Histol Histopathol (1997) 12: 719-727

Histology and Histopathology

Proliferation and migration kinetics of stem cells in the rat fundic gland

D-H. Yang, S. Tsuyama, Y-B. Ge, D. Wakamatsu, J. Ohmori and F. Murata
Department of Anatomy, Faculty of Medicine, Kagoshima University, Kagoshima, Japan

Summary. The proliferation and migration of stem cells in the developing and adult rat fundic gland have been studied using BrdU immunohistochemistry and BrdU-GSA II (*Griffonia-simplicifolia* agglutinin-II) double staining.

In the developing rat fundic gland, stem cells were first scattered throughout all levels of the epithelia and then concentrated in the depth of the pits. With the elongation and maturation of the fundic glands, stem cells left the gland base and moved upward. By 4 weeks after birth, the development of the fundic gland was completed and stem cells were confined to a narrow proliferative zone in the isthmus, reaching the adult distribution pattern.

In the adult rat fundic gland, stem cells in the isthmus differentiated and migrated upward and downward, replacing the surface mucous cells and glandular cells respectively. For upward migration, it took about one week for stem cells to migrate from the isthmus to the surface. For downward migration, it took about two weeks for stem cells to migrate from the isthmus to the neck, and it took 30-36 weeks to reach the gland unit's blind end. Finally stem cells were lost at the deepest level of the glands.

The results obtained by simple topographical distribution in the present experiment agreed well with those obtained by quantitative analysis, suggesting the usefulness of BrdU immunohistochemistry for cell kinetic studies.

Key words: Cell kinetics, Stem cell, Fundic gland, BrdU (bromodeoxyuridine), Ontogeny

Introduction

Gastric epithelium is a good tissue for cell kinetic study since it is characterized by rapid cell proliferation and by cells that differentiate from immature stem cells to terminal differentiated mature cells. Kinetic studies of gastric epithelium have been performed systematically in mouse (Kataoka, 1970; Karam, 1993; Karam and Leblond, 1993a-d), golden hamster (Hattori, 1974; Hattori and Fujita, 1976) and rat (Messier and Leblond, 1960; Hunt and Hunt, 1962; Yeomans and Trier, 1976). All these studies were carried out using ^{3}H-thymidine autoradiography.

^{3}H-thymidine autoradiography has long been a "gold-standard" for the determination of proliferation activity in tissues, because thymidine is incorporated specifically into S-phase DNA and thus "thymidine uptake" is a rigorous indicator of cell proliferation (Bacchi and Gown, 1993). This technique is much more precise and useful than the traditional "mitosis counting", but is time consuming and requires sophisticated equipment and radioactive materials.

To overcome these problems, ^{3}H-thymidine has been replaced with a different uridine analog, BrdU (bromodeoxyuridine), which has the advantage of being recognized by an antibody (Gratzner, 1982). In recent years, immunohistochemistry using BrdU has become a well established technique and has been widely used for cell kinetic studies instead of ^{3}H-thymidine autoradiography at both light and electron microscopic levels (Gratzner, 1982; Lacy et al., 1991; Murata, 1991; Tsuyama et al., 1994; Fernández-Suarez et al., 1996). A good correlation between ^{3}H-thymidine autoradiography and BrdU immunohistochemistry has been reported (Lacy et al., 1991). BrdU immunohistochemistry has been evaluated to be a simple, reliable and rapid method for labeling proliferating cells (Lacy et al., 1991; Yu et al., 1992).

To the best of our knowledge, there has been no report of a kinetic study of gastric epithelium using BrdU. Kinetic studies used to be performed by determining the number of labeled cells previously. The present study uses simple topographical distribution. BrdU immunohistochemistry and BrdU combined with GSA-II double staining which specifically labeled mucous neck cells (Ihida et al., 1988), were used to investigate the proliferation and migration of stem cells in the rat fundic gland. We focus on whether topographical distribution of labeling shares similar results of quantitation. In addition, an ontogenic study of

Offprint requests to: Dr. F. Murata, Department of Anatomy, Faculty of Medicine, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890, Japan

stem cells using BrdU immunohistochemistry was also undertaken.

Materials and methods

Reagents

Bromodeoxyuridine was purchased from Sigma Chemical Co. (St. Louis, MO, USA), monoclonal mouse anti-BrdU antibody from Becton Dickinson Inc. (San Jose, CA, USA), biotinylated anti-mouse immunoglobulin (IgG) antibody from Dako (Denmark), streptavidin-horseradish peroxidase conjugates from Gibco (Bethesda Research Laboratories, Gaithersburg, MD, USA), BSA (bovine serum albumin) from Sigma Chemical Co. (St. Luis, MO, USA) and GSA-II conjugated to horseradish-peroxidase (HRP) from E-Y Laboratories (San Mateo, CA, USA).

