail vein at appropriate time intervals.

The urine and feces specimens were collected for two days using metabolic cages. Each feces specimen was suspended thoroughly in 100 ml of water using a homogenizer and an aliquot of the suspension was applied to assay.

The specimens of tissues such as blood, brain, lung, liver, spleen, kidney, adipose tissue, muscle, rectum and anus were immediately taken after the rats were killed by a blow on the neck at appropriate time intervals, and rinsed with cold saline. In the case of the tissue of the rectum, the specimens were taken from the lower part of the rectum. To ensure that the rectum and anus specimens for rectal administration were clear of any part of the dosage form remaining unabsorbed, they were rinsed with methanol or chloroform after being washed in cold saline. Each tissue specimen was cut into small segments weighing 0.05–0.5 g as wet base and applied to assay.

To examine the pathway of the rectal absorption, the blood specimens were withdrawn from the portal vein, the abdominal aorta and the inferior vena cava 1, 2 and 24 h after administration.

Measurement of radioactivity

The blood samples (0.1 ml), the feces samples (1 ml) and the tissue samples (0.05–0.5 g) were placed in corn caps. When completely dry, they were combusted using a Packard model 306 oxidizer, and the radioactivities were measured in toluene-based scintillation fluid containing 1.5% of 2,5-diphenyl-oxazole (PPO) and 0.1% of 1,4-bis (2-(5-phenyl-oxazole)) benzne (POPOP).

The urine samples (0.1 ml) were mixed thoroughly with toluene-based scintillation fluid containing a detergent (Nissan Nonion NS-210, Nippon Yushi). All of the radioactivities were measured using a Nihon-Musen model 602 or a Aloka model 683 liquid scintillation counter. The counts were converted to dpm through quench correction.

Metabolism

In vitro experiment. The liver homogenate (2.5%) and small intestine homogenate (5%) were prepared with cold saline, using a tissue homogenizer equipped with a teflon pestle (Takashima Shoten, Tokyo). Ten μg of tribenoside (63.0 nCi) dissolved in 0.01 ml of ethanol and 0.2 ml of each fresh homogenate which was almost equivalent to 5 mg

protein were added to 0.8 ml of a buffer solution containing 1.5 μmol of NADP (nicotinamide adenine dinucleotide phosphate), 15 μmol of $MgCl_2$, 30 μmol of glucose-6-phosphate, 5 units of glucose-6-phosphate dehydrogenase, and 100 μmol of Tris (Hydroxymethyl) amino-ethane buffer (pH 7.4) (Kobayashi et al., 1974).

The mixture was incubated for 2 h at 37°C and then immediately cooled to a low temperature (about 5°C) to terminate the reaction. The whole mixture was extracted with ethyl acetate.

The radioactivities of unchanged [^{14}C]tribenoside and its metabolites were separated by thin layer chromatography (solvent system: chloroform–acetone = 9 : 1 v/v) and applied to assay.

In vivo experiment. The rectum tissue, 1 h after rectal administration of [^{14}C]tribenoside at a dose of 10 mg/kg (63.0 μCi/kg), was isolated and washed thoroughly as described above, and its homogenate was extracted with ethyl acetate. The radioactivities of unchanged [^{14}C]tribenoside and its metabolites were determined using thin layer chromatography as described above.

Microautoradiography

The rectum tissues, 1 h after oral and rectal administrations of [^{14}C]tribenoside at a dose of 10 mg/kg (63.0 μCi/kg), were isolated and washed. The blocks of the tissues (1–1.5 cm in length) were fixed in 0.1 M phosphate buffer solution (pH 7.4) containing 6% glutaraldehyde. Then, frozen sections (15 μm in thickness) were made by a Yamato-koki microtome. These sections were mounted on glass plates. After being thoroughly dried, they were coated by means of dipping method with a liquid photographic nuclear emulsion (Sakura NR-M2, Konishiroku), and kept in a dark box at 4°C for 5 weeks exposure. Then the plates were developed with Kodak D-19 (Kodak) for 4 min at 20°C, fixed in Super Fuji-Fix (Fuji Photo Film) for 2–3 min and rinsed with water. After staining with a nuclear fast red solution (0.1%) for 5 min, they were dehydrated in a graded series of ethanol through to xylene, and covered with Ceadax (Merk).

