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Radioautographic studies on radiosulfate incorporation in the digestive organs of mice

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Summary. The sulfate uptake and accumulation in mouse digestive organs were studied by light microscopic radioautography. Two litters of normal ddY mice 30 days after birth, each consisting of 3 animals, were studied. One litter of animals were sacrificed 30 min after the intraperitoneal injections with phosphate buffered $Na_2{}^{35}SO_4$, and the other litter animals were sacrificed 12 hr after the injections. Then several digestive organs, the parotid gland, the submandibular gland, the sublingual gland, antrum and fundus of the stomach, the duodenum, the jejunum, the ileum, the caecum, the ascending colon and the descending colon were taken out. The tissues were fixed, dehydrated, embedded in epoxy resin, sectioned, picked up onto glass slides, coated with radioautographic emulsion by a dipping method. After the exposure, they were developed, stained with toluidine blue and analyzed by light microscopy.

As the results, many silver grains were observed on serous cells of the salivary glands, mucosa and submucosa of the stomach, villous cells and crypt cells of the small intestines and whole mucosa of the large intestines at 30 min after the injection. Then at 12 hr after the injection silver grains were observed on mucous cells of the salivary glands, some of the stomach glands, and mucigen granules of goblet cells in the small intestines and the large intestines. The numbers of silver grains observed in respective organs at 30 min were less than those at 12 hr.

From these results, it is concluded that glycoprotein synthesis was demonstrated in several digestive organs by radiosulfate incorporation. In the salivary glands the silver grains were more observed in serous cells at 30 min, while in mucous cells more at 12 hr than 30 min after the injection. In other organs the silver grains were more at 30 min than at 12 hr. These results show the time difference of glycoprotein synthesis in respective organs.

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Introduction

The glycoproteins have recently been reported to hold the relationship between some kinds of microhabitants and the digestive organs such as human salivary glands (Murray et al., 1992), swine intestines (Katwa et al., 1991) and mouse small intestines (Laux et al., 1986). Spooner et al. (1985) demonstrated sulfate-rich glycosaminoglycan deposition in rat embryonic salivary gland. The distribution and synthesis of sulfate-containing glycoproteins were found in the stomach and the duodenum of rats (Smits and Kramer, 1981), goblet cells of the small intestines of mice (Merzel and Almeida, 1973), gastrointestinal epithelial cells of mice (Nagata et al., 1988), the small intestines of rats (Yeh and Moog, 1984), the colons of rats (Sawicki and Rowinski, 1969) and the stomach of dogs (Spicer et al., 1967). Sulfate is supposed to play some significant roles as precursor for the glycoproteins and glycosaminoglycans in several organs of the gastrointestinal system (Cutler, 1989; Madara, 1991).

In this paper, we have studied the uptake and accumulation of radiosulfate $^{35}SO_4$ in several digestive organs of mice by means of light microscopic radioautography.

Materials and methods

Animals

Two litters of normal ddY mice, each consisting of 3 individuals, bred and maintained in our laboratory for 30 days after birth, weighing about 150 gm, were used. They were fed on a standard diet (Clea EC-2, Clea Co., Tokyo, Japan) every morning with access to water ad libitum and were not fasted until sacrifice.

Tissue Preparation

Animals were injected with radiosulfate solution, phosphate-buffered (pH 7.2) $Na_2{}^{35}SO_4$ (NEN, New England, USA, specific activity 185 GBq /mM, 740 kBq/g body weight), before sacrifice. At 30 minutes after the intraperitoneal injection, one group of animals were sacrificed under deep anesthesia with Nembutal (Abbott Laboratories, Chicago, Ill., USA), and the other group of animals were sacrificed at 12 hr after the injection. Then the digestive organs, i.e., the parotid gland, the submandibular gland, the sublingual gland, the antrum and the fundus of the stomach, the duodenum, the jejunum, the ileum, the caecum, the proximal colon and the distal colon, were quickly taken out from each animal. The tissues were cut into small pieces (1 x 1 x 1 mm) with blades and 3 pieces of tissue blocks from each organ of each animal were washed in Hanks' solution (Nissui, Tokyo, Japan), fixed in phosphate-buffered (pH 7.2) 2.5% glutaraldehyde at 4 °C for 3 hr and postfixed in 1% osmium tetroxide at 4 °C for 1 hr, dehydrated in ascending series of ethanol and acetone, and finally embedded into Luveak 812 epoxy resin (Oken Co., Tokyo, Japan).

