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Decrease of Gastrointestinal Mucosal Damage by Salicyluric Acid Compared with Salicylic Acid in Rabbits

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Abstract—The gastrointestinal mucosal damage following the oral administration of salicylic acid or salicyluric acid was examined in rabbits using a scanning electron microscope. Six and 24 h after treatment with salicylic acid, morphological changes of gastric mucosa were recognized. In rabbits treated with salicyluric acid, however, severe damage in the gastric mucosa was not found after 24 h compared with the treatment with salicylic acid. Following the treatment with salicylic acid, some mucosal damage in the duodenum, jejunum and ileum was observed after 24 h. The surface character of the duodenal, jejunal, ileal, caecal and colonic mucosa were almost identical compared with the control following the treatment with salicyluric acid. It was reported that salicyluric acid is metabolized to salicylic acid by the intestinal microorganisms. From these results, it was suggested that prodrugs utilizing the metabolism of salicyluric acid to salicylic acid by intestinal microorganisms may be useful in reducing gastrointestinal mucosal damage.

The damaging effects of drugs on the gastrointestinal tract are considered in terms of the gastrointestinal mucosal barrier, gastrointestinal erosions and microbleeding and whether peptic ulcers are caused. Gastrointestinal mucosal damage is often produced in experimental animals and humans by nonsteroidal anti-inflammatory drugs including indomethacin, aspirin and phenylbutazone, and by secretagogues. The ingestion of salicylic acid may result in epigastric distress, nausea and vomiting. Salicylic acid may also cause gastric ulceration and even haemorrhage. In previous reports (Shibasaki et al 1985; Nakamura et al 1986, 1988a, b, 1989a, b, 1990), we examined the blood concentration of salicyluric acid and salicylic acid following the i.v., oral, intracaecal and rectal administration of salicyluric acid in rabbits, rats and dogs. In these species, salicyluric acid is metabolized to salicylic acid by intestinal microorganisms. Following rectal administration of salicyluric acid, prolonged blood concentrations of salicylic acid were observed. However, species differences in the metabolic fate of salicyluric acid following oral and i.v. administration of salicyluric acid were recognized. This study was undertaken to examine the gastrointestinal mucosal damage following the oral administration of salicyluric acid or salicylic acid using a scanning electron microscope in rabbits.

Materials and Methods

Materials. Salicyluric acid was obtained from Sigma Chemical Co. (St Louis, USA). Salicylic acid and glutaraldehyde (25% in water) were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Carboxymethylcellulose sodium salt (CMC) was obtained from Hayashi Pure Chemical Industries Ltd. (Osaka, Japan). All other chemicals used in these experiments were of reagent grade.

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Animal experiments. Male albino rabbits, 2–3 kg, were individually housed in cages in an air-conditioned room and maintained on a standard laboratory diet (ORC4, Oriental Yeast Co. Ltd, Tokyo, Japan). Salicyluric acid (1 g kg^{-1}, salicylic acid equivalent) or salicylic acid (1 g kg^{-1}) suspended in 1% CMC solution were administered by gastric intubation in at least two rabbits and animals were allowed free access to water only. Six or 24 h later, the gastrointestinal tract was removed under pentobarbitone anaesthesia. Two or 3 specimens of gastric and intestinal mucosa in each rabbit were placed in 1% glutaraldehyde solution diluted with pH 7·3 phosphate buffer solution for 1 h at 4°C. Gastrointestinal mucosal damage was observed using a scanning electron microscope (model WS-250, Akashi Beam Technology Co., Tokyo, Japan).

Results and Discussion

Many studies have pointed to the importance of intestinal micro-organisms in metabolizing a wide variety of drugs and other foreign compounds (Scheline 1968, 1973; Williams 1972; Boxenbaum et al 1974, 1979; Goldman 1978; Rowland 1986). In the previous report (Shibasaki et al 1985), we demonstrated the conversion of orally administered salicyluric acid to salicylic acid by gut microflora after oral administration in rabbits. In the present study, the gastrointestinal mucosal damage following the oral administration of salicyluric acid or salicylic acid to rabbits was examined using a scanning electron microscope.

Fig. 1 shows scanning electron micrographs of gastric mucosa. In control rabbits (Fig. 1a), the cobblestone appearance of the surface at this magnification was attributable to the convex surfaces of individual epithelial cells, covered with gastric mucus. The polyhedral shape of the apical, lateral boundaries in the rabbit stomach is common to most species, including man. Stomach treated with salicylic acid, 6 and 24 h previously had widely-spaced gastric pits (Fig. 1b, d). Individual epithelial cells could not be clearly observed.