RESULTS

Blood level and urinary and fecal recovery

Plots of the mean blood levels of [^{14}C]radioactivity vs time after oral and rectal administrations of [^{14}C]tribenoside at a dose of 10 mg/kg (63.0 μCi/kg) are shown in Fig. 2.

For the oral administration, the peak blood level of the radioactivity (0.82 μg · equiv./ml) was observed 15 min after dosing; a rapid decrease was observed from 15 min to 1 h; the blood level from 1 to 6 h was almost constant at 0.35 μg · equiv./ml. For the rectal administration, the peak blood level of the radioactivity (0.27 μg · equiv./ml) was observed 1 h after dosing; a very slow decrease was observed from 1 to 6 h; the blood level at 6 h was 0.14 μg · equiv./ml.

The AUCs (area under the curves) from 0 to 24 h for the oral and rectal administrations calculated with trapezoidal rule were 5.75 μg · equiv. · h/ml and 3.26 μg · equiv. · h/ml, respectively. The bioavailability of tribenoside for the rectal suppository calculated with the orally administered solution as the reference standard was found to be 56.7%.

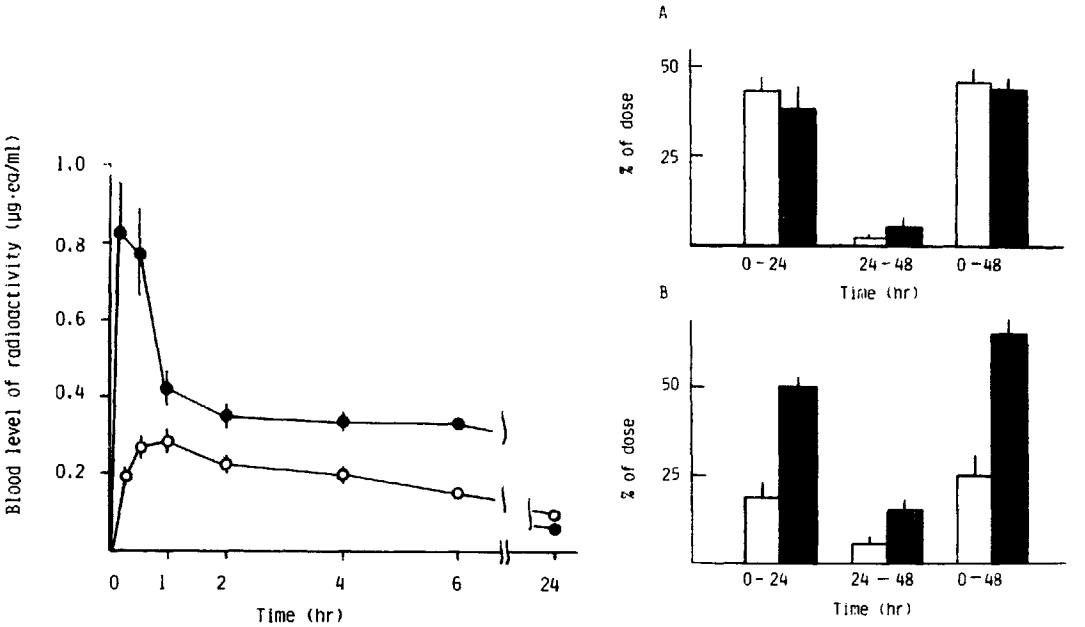

Fig. 2. Plots of the mean blood levels of the radioactivity vs time after oral (●) and rectal (○) administration of [^{14}C]tribenoside at a dose of 10 mg/kg (63.0 μCi/kg) in rats. Each point represents the mean with standard error of 6 experiments.

Fig. 3. A: urinary and fecal recoveries of the radioactivity after oral administration of [^{14}C]tribenoside at a dose of 10 mg/kg (63.0 μCi/kg) in rats. B: urinary and fecal recoveries of the radioactivity after rectal administration of [^{14}C]tribenoside at a dose of 10 mg/kg (63.0 μCi/kg) in rats. Open column is for urinary recovery and closed column for fecal recovery. Vertical bars indicate the standard error of 4 experiments.