Radioautography

The embedded tissue blocks, 3 pieces from each organ of each animal, were sectioned at 2μm thickness with a Porter-Blum MT-2B ultramicrotome (DuPont-Sorvall, Newtown, CA, USA) using glass knives. The 15 to 18 sections obtained from each animal were collected onto 3 glass slides, and coated with Konica NR-M2 emulsion (Konica Photo Industry Co., Tokyo, Japan) by a dipping method (Nagata, 1992, 1997). After the exposure in dark boxes at 4 °C for 10 months, they were developed with SDX-1 developer at 20 °C for 5 minutes and fixed with an acid hardening fixer (sodium sulfate) for 10 minutes. They were stained with 0.1% toluidine blue in 0.1M sodium phosphate buffer (pH 7.4) for 3 min, and air dried. As controls, tissues obtained from normal mice without any RI-injection were processed through the same procedure for radioautography. All the specimens were observed and photographed with an Olympus Vanox AHB-LB light microscope (Olympus Optical Industries Co., Tokyo, Japan).

Results

When the light microscopic radioautograms prepared from all the organs obtained from the animals receiving radiosulfate injections were observed with transmission light microscopy using x100 oil immersion objectives, many silver grains were found over various kinds of cells in the respective organs. The detailed findings will be described in the following organs respectively. On the other hand, no silver grain was observed in the control radioautograms prepared from the normal animals without radiosulfate injection.

The salivary glands

The radioautograms prepared from the salivary glands showed the silver grains over the cytoplasm of all kinds of cells of the parotid, the submandibular and the sublingual glands of all the animals. At 30 min after the radiosulfate injection (Fig. 1A, C, D), many silver grains were observed on all the parotid gland cells (Fig. 1A) and on serous cells of the submandibular gland (Fig. 1C) and the sublingual gland (Fig. 1E). Some numbers of silver grains were also found on the mucous cells of the submandibular gland and the sublingual gland (Fig. 1C, E). On the other hand, at 12 hr after the injection (Fig. 1B, D, F) many silver grains were observed on the mucous cells of the submandibular gland (Fig. 1D) and the sublingual gland (Fig. 1F), while very small numbers of silver grains were found on the parotid gland (Fig. 1B) and on the serous cells of the submandibular and sublingual gland (Fig. 1D, F). Some constant numbers of silver grains were observed on the ductal cells and the connective tissues of all the salivary glands during the two time intervals, 30 min and 12 hr after the injections.

The stomach

The silver grains were observed on all kinds of cells in the glands of the fundus and the antrum. At 30 minutes after the radiosulfate injection (Fig. 2A, B, E, F), many silver grains were observed on various cells of the mucosa, i.e. the chief cells, the parietal cells, the basement granular cells, the undifferentiated cells and the mucous neck cells, as well as the submucosal tissue and the smooth muscle layer. The number of silver grains on the fundus (Fig. 2A, B, C, D) was lower than that of the antrum (Fig. 2E, F, G, H). However, at 12 hr after the injection (Fig. 2C, D, G, H) silver grains were scarcely observed on either the fundus (Fig. 2C, D) or the antrum (Fig. 2G, H).

The small intestine

The silver grains were observed on cytoplasm of all kinds of epithelial cells, smooth muscle cells and connective tissue. At 30 min after the radiosulfate injection (Fig. 3A, B, E, F, I, J), many silver grains were observed on villi cells, especially on the Golgi regions of goblet cells and basement granular cells. The number of the silver grains changed along the intestine, more in the duodenum (Fig. 3A, B), moderate in the jejunum (Fig. 3E, F) and less in the ileum (Fig. 3I, J). At 12 hr after the injection (Fig. 3C, D, G, H, K, L), silver grains were located on mucous granules of goblet cells and cytoplasm of basement granular cells on villi (Fig. 3D, H, L), and only some numbers of silver grains were observed on crypts (Fig. 3C, G, K). There was not as much difference among the number of silver grains on the duodenum (Fig. 3C, D), the jejunum (Fig. 3G, H) and the ileum (Fig. 3K, L).