Tissue preparation

1. Stem cells in the developing rat fundic gland

Wistar rats were used. Developing rats were prepared as previously described (Yang et al., 1996). Briefly, mating was performed overnight by housing a male and a female rat together in the same cage. The presence of a vaginal plug in the morning determined day 0.5 of gestation. Pregnant rats and postnatal rats of certain development ages were intraperitoneally administered with BrdU at a dose of 50 mg/kg body weight under diethyl ether anesthesia. One hour after the injection, the animals were sacrificed by an overdose of anesthetic. Fetuses were removed by hysterectomy. Stomachs of fetuses from day 17.5, 18.5, 19.5, 20.5 and 21.5 of gestation were taken, fixed in 10% formalin in neutral PBS, dehydrated through a graded series of ethanol and xylene, and embedded in paraffin. Stomachs of postnatal 0, 1 and 3 day and 1, 2, 3, 4, 5 and 8 week rats were removed, incised along the greater curvature and the luminal contents washed out. The corpora were cut, fixed and embedded as with the fetuses.

2. Proliferation and migration of stem cells in the adult rat fundic gland

Adult rats were given 3 intraperitoneal injections of BrdU (50 mg/Kg body weight) per day at 8-hour intervals for 3 consecutive days. They were sacrificed at 1, 3, 6 and 12 hours, at 1 and 3 days, and at 1, 2, 3, 6, 8, 12, 18, 24, 30, 36, 42 and 48 weeks after the last injection. The stomachs were taken and processed as described above.

Immunohistochemistry

1. BrdU immunohistochemistry

Paraffin sections were cut at 4 μm-thick and mounted on silanizied slides. After deparaffinization and hydration, sections were incubated with 0.3% hydrogen peroxide in absolute methanol for 20 min at room temperature to inhibit endogenous peroxidase activity, denatured by immersion in 2N HCl for 90 min, neutralized by borate buffer (pH 9.0) and treated with 0.2% trypsin for 20 min at 37 °C and 1% BSA for 10 min at room temperature. Sections were then incubated with a 1:100 dilution of anti-BrdU monoclonal antibody in PBS containing 1% BSA at 4 °C overnight, incubated with a 1:100 dilution of biotinylated anti-mouse IgG and a 1:300 dilution of streptavidin-horseradish peroxidase at room temperature for 30 min each. Between each step, sections were washed three times for 5 min in PBS (pH 7.4). The peroxidase reaction was developed by adding 0.05% 3-3'-diaminobenzidine tetrahydrochloride (DAB) and 0.001% H_2O_2 in 0.05M Tris buffer (pH 7.6). Sections were counterstained with Mayer's hematoxylin, dehdyrated, cleared and mounted.

2. GSA-II- BrdU double staining

4 μm-thick paraffin sections were deparaffinized, dehydrated and immersed in 0.3% hydrogen peroxide in methanol for 20 min, treated with 1% BSA for 10 min, incubated in HRP-labeled GSA-II (25 μg/ml) for 120 min at room temperature and developed in the above DAB solution. Between each step, sections were washed three times for 5 min in PBS (pH 7.4). Sections were then stained with anti-BrdU antibody as described above, except that DAB reaction was intensified by adding nickel ammonium sulfate to the solution.

3. Control experiments

The specificity of anti-BrdU monoclonal antibody was determined by replacing it with 1% BSA in PBS. Specimens from rats that did not receive BrdU injection were processed as above and also served as negative controls.

Results

1. Ontogeny of stem cells

According to the morphological changes, the development of stem cells in the rat fundic gland was divided into 4 stages which corresponded to our previous ontogenic classification (Yang et al., 1996). (1) The first stage was from the late fetal period extending to day 0.5 after birth. From day 17.5-18.5 of gestation, the mucosa of rat fundic gland were stratified and composed of abundant BrdU-positive cells. These cells were scattered throughout all levels of the epithelia as well as the submucosa and muscular layer in a random fashion. At day 19.5 of gestation, with the appearance of primitive gastric pits, most of the BrdU-positive cells were present in the depth of the pits and significantly fewer were present on the epithelial surface (Fig. 1). At day 21.5 of

gestation, as fundic gland development proceeded, a shift in the distribution of labeled cells occurred. The majority of labeled cells now occupied the gland base, which was also the depth of the pits, and were seldom seen in the epithelial surface (Fig. 2). (2) The second stage was from 1 day to 2 weeks after birth. From 1 day to 1 week after birth, the distribution pattern of labeled cells was similar to that just before birth. BrdU-positive cells remained concentrated in the gland base in spite of the elongation and maturation of the fundic gland (Figs. 3, 4). (3) The third stage was from 2 to 4 weeks after birth. From 2 weeks after birth, BrdU-positive cells showed a tendency to move upward (Fig. 5). They gradually extended from the gland base toward the neck by postnatal week 3 (Fig. 6) and some of them eventually reached the isthmus. (4) The final stage was from 4 to 8 weeks after birth. By 4 weeks, most labeled cells disappeared from the bottom of the gland and moved upward to the isthmus between the pits and the glands, which was similar to the adult distribution pattern (Fig. 7). From 4 weeks onward, the distribution of BrdU-positive cells remained confined to a narrow