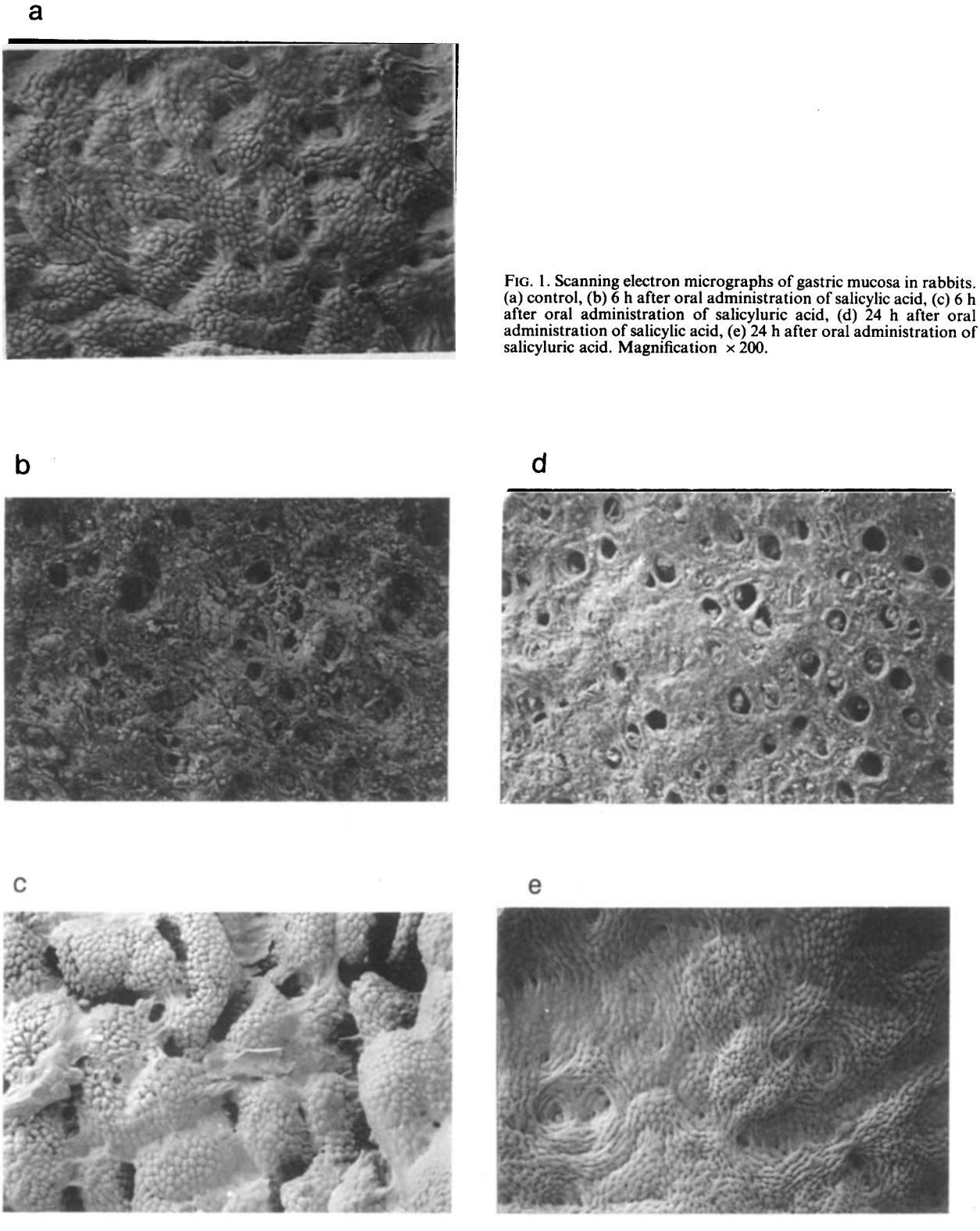

FIG. 1. Scanning electron micrographs of gastric mucosa in rabbits. (a) control, (b) 6 h after oral administration of salicylic acid, (c) 6 h after oral administration of salicyluric acid, (d) 24 h after oral administration of salicylic acid, (e) 24 h after oral administration of salicyluric acid. Magnification × 200.