The mean recoveries of the radioactivity in the urine and the feces after oral and rectal administrations of [^{14}C]tribenoside at a dose of 10 mg/kg (63.0 μCi/kg) are shown in Fig. 3.

For the oral administration, the urinary and fecal recoveries during the first 24 h were 43.6% and 38.1% and during the next 24 h, 1.8% and 5.3%, respectively. The radioactivity recovered in both the urine and feces up to 48 h was almost 90%. For the rectal administration, the urinary and fecal recoveries during the first 24 h were 19.0% and 49.8% and during the next 24 h, 5.8% and 15.2%, respectively. The radioactivity recovered in both the urine and feces up to 48 h was almost 90%.

A study of i.v. injection of [^{14}C]tribenoside to rats has shown a small amount of the radioactivity was excreted via the bile into the feces (Kobayashi et al., 1974). This indicates only the data of the urinary recovery are comparable in regard to the systemic bioavailability. The bioavailability of tribenoside for the rectal administration of the suppository calculated by the urinary recovery up to 48 h was 54.7% with the orally administered solution as the reference standard. The result is well consistent with those from the blood level data.

TABLE 2

TISSUE LEVELS OF THE RADIOACTIVITY AFTER ORAL AND RECTAL ADMINISTRATIONS OF [^{14}C]TRIBENOSIDE AT A DOSE OF 10 mg/kg (63.0 μCi/kg) IN RATS

	1 h	2 h	4 h	6 h	24 h
Oral administration					
blood	0.37 ± 0.03	0.28 ± 0.06	0.28 ± 0.07	0.28 ± 0.03	0.10 ± 0.00
brain	0.06 ± 0.00	0.06 ± 0.01	0.04 ± 0.01	0.05 ± 0.00	0.01 ± 0.00
lung	0.24 ± 0.05	0.50 ± 0.23	0.19 ± 0.06	0.20 ± 0.01	0.03 ± 0.00
liver	2.12 ± 0.56	2.00 ± 0.07	2.15 ± 0.54	1.81 ± 0.36	0.90 ± 0.03
spleen	0.22 ± 0.02	0.18 ± 0.04	0.17 ± 0.03	0.18 ± 0.00	0.03 ± 0.00
kidney	2.28 ± 0.25	2.29 ± 0.22	2.50 ± 1.11	1.72 ± 0.17	0.11 ± 0.01
adipose tissue	0.12 ± 0.03	0.15 ± 0.02	0.12 ± 0.03	0.12 ± 0.01	0.15 ± 0.03
muscle	0.19 ± 0.02	0.16 ± 0.03	0.11 ± 0.02	0.13 ± 0.01	0.02 ± 0.00
rectum	1.70 ± 0.17	0.47 ± 0.07	0.19 ± 0.03	0.22 ± 0.03	0.26 ± 0.06
anus	1.07 ± 0.39	0.44 ± 0.04	0.24 ± 0.07	0.26 ± 0.03	0.19 ± 0.03
Rectal administration					
blood	0.20 ± 0.04	0.19 ± 0.01	0.14 ± 0.01	0.13 ± 0.02	0.11 ± 0.03
brain	0.08 ± 0.02	0.07 ± 0.01	0.08 ± 0.02	0.06 ± 0.00	0.02 ± 0.00
lung	0.16 ± 0.02	0.17 ± 0.02	0.15 ± 0.00	0.16 ± 0.05	0.05 ± 0.01
liver	0.60 ± 0.17	0.56 ± 0.07	0.49 ± 0.05	0.55 ± 0.06	0.26 ± 0.06
spleen	0.16 ± 0.05	0.12 ± 0.00	0.11 ± 0.02	0.17 ± 0.06	0.05 ± 0.00
kidney	1.00 ± 0.30	0.87 ± 0.15	0.60 ± 0.07	0.70 ± 0.04	0.21 ± 0.06
adipose tissue	0.54 ± 0.14	0.31 ± 0.02	0.44 ± 0.10	0.41 ± 0.10	0.24 ± 0.10
muscle	0.12 ± 0.03	0.15 ± 0.01	0.11 ± 0.01	0.17 ± 0.07	0.04 ± 0.00
rectum	23.43 ± 8.56	37.64 ± 9.30	50.40 ± 9.60	32.07 ± 5.49	0.43 ± 0.14
anus	29.46 ± 5.00	22.28 ± 4.87	21.41 ± 6.25	12.73 ± 3.69	5.25 ± 1.32

Each value (μg · equiv. tribenoside/g or ml) is expressed as mean ±S.E. of 3 experiments.