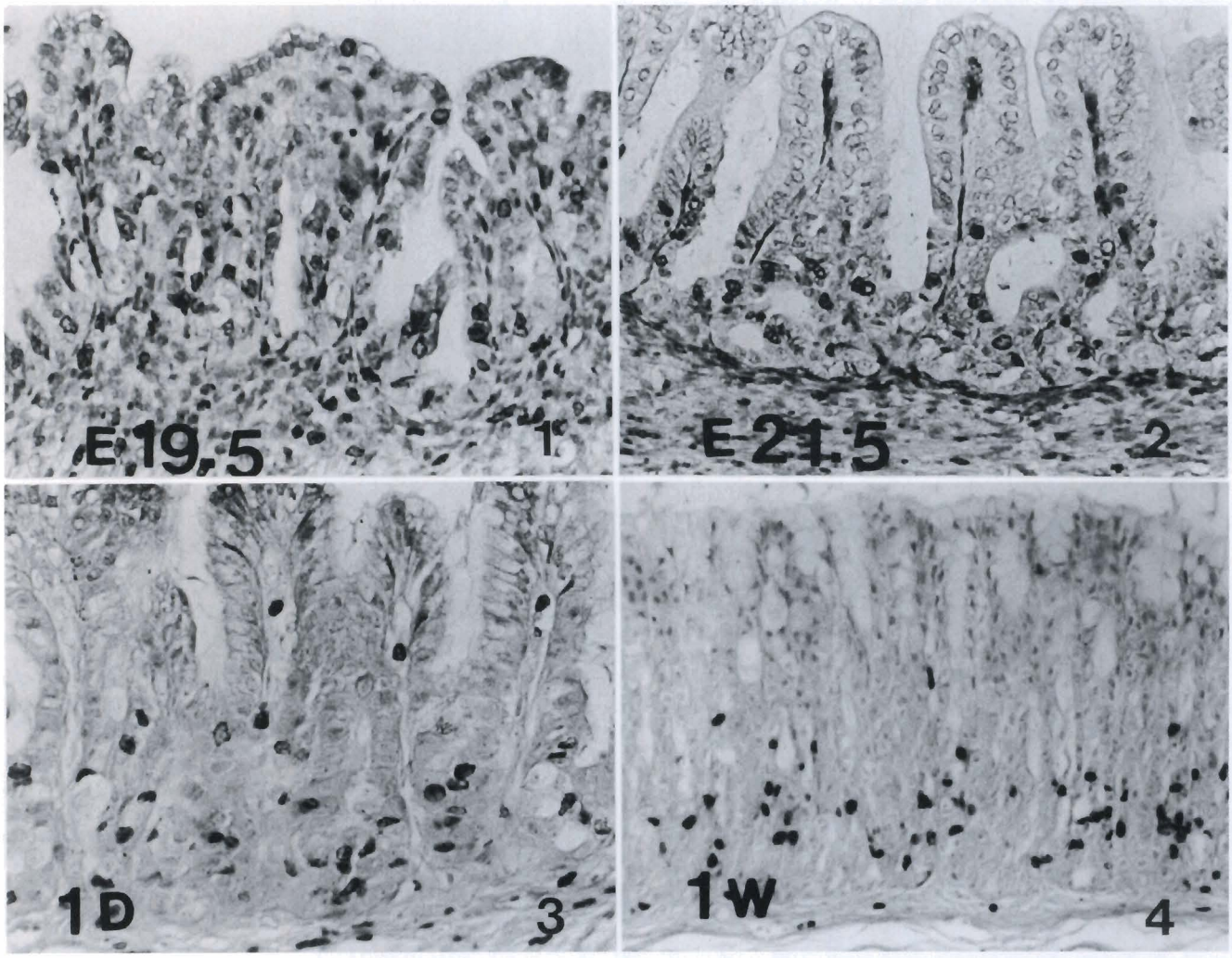

Fig. 1. BrdU staining of rat fundic gland at day 19.5 of gestation. Labeled cells are fewer in the epithelial surface than in the depth of the primitive pits. x 300

Fig. 2. BrdU staining of rat fundic gland at day 21.5 of gestation. Labeled cells are rare in the epithelial surface and are concentrated in the lower half of the primitive gastric gland. x 240

Fig. 3. BrdU staining of rat fundic gland at day 1 after birth. Labeled cells remain concentrated in the lower half of the fundic gland. x 300

Fig. 4. BrdU staining of rat fundic gland at 1 week after birth. The fundic glands become elongated and mature; labeled cells remain concentrated in the gland base. x 200