As shown in Fig. 1c, the stomach treated with salicyluric acid 6 h previously appeared to have mucosal damage, but had less irritation compared with the treatment with salicylic acid. The sample obtained 24 h after salicyluric acid treatment (Fig. 1e) had a surface character of the epithelial cells almost identical to that of the control.

Scanning electron micrographs of duodenal mucosa are shown in Fig. 2. In control rabbits (Fig. 2a), the duodenal villi were broad and occasionally folded on the longitudinal axis. Individual cells could not be discriminated at this magnification. Six and 24 h after treatment with salicylic acid (Fig. 2b, d), some change of the mucosal surface was

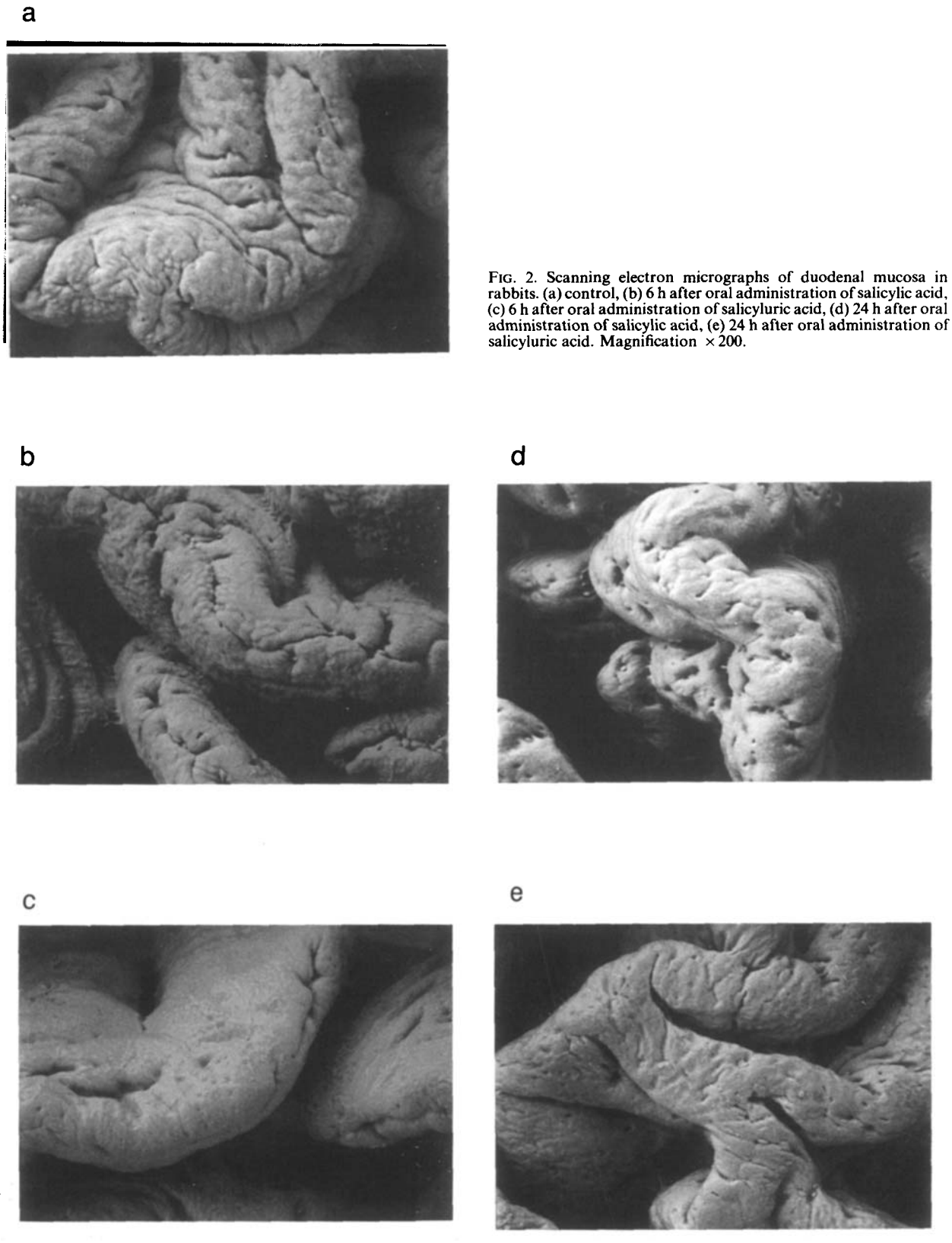

FIG. 2. Scanning electron micrographs of duodenal mucosa in rabbits. (a) control, (b) 6 h after oral administration of salicylic acid, (c) 6 h after oral administration of salicyluric acid, (d) 24 h after oral administration of salicylic acid, (e) 24 h after oral administration of salicyluric acid. Magnification ×200.

recognized. However, following treatment with salicyluric acid the surface character of the duodenal mucosa was almost identical to that of the control (Fig. 2c, e).