The large intestine

The silver grains were observed on cytoplasm of all kinds of epithelial cells, smooth muscle cells and connective tissue of the caecum (Fig. 4A, B, C, D), proximal colon (Fig. 4E, F, G, H) and distal colon (Fig. 4I, J, K, L). At 30 min after the radiosulfate injection (Fig. 4A, B, E, F, I, J), some silver grains were observed on the cells in the intestinal gland, especially on the Golgi regions of the goblet cells (Fig. 4A, B, E, F, I, J). At 12 hr after the injection (Fig. 4C, D, G, H, K, L), fewer silver grains were located on the mucigen granules of the goblet cells. There were small differences between the number of silver grains on the caecum (Fig. 4C, D), and proximal (Fig. 4G, H) and distal colons (Fig. 4K, L).

Discussion

From the results obtained at present, it is clear that the silver grains demonstrated in the radioautograms of respective organs are due to the glycoproteins incorporating radiosulfate, since the control radioauto-

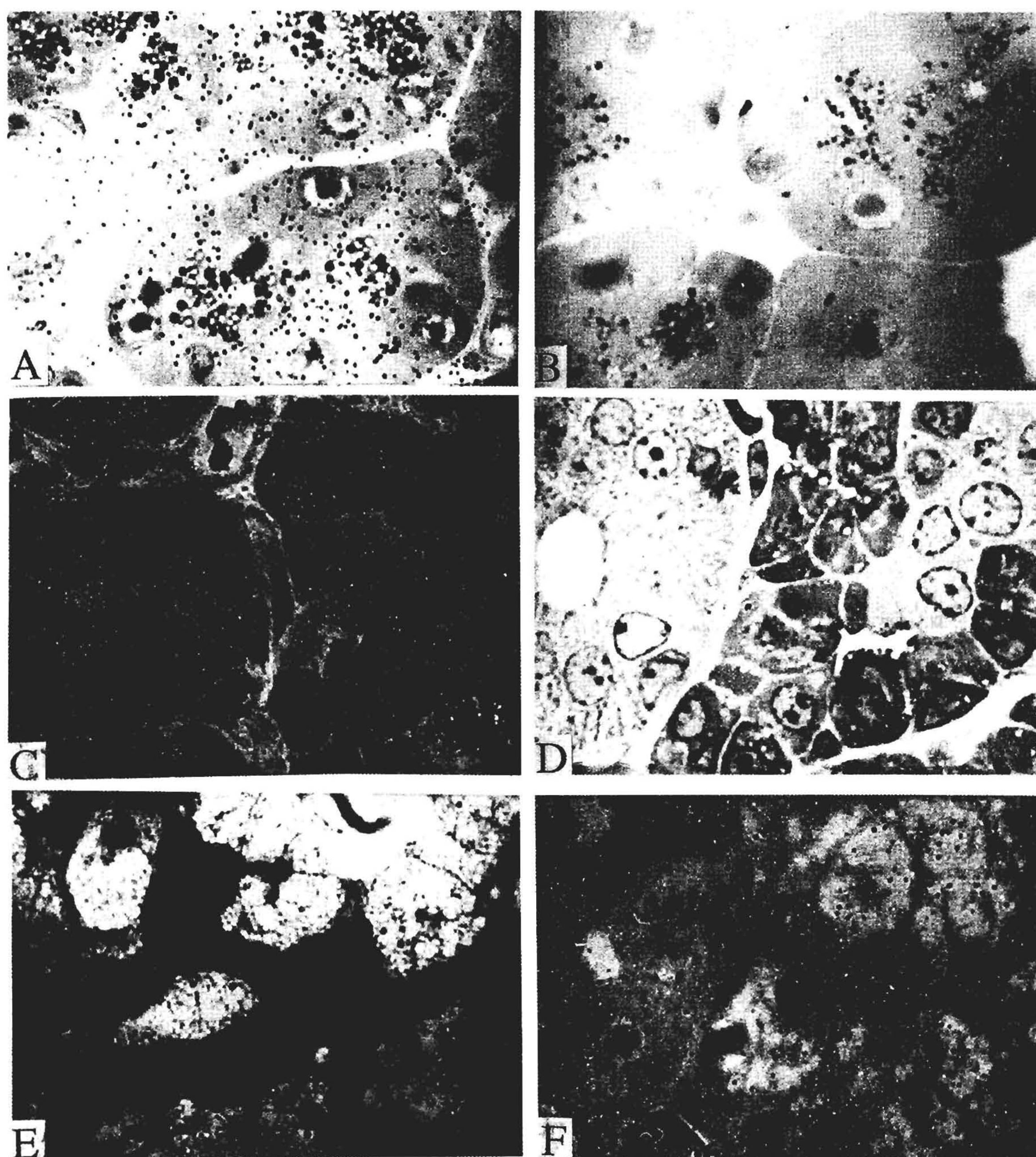

Fig. 1. Light microscopic radioautogram of the salivary glands. **A, B:** parotid gland; **C, D:** submandibular gland; **E, F:** sublingual gland. A, C, E: 30 min after $^{35}SO_4$ injection; B, D, F: 12 hr after $^{35}SO_4$ injection. x 400

grams without radiosulfate injection did not show any silver grains. From the results, glycoprotein synthesis was demonstrated in several digestive organs simultaneously by radioautography using $^{35}SO_4$. It is interesting to observe that in the salivary glands silver grains were found more in serous cells at 30 minutes than 12 hr but in mucous cells at 12 hr rather than 30 min after the injection. In other organs, such as the stomach, and the small and large intestines, the silver grains were greater at 30 minutes than at 12 hr.

The chemical composition of glycoproteins synthesized in the digestive organs has been studied