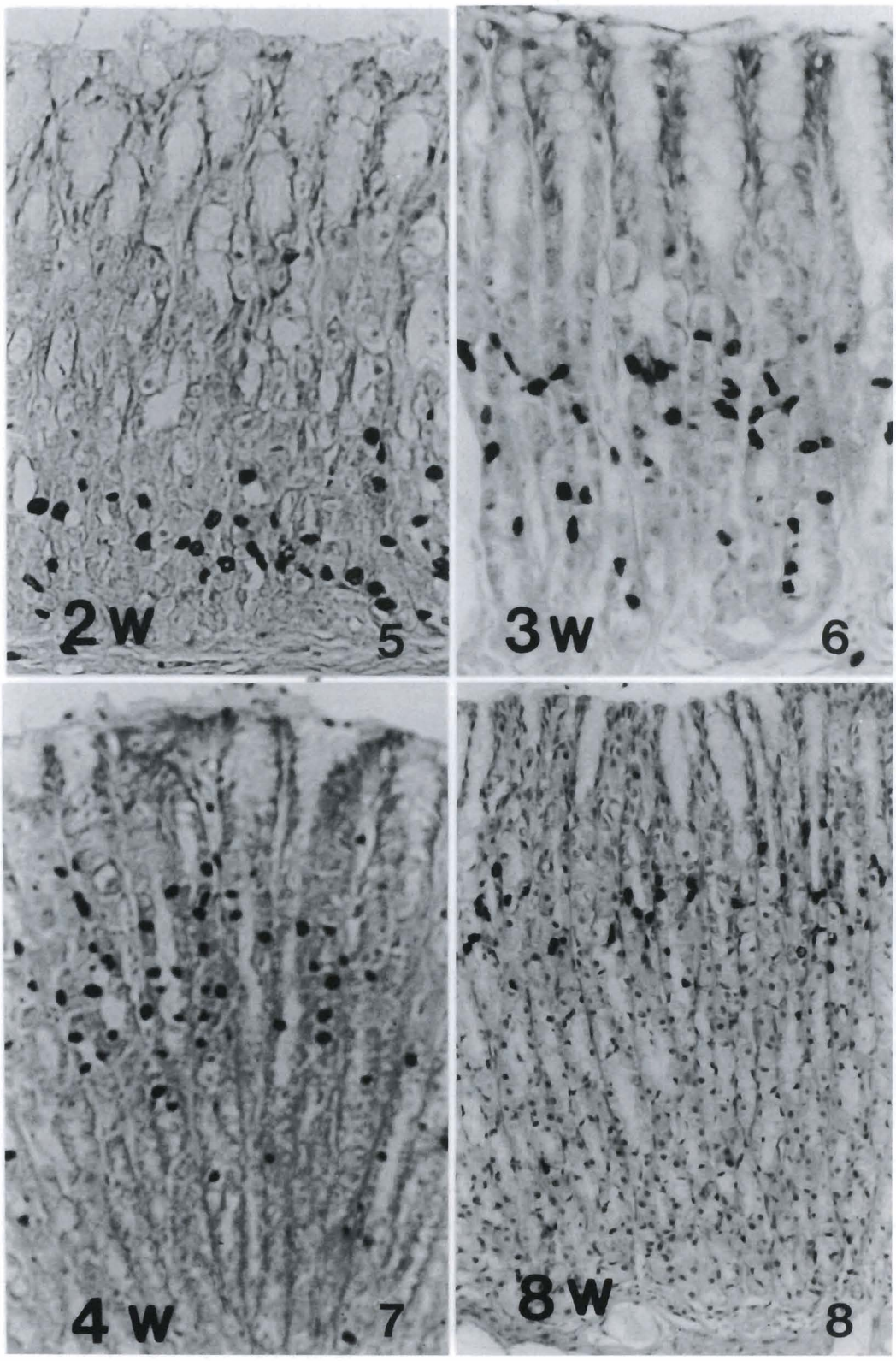

Fig. 5. BrdU staining of rat fundic gland at 2 weeks after birth. Labeled cells are still concentrated in the gland base, but there is a tendency to move upwards. x 300

Fig. 6. BrdU staining of rat fundic gland at 3 weeks after birth. Some of the labeled cells leave the gland base and move towards the neck. x 300

Fig. 7. BrdU staining of rat fundic gland at 4 weeks after birth. Labeled cells move further upwards. Most of them reach the neck and isthmus. x 200

Fig. 8. BrdU staining of rat fundic gland at 8 weeks after birth. Most of the labeled cells are confined in the isthmus. The number of the labeled cells is fewer than that at 4 weeks. x 200