Fig. 3 shows scanning electron micrographs of jejunal mucosa. Broad and tongue-shaped villi were found in the jejunum. Twenty-four h after treatment with salicylic acid (Fig. 3d), morphological change of the jejunal mucosa was observed, and mucus was secreted. After twenty-four h

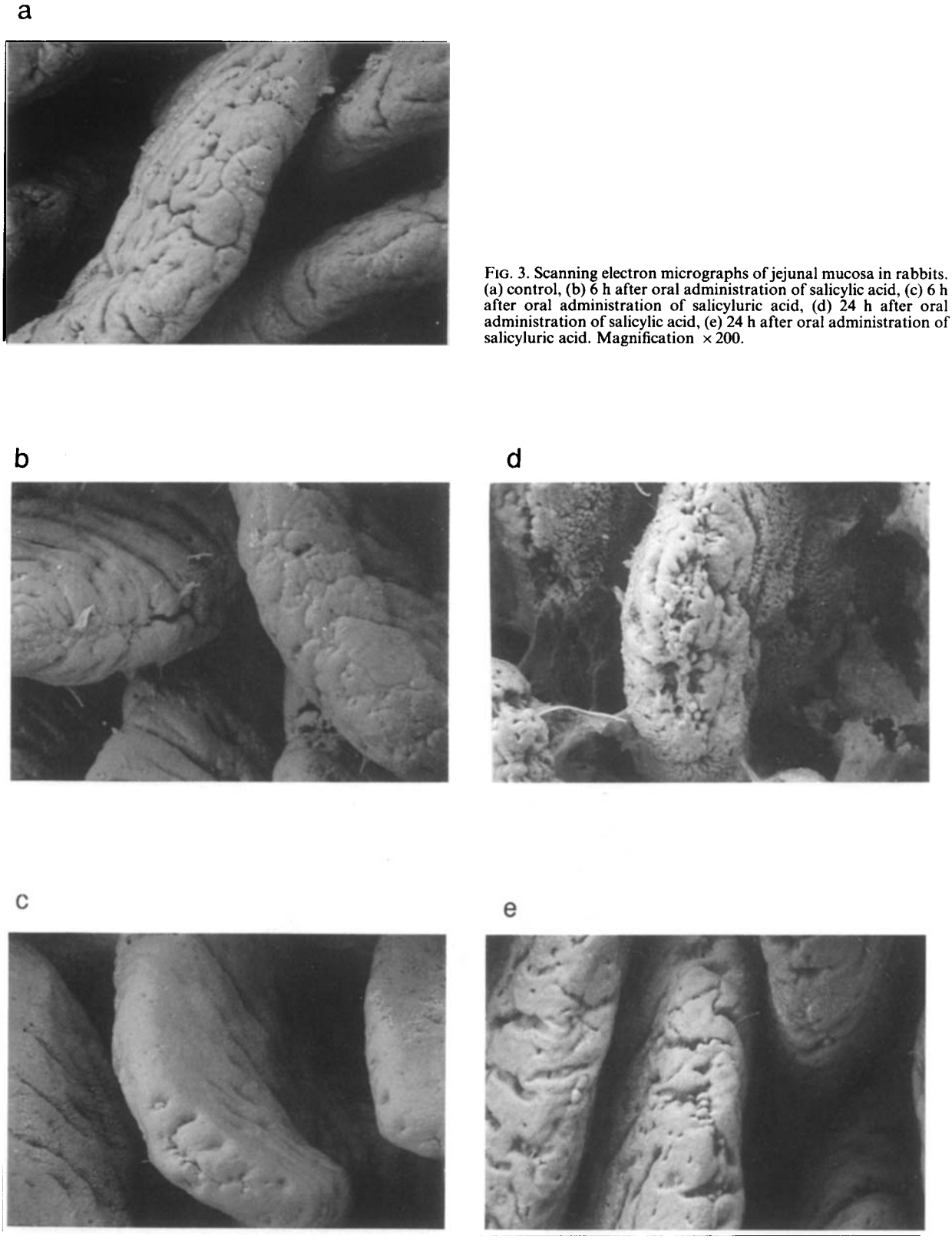

FIG. 3. Scanning electron micrographs of jejunal mucosa in rabbits. (a) control, (b) 6 h after oral administration of salicylic acid, (c) 6 h after oral administration of salicyluric acid, (d) 24 h after oral administration of salicylic acid, (e) 24 h after oral administration of salicyluric acid. Magnification ×200.

treatment with salicyluric acid (Fig. 3e), the surface character of the jejunal mucosa was almost identical to that of the control.