Tissue distribution

The mean tissue levels of the radioactivity after oral and rectal administrations of [^{14}C]tribenoside at a dose of 10 mg/kg (63.0 μCi/kg) were shown in Table 2.

For the oral administration, larger amounts of the radioactivity were observed in the liver, kidney, rectum and anus than in the blood at 1–6 h; the radioactivities in the rectum and the anus 1 h after dosing were 3–5 times higher than in the blood. The radioactivities in the other tissues were observed as high as, or lower than in the blood.

For the rectal administration, much larger amounts of the radioactivity were observed in the rectum and the anus for a longer period of time. These are plotted against time as compared to those in the oral administration in Fig. 4. The average radioactivities in the rectum and anus for the rectal administration were almost 50 times higher than those for the oral administration. The radioactivities in the adipose tissue were found to be a little higher, and the radioactivities in the other tissues were lower than those for the oral administration, corresponding to the blood levels.

Pathway of rectal absorption

To examine the absorption pathway of the drug, the blood levels in the portal vein,

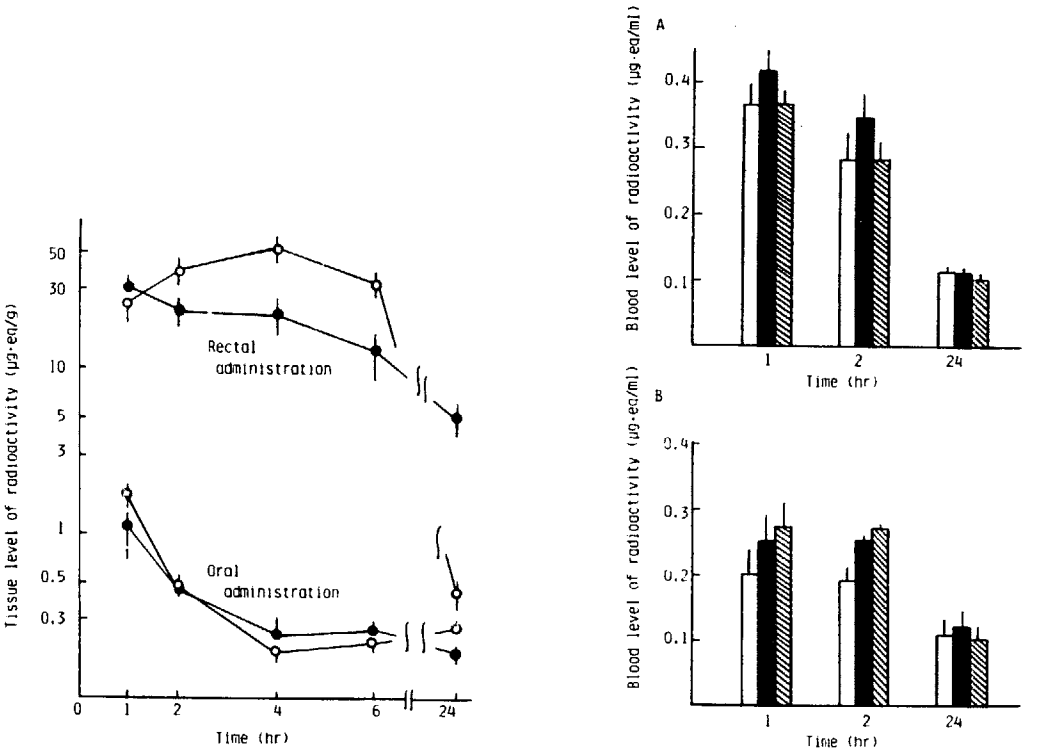

Fig. 4. Plots of the mean rectum (○) and anus (●) tissue levels of the radioactivity vs time after oral and rectal administrations of [^{14}C]tribenoside at a dose of 10 mg/kg (63.0 μCi/kg) in rats. Each point represents the mean with standard error of 3 experiments.