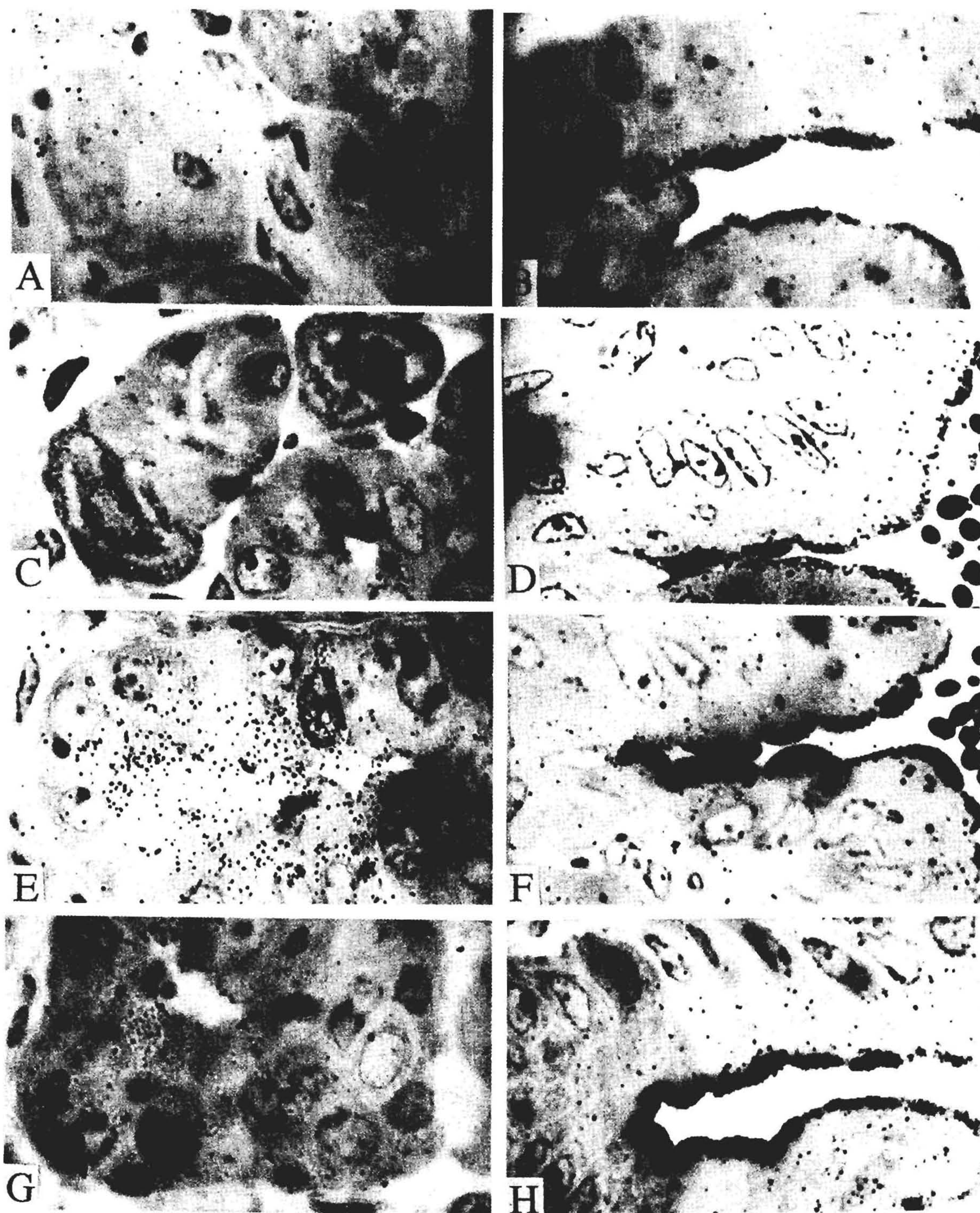

Fig. 2. Light microscopic radioautogram of mucosal epithelium of the stomach. **A-D:** mucosal epithelium of the fundus; **E, F, G, H:** mucosal epithelium of the antrum; A, C, E, G: crypt of the gland; B, D, F, H: neck and free surface of the stomach; A, B, E, F: 30 min after $^{35}SO_4$ injection; C, D, G, H: 12 hr after $^{35}SO_4$injection. x 400