Scanning electron micrographs of ileal mucosa are shown in Fig. 4. In control rabbits (Fig. 4a), the ileal villi were broad and tongue-shaped structures. Following six and 24 h treatment with salicylic acid (Fig. 4b, d), the villi of ileal mucosa were broken down and the villus surface was covered

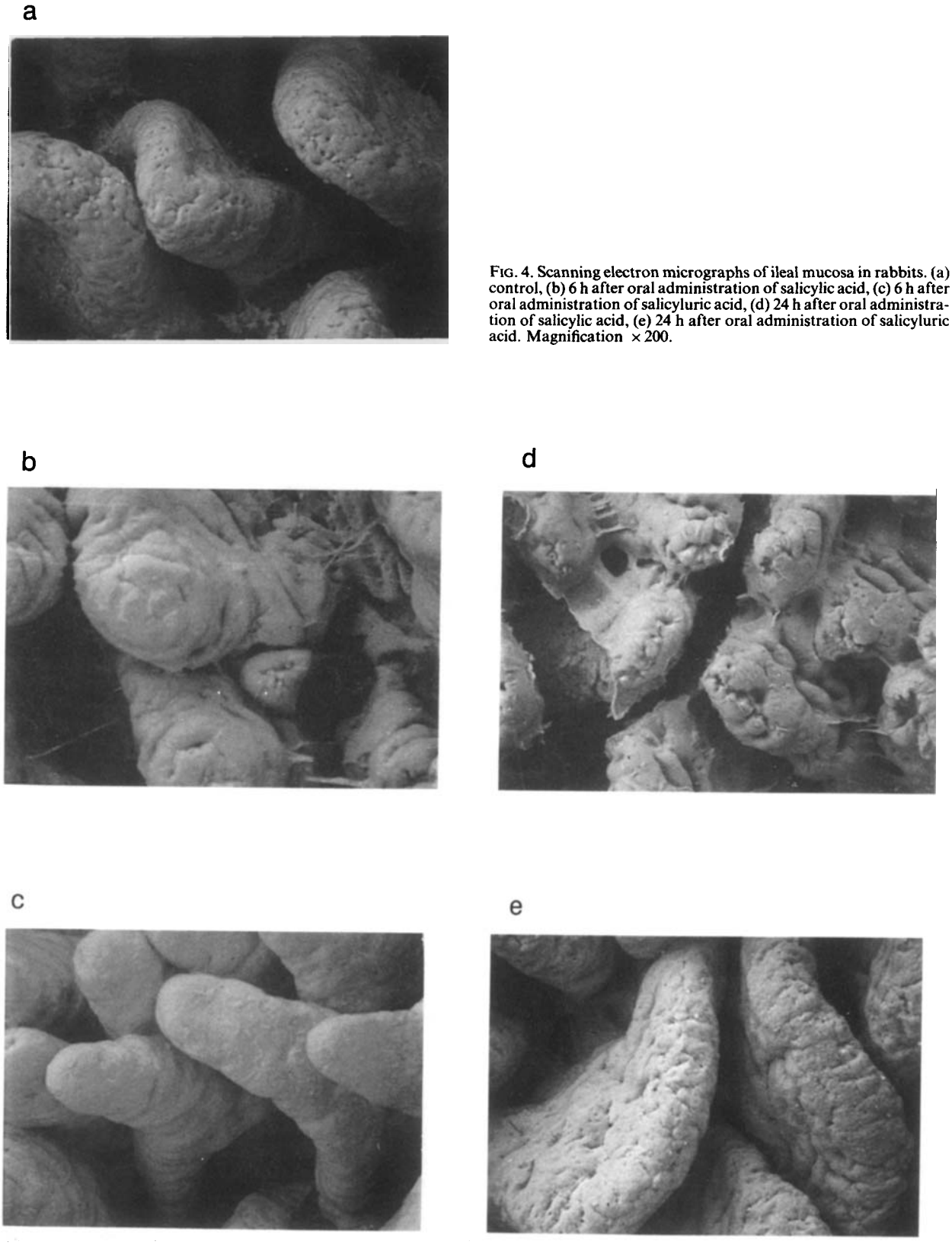

FIG. 4. Scanning electron micrographs of ileal mucosa in rabbits. (a) control, (b) 6 h after oral administration of salicylic acid, (c) 6 h after oral administration of salicyluric acid, (d) 24 h after oral administration of salicylic acid, (e) 24 h after oral administration of salicyluric acid. Magnification × 200.