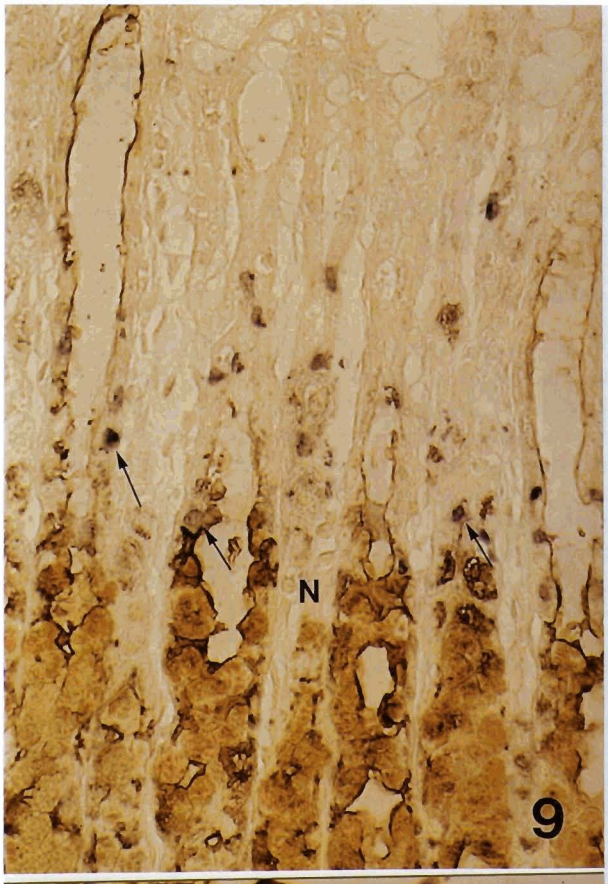

Fig. 9. GSA II-BrdU double staining of rat fundic gland 3 hours after the last injection. Labeled cells (arrows) are confined to the isthmus (proliferative zone) which is above the GSA II-positive mucous neck region (N). x 240

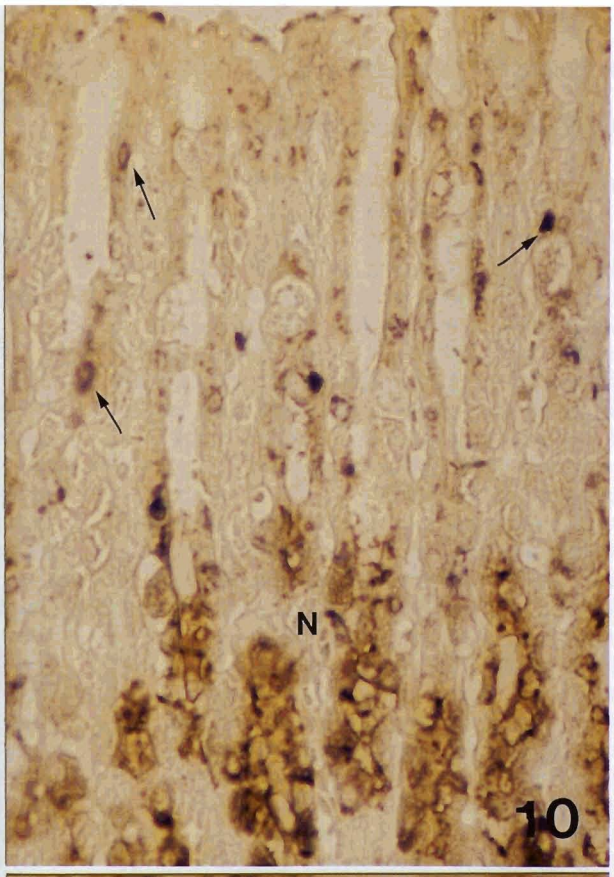

Fig. 10. GSA II-BrdU double staining of rat fundic gland 3 days after the last injection. Labeled cells (arrows) are seen in the upper part of gastric pits. N: GSA II-positive mucous neck region. x 300

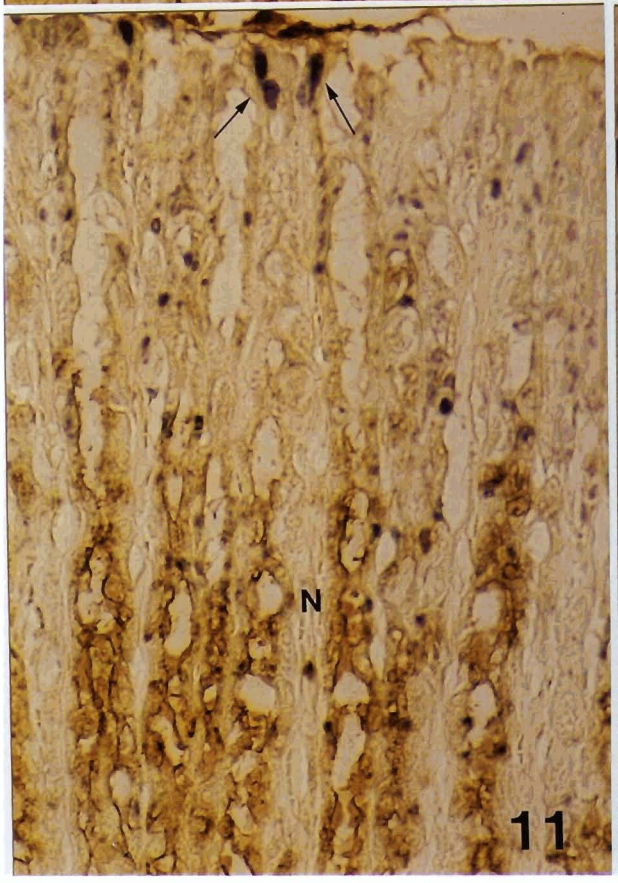

Fig. 11. GSA II-BrdU double staining of rat fundic gland 1 week after the last injection. Labeled cells (arrows) move higher up to the surface. N: GSA II-positive mucous neck cell region. x 240