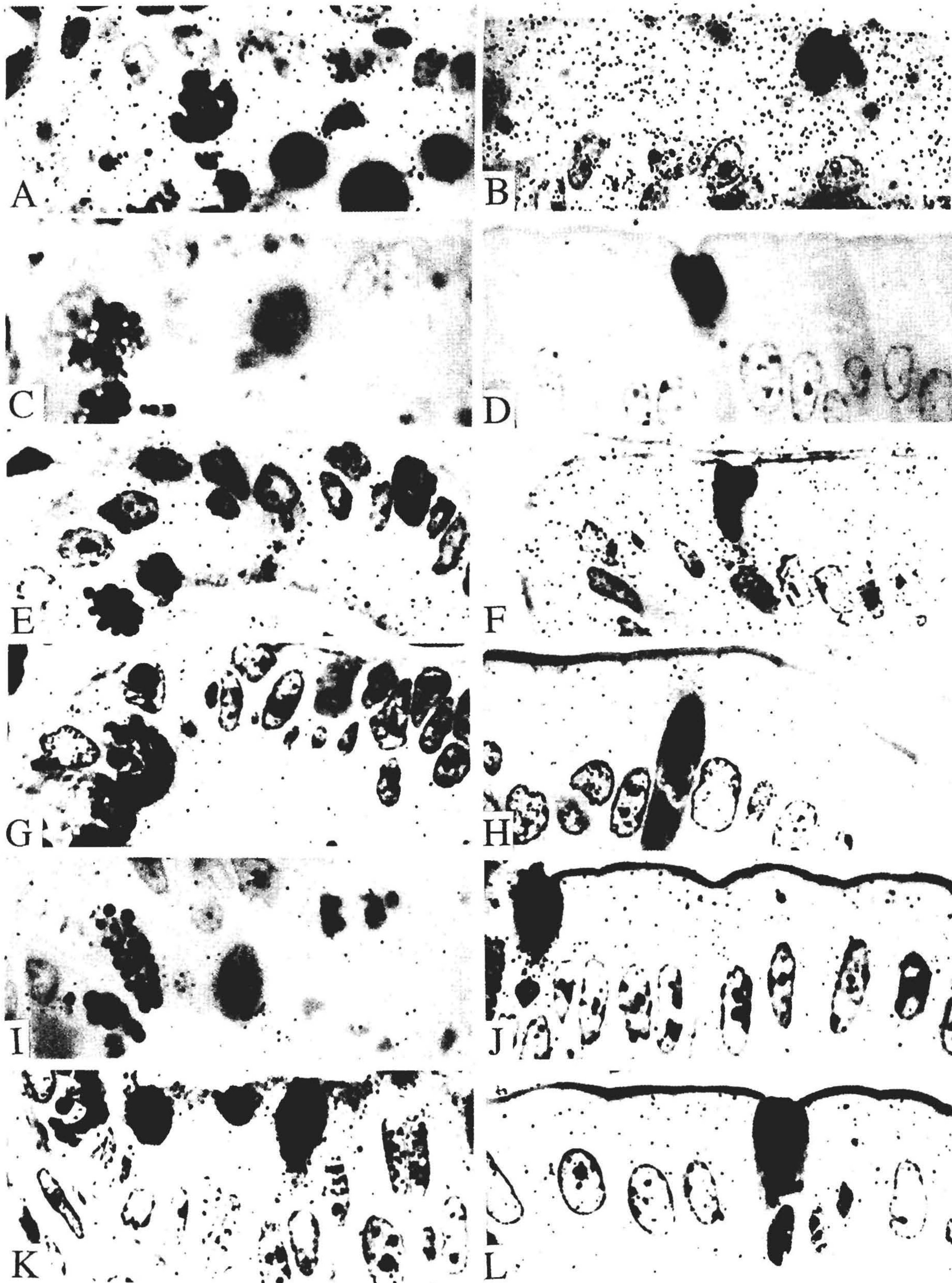

Fig. 3. Light microscopic radioautogram of the small intestines. **A-D:** mucosal epithelium of the duodenum; **E-H:** mucosal epithelium of the jejunum; **I, J, K, L:** mucosal epithelium of the ileum. A, C, E, G, I, K: crypt of the gland; B, D, F, H, J, L: villous cells; A, B, E, F, I, J: 30 min after $^{35}SO_4$ injection; C, D, G, H, K, L: 12 hr after $^{35}SO4$ injection. x 400