with mucus. However, severe mucosal damage was not found following treatment with salicyluric acid (Fig. 4c, e).

Fig. 5 shows scanning electron micrographs of caecal mucosa. The mucosal surface of the caecum in control rabbits showed a folded structure (Fig. 5a). In most rodents and herbivorous mammals, the caecum is a highly-developed, morphologic specialization and is important in the bacterial degradation of macromolecular carbohydrates of

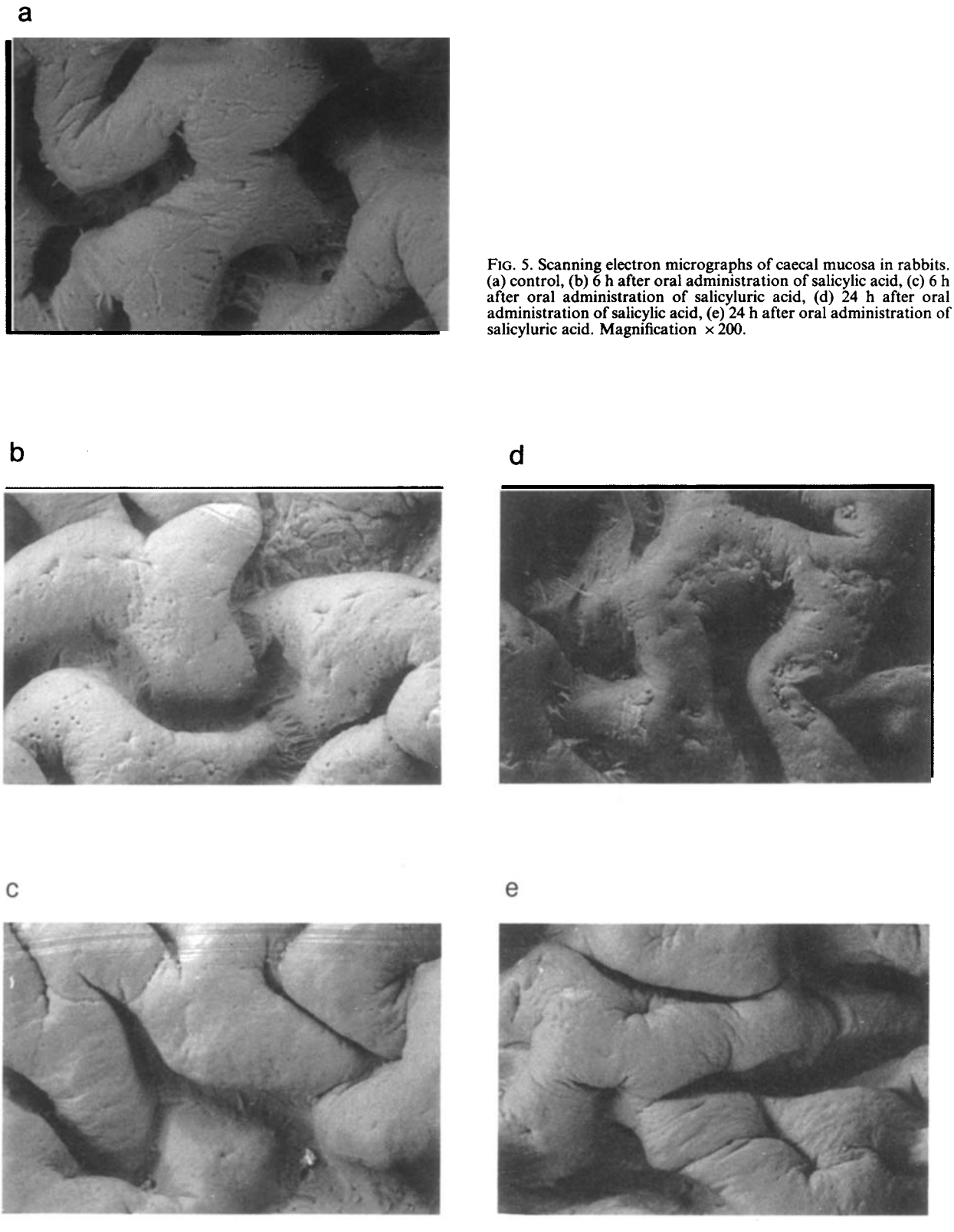

FIG. 5. Scanning electron micrographs of caecal mucosa in rabbits. (a) control, (b) 6 h after oral administration of salicylic acid, (c) 6 h after oral administration of salicyluric acid, (d) 24 h after oral administration of salicylic acid, (e) 24 h after oral administration of salicyluric acid. Magnification ×200.

dietary origin. No mucosal damage was seen either six or 24 h after treatment with salicylic acid (Fig. 5b, d) or salicyluric acid (Fig. 5c, e).

Scanning electron micrographs of colonic mucosa are shown in Fig. 6. The mucosal surface of the colon was relatively flat and characterized by numerous rugosities. Following the treatment with salicylic acid (Fig. 6b, d) or salicyluric acid (Fig. 6c, e) severe mucosal damage was not observed.