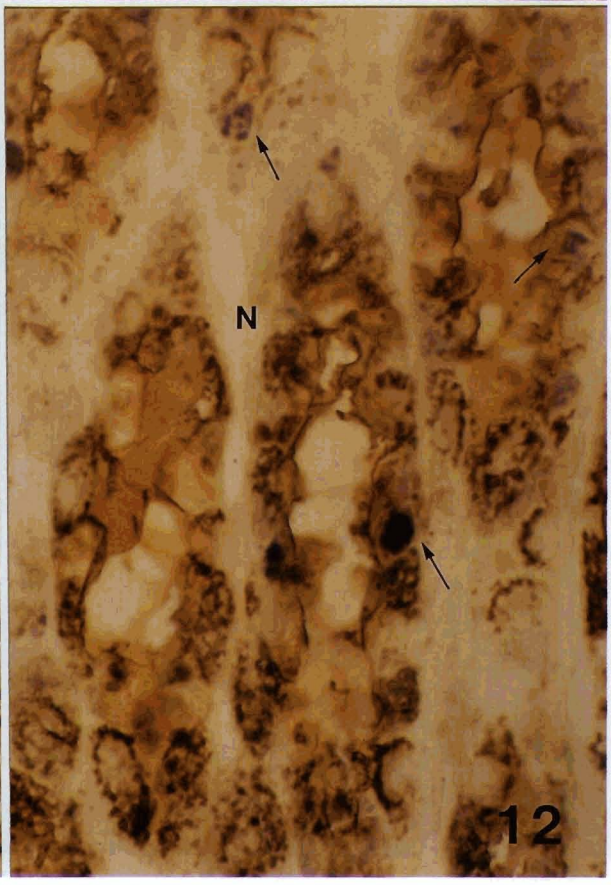

Fig. 12. GSA II-BrdU double staining of rat fundic gland 2 weeks after the last injection. Arrow indicates the doubly labeled cells in the neck region. x 600

proliferative region in the isthmus (Fig. 8).

2. Proliferation and migration of stem cells

BrdU-positive cells were present in a narrow proliferative zone at times varying from 1, 3, 6, 12 and 24 hours after the last injection. At these times, the BrdU-positive proliferating zone corresponded to the isthmus, which is just above the GSA II-positive neck region in GSA II-BrdU double staining (Fig. 9). From 3 days after the last injection, most of the BrdU-labeled cells left the isthmus and migrated upward and downward. Some of the labeled cells were seen in the upper part of the pits (Fig.10). By 1 week after the last injection, some of the labeled cells reached the pit top and were seen on the surface (Fig. 11). By 2 weeks after the last injection, significant labeled cells were present in the neck region but were hardly seen in the surface of the gastric mucosa. They were doubly labeled by GSA II and BrdU (Fig. 12). BrdU-labeled cells migrated further downward to the base and only a few were seen in the neck region by 6 weeks after the last injection (Fig. 13). At 8-12 weeks after the last injection, most of the labeled cells were still migrating downward and the number of labeled cells decreased (Figs. 14-15). At 18-24 weeks after the last injection, labeled cells were almost entirely confined to the base and the number decreased further (Fig. 16). At 30-36 weeks after the last injection, only a few labeled cells were recognized at the gastric unit's blind end (Figs. 17, 18). Very few labeled cells were seen after 40 weeks. The migration of BrdU-labeled cells is summarized in Fig. 19.

3. Control experiments

No labeled cells was found in any of the control experimental groups.

Discussion

In the present study of a single BrdU injection followed by BrdU immunohistochemistry, labelling was first scattered throughout all levels of the epithelium in the fetal rat fundic gland and then appeared in the lower part of the primitive gastric pits, which was suggested to be the initial progenitor zone (stem cell zone) by Yeomans and Trier (1976). The labelling subsequently moved upward as the mucosa matured until 4 weeks after birth, when the distribution of labeled cells was similar to that of adult. These findings agreed well with those observed in the developing rat stomach using ^{3}H-thymidine autoradiography (Yeomans and Trier, 1976). The changes of location of BrdU-labeled cells were analogous to other cells in the developing rat fundic gland (Ihida et al., 1988; Yang et al., 1996). In the adult rat fundic gland, the labelling was confined to the progenitor zone in the isthmus, as observed by ^{3}H-thymidine autoradiography in mouse stomach (Karam and Leblond, 1993a) and BrdU immunoelectron microscopic study in rat stomach (Tsuyama et al., 1994).