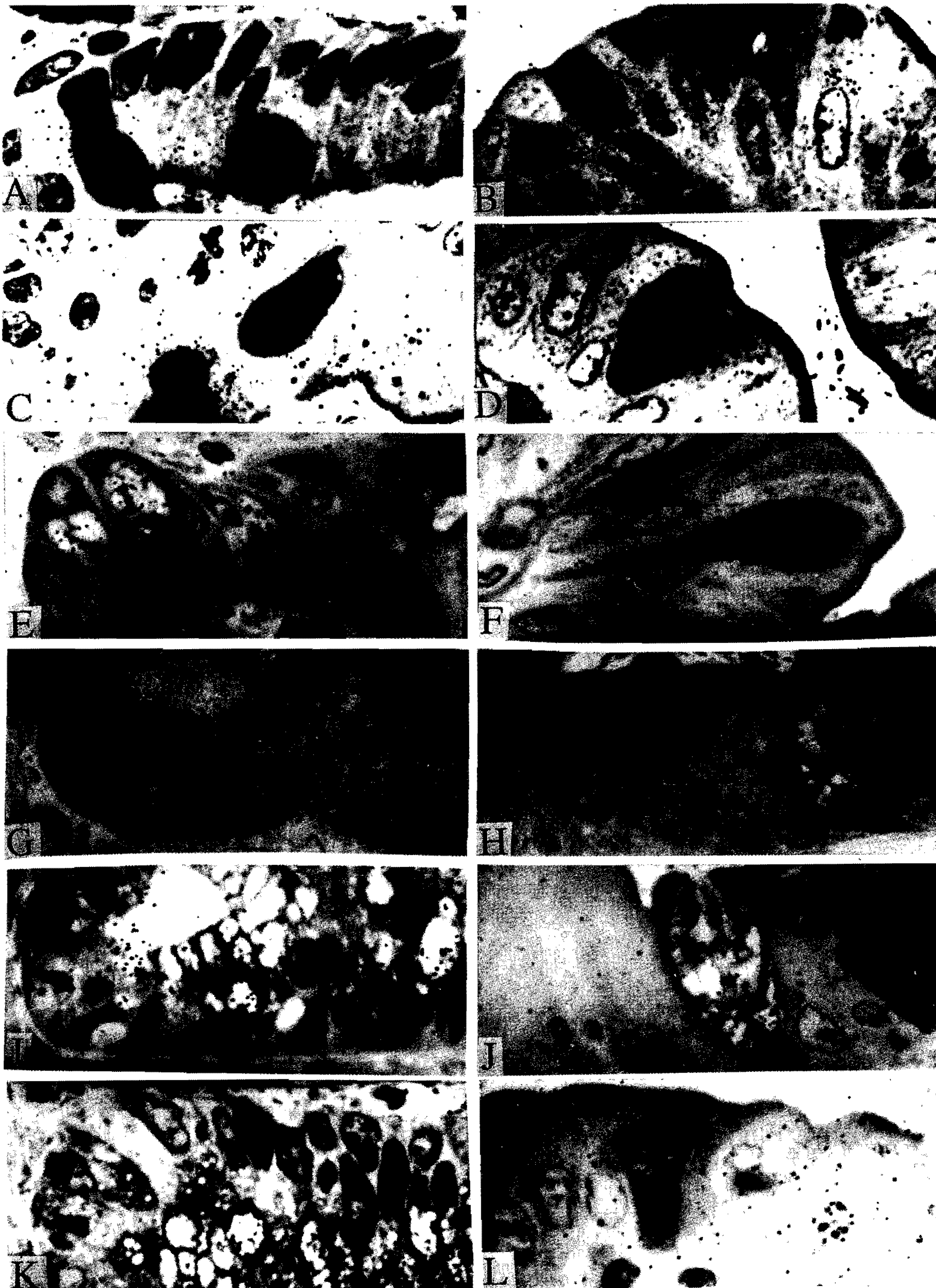

Fig. 4. Light microscopic radioautogram of the large intestines. **A-D:** mucosal epithelium of the caecum; **E-H:** mucosal epithelium of the proximal colon; **I-L:** mucosal epithelium of the distal colon; A, C, E, G, I, K: crypt of the gland; B, D, F, H, J, L: free surface; A, B, E, F, I, J: 30 min after $^{35}SO_4$ injection; C, D, G, H, K, L: 12 hr after $^{35}SO_4$ injection. x 400

biochemically by some authors. Bouziges et al. (1991) reported the synthesis of glycosaminoglycan chains studied with incorporations of ^{3}H-glucosamine and ^{35}S-sulfate in developing rat intestinal segments at various developmental stages and found a clear age-dependence with a broad maximum in the fetal period when dramatic growth and morphogenesis occurred. Kim and Perdomo (1974) investigated chemical composition of rat small intestines and analyzed the glycoprotein content of brush border and submicrosomal membranes.

Histochemical studies to visualize glycoproteins were also reported by many authors (Ohara et al., 1993; McMahon et al., 1994; Menghi et al., 1994). Therkildsen et al. (1994) studied simple mucin-type carbohydrate antigen in human salivary glands immunohistochemically. Bennett and Leblond (1977) demonstrated by radioautography that ^{3}H-N-acetyl mannosamine, ^{3}H-glucose, and ^{3}H-fucose were incorporated into Golgi apparatus and secreted to the glycoproteins present in plasma membranes and secretory materials in rat colon. Sakata and Engelhardt (1981) studied the luminal and epithelial mucin in the large intestines of mice, rats and guinea pigs and found that neutral mucin decreased but acid mucin increased in the epithelium from the caecum to the distal colon. Sheahan and Jervis (1976) studied the gastrointestinal mucosubstances in 11 species of animals by histochemical sequential staining and reported that the distribution of gastrointestinal glycoproteins varied among the animal species.