Colonic delivery of drugs from prodrug depends on the

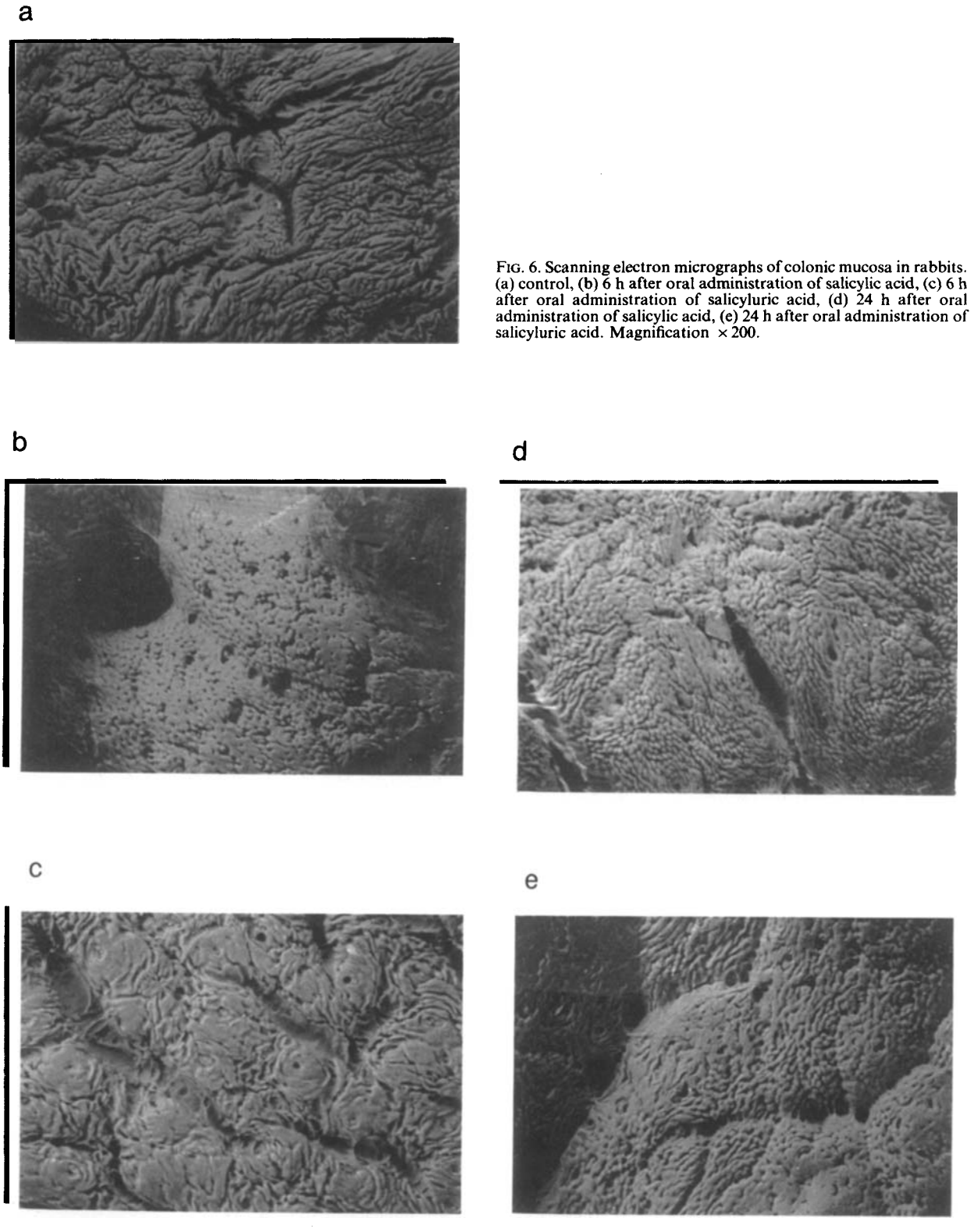

FIG. 6. Scanning electron micrographs of colonic mucosa in rabbits. (a) control, (b) 6 h after oral administration of salicylic acid, (c) 6 h after oral administration of salicyluric acid, (d) 24 h after oral administration of salicylic acid, (e) 24 h after oral administration of salicyluric acid. Magnification ×200.

sharp gradient of enzyme activity in the gastrointestinal tract. In addition to the colon-specific drug delivery, the evaluation of intestinal bacterial growth and the prolonged blood concentration of drug, the present results suggest that prodrugs utilizing metabolism in intestinal microorganisms may be useful in reducing gastrointestinal mucosal damage.

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References

Boxenbaum, H. G., Jodhka, G. S., Ferguson, A. C., Riegelman, S., MacGregor, T. R. (1974) The influence of bacterial gut hydrolysis on the fate of orally administered isonicotinuric acid in man. J. Pharmacokinet. Biopharm. 2: 211–237

Boxenbaum, H. G., Bekersky, I., Jack, M. L., Kaplan, S. A. (1979) Influence of gut microflora on bioavailability. Drug Metab. Rev. 9: 259–279

Goldman, P. (1978) Biochemical pharmacology of the intestinal flora. Ann. Rev. Pharmacol. Toxicol. 18: 523–539

Nakamura, J., Inoue, Y., Sasaki, H., Shibasaki, J. (1986) Prolonged blood concentration of salicylic acid following the simultaneous oral administration of salicylic acid and salicyluric acid in rabbits. Chem. Pharm. Bull. 34: 2624–2627