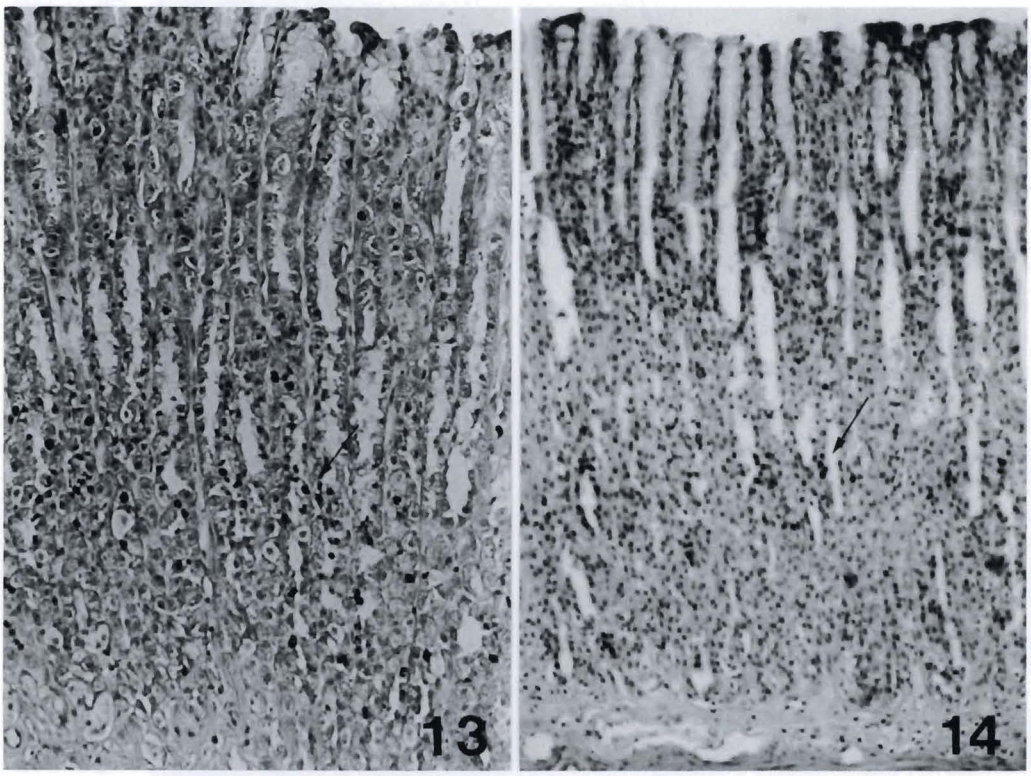

Fig. 13. BrdU staining of rat fundic gland 6 weeks after the last injection. Most of the labeled cells (arrow) migrate downward to the base. x 150

Fig. 14. BrdU staining of rat fundic gland 8 weeks after the last injection. Labeled cells (arrow) migrate downward to the base; the number of labeled cells decreases. x 120