Fig. 5. A: the blood levels of the radioactivity in the abdominal aorta, the portal vein and the inferior vena cava after oral administration of [^{14}C]tribenoside at a dose of 10 mg/kg (63.0 μCi/kg) in rats. B: the blood levels of the radioactivity in the abdominal aorta, the portal vein and the inferior vena cava after rectal administration of [^{14}C]tribenoside at a dose of 10 mg/kg (63.0 μCi/kg) in rats. Open column is abdominal aorta. Closed column portal vein. Hatched column inferior vena cava. Vertical bars indicate the standard error of 6 experiments.

abdominal aorta and inferior vena cava after oral and rectal administrations were compared to one another. As shown in Fig. 5, the blood levels of tribenoside in the portal vein 1 and 2 h after the oral administration were higher than those in the inferior vena cava and the abdominal aorta, and the levels in the inferior vena cava and the abdominal aorta were the same. On the other hand, the blood levels in the inferior vena cava 1 and 2 h after rectal administration were higher than those in the portal vein and the abdominal aorta, and the levels in the portal vein were higher than those in the abdominal aorta.

These results indicate that when tribenoside is administered rectally, the drug absorbed from the rectum enters both the inferior vena cava and the portal vein.

Metabolism

To examine the metabolism of tribenoside after rectal administration, first the in vitro metabolism of [^{14}C]tribenoside in the rectum homogenate was determined as compared

TABLE 3

IN VITRO METABOLISM OF [^{14}C]TRIBENOSIDE IN THE HOMOGENATES OF THE LIVER, SMALL INTESTINE AND RECTUM OF RATS

	Metabolized tribenoside (μg · equiv./mg protein/h)
Liver homogenate	2.39
Small intestine homogenate	0.22
Rectum homogenate	0.17

Homogenate (5 mg protein), tribenoside 10 μg, NADP 1.5 μmol, G-6-P 30 μmol, G-6-P-D 5 units, $MgCl_2$ 15 μmol, Tris buffer (pH 7.4) 100 μmol incubation temperature: 37°C, incubation time: 2 h.

to that in the liver homogenate and small intestine in rats.

As shown in Table 3, tribenoside was hardly metabolized in the rectum homogenate. In the rectum mucosa 1 h after rectal administration, however, almost 70% of the radioactivity was due to unchanged tribenoside (Table 4).

To estimate the metabolites of tribenoside in the systemic circulation, the radioactivities of unchanged tribenoside in the blood 1 h after oral and rectal administrations were determined. The data in Table 4 show that the radioactivities appearing in the systemic circulation for both the administrations were due almost entirely to the metabolites.

Microautoradiography

In order to visualize the distribution of tribenoside in the rectum tissue for the oral and rectal administrations, the microautoradiography was performed. Figs. 6 and 7 show the microautoradiographs of the rectum mucosa and muscularis after oral and rectal administrations of [^{14}C]tribenoside, where the distribution of the radioactivity was presented by silver grains. One hour after the oral administration, most of silver grains were localized in the blood capillaries in the mucosa and the muscularis (Fig. 6). On the other hand, 1 h after the rectal administration, many silver grains were observed diffusively in

TABLE 4

IN VIVO METABOLISM OF [^{14}C]TRIBENOSIDE IN THE RECTUM MUCOSA AND IN THE BLOOD 1 h AFTER ITS ORAL AND RECTAL ADMINISTRATIONS AT A DOSE OF 10 mg/kg (63.0 μCi/kg) IN RATS

Sample	Administration route	Total radioactivity (μg · equiv./g or ml)	Radioactivity of unchanged tribenoside (μg/g or ml)
Rectum mucosa	rectal	23.43	16.30
Blood	rectal	0.37	0.009
Blood	p.o.	0.20	0.009