Nakamura, J., Shiota, H., Haraguchi, Y., Sasaki, H., Shibasaki, J. (1988a) Further studies on the hydrolysis of salicyluric acid in intestinal microorganisms and prolonged blood concentration of salicylic acid following rectal administration of salicyluric acid in rabbits. J. Pharmacobio-Dyn. 11: 53–57

Nakamura, J., Shiota, H., Sasaki, H., Shibasaki, J. (1988b) Hydrolysis of salicyluric acid in intestinal microorganisms and prolonged blood concentration of salicylic acid following rectal administration of salicyluric acid in rats. Ibid. 11: 625–629

Nakamura, J., Shiota, H., Sasaki, H., Shibasaki, J. (1989a) Sustained blood concentration of salicylic acid following rectal administration of salicyluric acid in dogs. Chem. Pharm. Bull. 37: 2537–2538

Nakamura, J., Haraguchi, Y., Sasaki, H., Shibasaki, J. (1989b) Effect of fasting on the hydrolysis of salicyluric acid in rabbit intestinal microorganisms. J. Pharmacobio-Dyn. 12: 602–607

Nakamura, J., Haraguchi, Y., Asai, K., Sasaki, H., Shibasaki, J. (1990) Effect of pretreatment with antibiotics on the hydrolysis of salicyluric acid in rabbit intestinal microorganisms. Ibid. 13: 461–467

Rowland, I. R. (1986) Reduction by the gut microflora of animals and man. Biochem. Pharmacol. 35: 27–32

Scheline, R. R. (1968) Drug metabolism by intestinal microorganisms. J. Pharm. Sci. 57: 2021–2037

Scheline, R. R. (1973) Metabolism of foreign compounds by gastrointestinal microorganisms. Pharmacol. Rev. 25: 451–523

Shibasaki, J., Inoue, Y., Kadosaki, K., Sasaki, H., Nakamura, J. (1985) Hydrolysis of salicyluric acid in rabbit intestinal microorganisms. J. Pharmacobio-Dyn. 8: 989–995

Williams, R. T. (1972) Toxicologic implications of biotransformation by intestinal microflora. Toxicol. Appl. Pharmacol. 23: 769–781