With regard to the gastrointestinal histochemical composition related to differentiation and aging, Maruyama and Nagata (1987), Kametani et al. (1998) and Nagata et al. (1998) studied the aging changes of sulfur content in colonic goblet cells of mice by X-ray microanalysis. Taatjes and Roth (1990) investigated the aging changes of sialic acid and fucose residues in rat small intestines by lectin-gold technique. Chen et al. (1993) reported the aging changes of glycoconjugates in mouse goblet cells and vacuolated cells by lectin, Alcian blue and PAS staining. Morita (1993) studied the aging changes of ^{3}H-glucosamine uptake of mouse ileum by radioautography. Yeh and Moog (1984) reported the glycoprotein synthesis in the small intestines of hypophysectomized rats by radioautography using ^{3}H-galactase, ^{3}H-fucose and ^{3}H-mannose. Nagata et al. (1988) studied radiosulfate incorporation in gastrointestinal epithelial cells at 1 and 3 hr after radiosulfate injection and found the difference in the rate of transport and secretion of sulfomucin in respective organs from 1 to 3 hr. From the literature, the difference between the intestinal segment incorporating glycoprotein precursors was not yet clear.

From the results obtained at present it was concluded that radiosulfate incorporation was more observed in the parotid gland at 30 min, in the sublingual gland at 12 hr, in the intestinal columnar epithelium at 30 min, and in the intestinal goblet cells in the small and large intestines at 12 hr. Thus, it should be noteworthy that the organ differences and the time differences in glycoprotein synthesis of several digestive organs, the salivary glands and the gastrointestinal tract, were demonstrated in the present study.

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