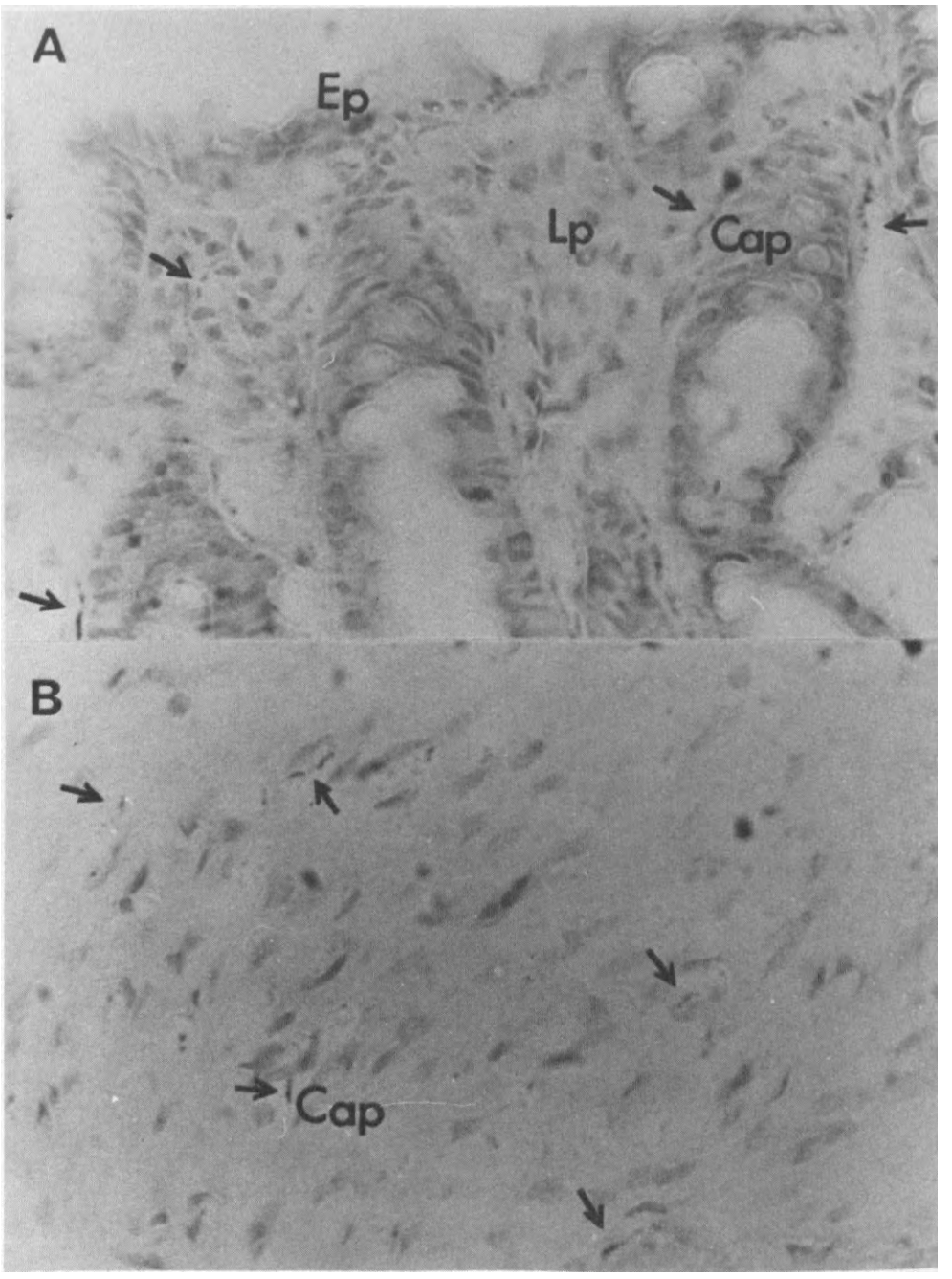

Fig. 6. A: microautoradiograph of the rectum mucosa 1 h after oral administration of [^{14}C]tribenoside at a dose of 10 mg/kg (63.0 μCi/kg) in rats. ×400. Silver grains (arrows) are distributed in the blood capillaries (Cap), but few in the lamina propria (Lp) and on the epithelium (Ep). B: microautoradiograph of the rectum muscularis 1 h after oral administration of [^{14}C]tribenoside at a dose of 10 mg/kg (63.0 μCi/kg) in rats. ×400. Silver grains (arrows) are distributed in the muscularis and the blood capillaries (Cap).

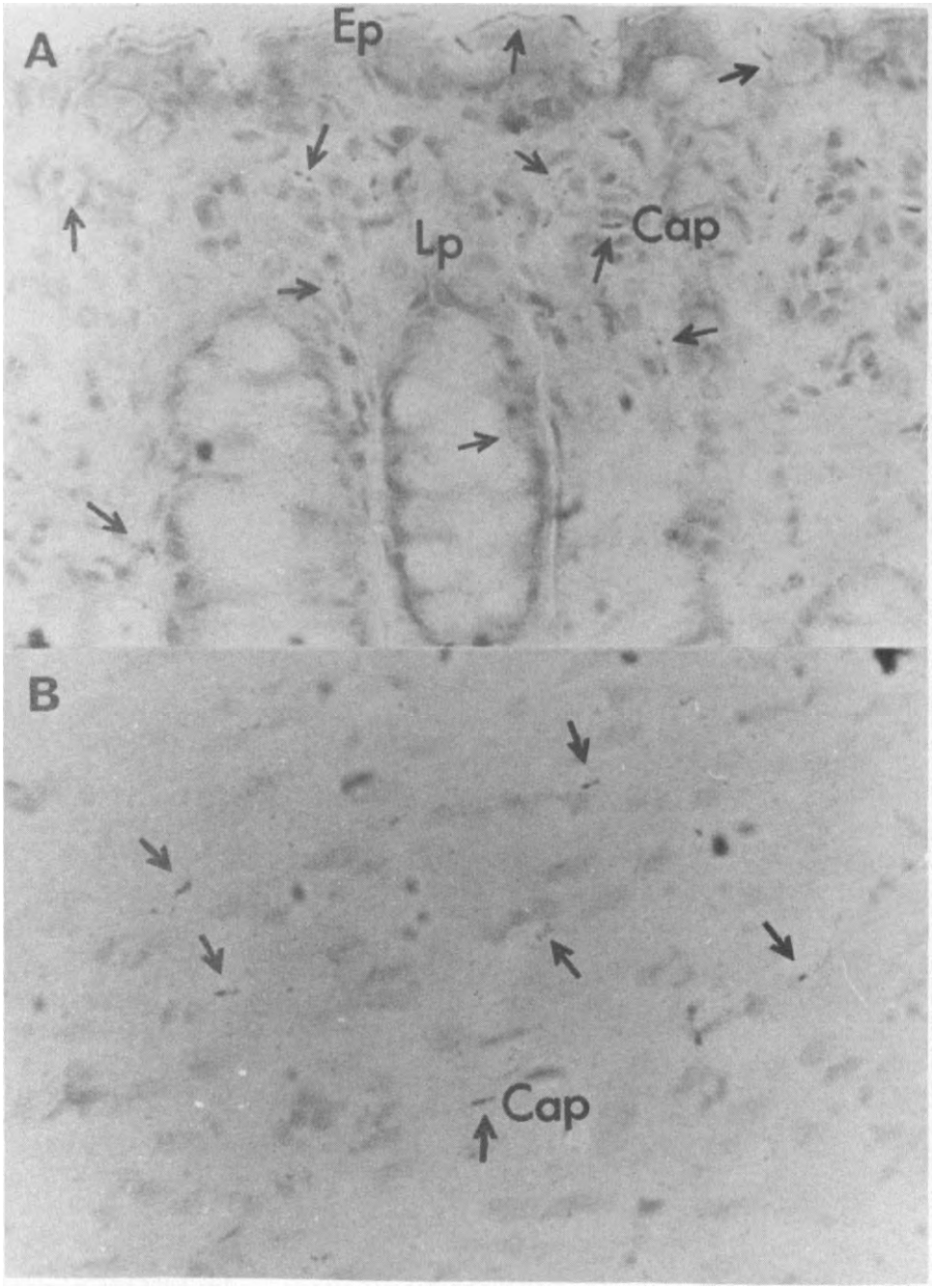

Fig. 7. A: microautoradiograph of the rectum mucosa 1 h after rectal administration of [^{14}C]tribenoside at a dose of 10 mg/kg (63.0 μCi/kg) in rats. ×400. Many silver grains (arrows) are widely distributed in the lamina propria (Lp), and some are also in the blood capillaries (Cap) and on the epithelium (Ep). B: microautoradiograph of the rectum muscularis 1 h after rectal administration of tribenoside at a dose of 10 mg/kg (63.0 μCi/kg) in rats. ×400. Silver grains (arrows) are distributed in the muscularis and the blood capillaries.

the rectum mucosa, particularly in the rectum lamina propria, and some were also observed in the blood capillaries and the muscularis (Fig. 7).

DISCUSSION

The present study in rat also shows that tribenoside is absorbed more slowly and less in the rectal administration than in the oral administration but confirms that a significant amount of tribenoside is absorbed from the rectum. The relative bioavailability estimated from the AUCs and urinary excretion is almost 60%.

To assess the availability in the site of the rectum and anus where hemorrhoids often occur, first the total radioactivity in the tissues was determined both in the oral and rectal administrations. The levels of the radioactivities in the various tissues after oral administration are similar to those obtained by Tanayama et al. (1974), but the levels in the rectum and anus are only 3–5 times higher than in the blood. On the other hand, when tribenoside is administered rectally, the levels of the radioactivities are much higher in those tissues than in the other tissues for longer periods of time, and the average concentrations of the drug in those tissues are almost 50 times higher than for the oral administration as illustrated in Fig. 3. This indicates that the rectal suppository gives a higher local availability than the orally administered solution with respect to the levels in the rectum and anus.

The metabolism of tribenoside has been extensively studied (Keberle and Schmid, 1971; Tanayama et al., 1974; Kobayashi et al., 1974), showing that the drug absorbed was metabolized substantially by the first liver passage, partly in the intestinal mucosa after oral administration. In relation to the metabolic fate, Keberle and Schmid, have proposed 28 different metabolites which are theoretically possible, and they have found that the primary metabolites exhibited effects similar to those of unchanged tribenoside itself, but most of them were only one-half to one-third as active (Keberle and Schmid, 1971).

First-pass effect in the rectal administration is known to be dependent on the pathways through which an absorbed drug enters the systemic circulation; a drug absorbed from the lower or middle parts of the rectum appears first in the inferior or middle rectal veins which go directly to the inferior vena cava, whereas a drug absorbed from the upper part of the rectum appears first in the superior rectal vein which goes to the portal vein to be subjected to metabolism by the first liver passage (Kitahara, 1974; Jonkman et al., 1976). The present study of such pathways shows that tribenoside absorbed from the rectum enters both the inferior vena cava and the portal vein. This indicates that the metabolism by the first liver passage is partly avoided by the rectal administration. But, as shown in Table 4, the metabolism of tribenoside during the systemic circulation is very rapid even by this route of administration. Therefore, the rectal application of tribenoside might not be promising in improving the bioavailability of unchanged tribenoside in the systemic circulation.

The present in vitro results, however, show that tribenoside is hardly metabolized in the rectum homogenate. The study on the metabolism in the rectal mucosa 1 h after rectal administration shows that almost 70% of the radioactivity is due to unchanged tribenoside. These results indicate that unchanged tribenoside after being absorbed from the rectum stays for a longer period of time, which is favourable for the treatment of hemorrhoids.

To further assess the local availability in the rectum, the distribution of the radioactivity in the tissue was measured by means of microautoradiography after oral and rectal administrations. Tribenoside rectally administered, first distributes from the molten suppository base widely into the rectum lamina propria via the epithelium, and then gradually into the rectum plexus in the mucosa, or into the muscularis, while tribenoside orally administered distributes via the blood stream from the systemic circulation into the rectal mucosa or into the muscularis. The results confirm that the retention of the radioactivity in the rectum and anus is not due to the drug staying at the surface of the rectum epithelium, but due to the drug which is actually absorbed into the inner part of the tissues. Moreover, the slower absorption of the rectal suppository found in the blood level data might result from the slow diffusion of the drug from the rectum tissue into the blood stream.

These findings strongly suggest that rectal application of tribenoside for such a treatment of hemorrhoids as a suppository, could offer a greater advantage with regard to the distribution of the drug close to the actual site of the action before being diluted in the systemic circulation and metabolized by the liver passage, or with regard to the duration of the drug action.

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