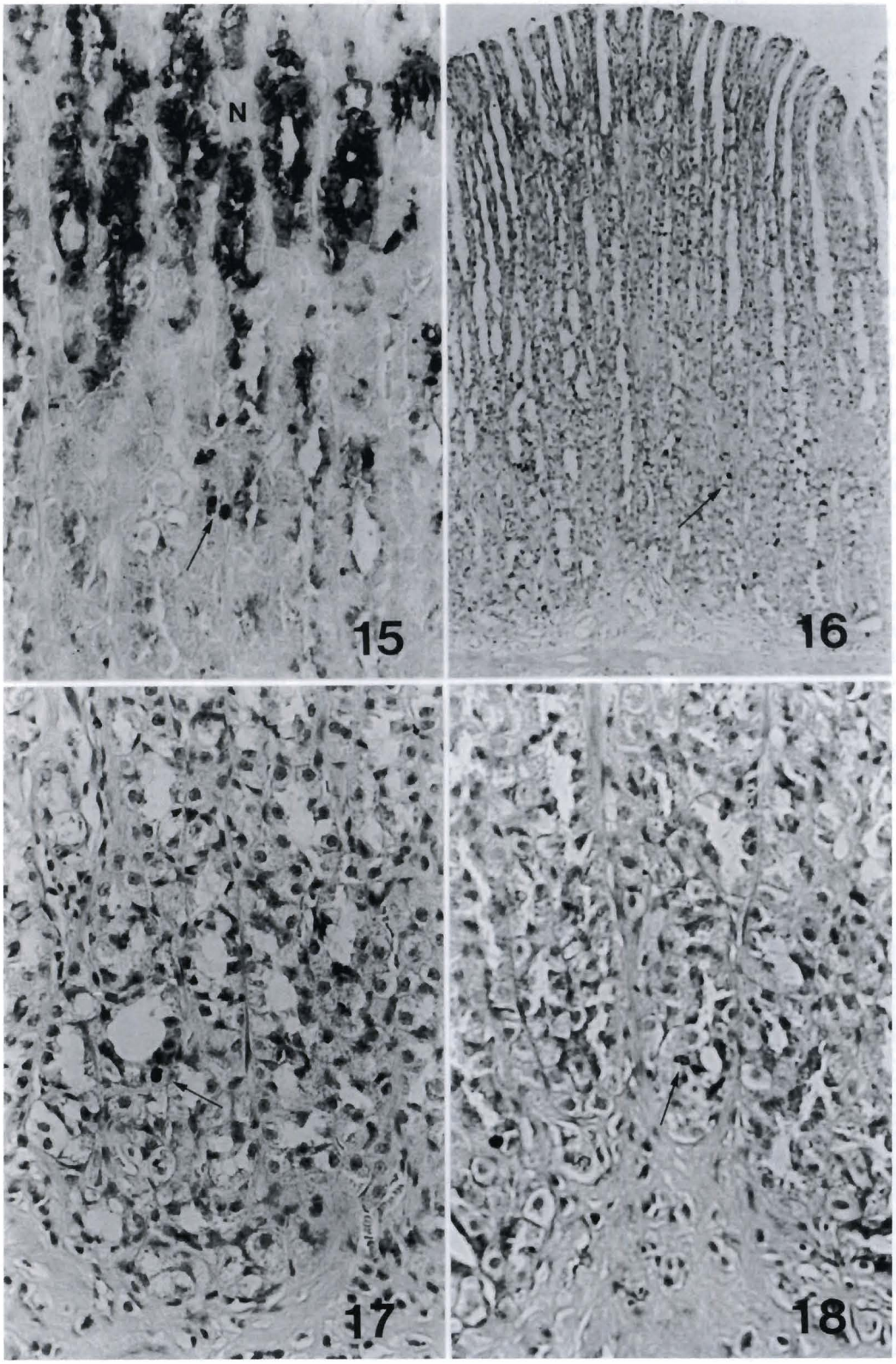

Fig. 15. GSA II-BrdU double staining of rat fundic gland 12 weeks after the last injection. BrdU-labeled cells (arrow) appear in the base under the neck region (N). x 300

Fig. 16. BrdU staining of rat fundic gland 18 weeks after the last injection. Labeled cells (arrow) migrate further downward to the base; the number of labeled cells decreases further. x 100

Fig. 17. BrdU staining of rat fundic gland 30 weeks after the last injection. Only a few labeled cells (arrow) are recognized at the gastric unit's blind end. x 300

Fig. 18. BrdU staining of rat fundic gland 36 weeks after the last injection. Arrow indicates labeled cells in the unit's blind end. x 300

The migration and turnover time of cells is usually assessed on the basis of cell number rather than topographical distribution. However, the quantitation of results obtained by autoradiography or immunocytochemistry is relatively imprecise and often causes controversy. It is well known that stem cells in the adult isthmus differentiate and migrate upward and downward to form surface mucous cells and other glandular cell types respectively (Messier and Leblond, 1960; Kataoka, 1970; Hattori and Fukita, 1976; Karam and Leblond, 1993a). For upward movement, the migration time from the isthmus to the mucosal surface is estimated to be about 1 week by some investigators (Messier and Leblond, 1960; Hattori and Fujita, 1976) and 3 days by others (Karam and Leblond, 1993b). In our present experiment, following a series of 3 BrdU injections per day for 3 days, we estimated the migration time of stem cells from isthmus to the mucosal surface to be about 1 week. This result is similar to most of the previous studies (Messier and Leblond, 1960; Hattori and Fujita, 1976). For downward movement, we observed BrdU-labeled cells progressing with time in the direction of the blind end of the unit. The number of labeled cells decreased in an irregular manner. By 2 weeks, most of the BrdU-labeled cells appeared in the neck region, superinposed with GSA II-positive cells. This result agrees with the estimation of migration time of about 2 weeks for mucous neck cells (Hattori and Fujita, 1976; Karam and Leblond, 1993c). There are reports that mucous neck cells transform to zymogenic cells through intermediate pre-zymogenic cells while they migrate downward to the unit's blind end (Sato and Spicer, 1980; Suzuki et al., 1983; Murata et al., 1988; Madrid et al., 1990; Karam and Leblond, 1992). The differentiation of chief cells takes place in the middle level of the gastric gland (Kataoka, 1970; Hattori and Fujita, 1976).

According to our observation, from 6 weeks onward labeled cells appeared to migrate from the neck region to the base. This coincides with the earlier findings of Hattori and Fujita who reported that from day 42 to day 100, the labeled mucous neck cells migrated downward to transform to chief cells, and the labeled chief cells migrated further downward (Hattori and Fujita, 1976). The turnover times were estimated to be 42 days for prezymogenic cells and 194 days for zymogenic cells (Karam and Leblond, 1993c). It is estimated to be 20-36 weeks for parietal cells (Murata, 1991) or 31 days for parietal cells in the isthmus, 35 days for parietal cells in the pit, 54 days for parietal cells in the neck and 189 days for parietal cells in the base (Karam, 1993). The turnover time is estimated to be 45-60 days for endocrine cells by Thompson et al. (1990) and 2-4 months by Lehy and Willems (1976). In our present study, BrdU-positive cells were at first concentrated at the upper level of the gland, and gradually appeared deeper in the gland, reaching the blind end at 30-36 weeks. It is impossible in our present tissue preparations to study the kinetics of individual glandular cells separately. It would seem, however, that the tendency of our results coordinates essentially with the previous studies. In order to define the differentiation and migration of glandular cell types, further experiments, such as BrdU combination with GSA1-B_4 double staining to identify parietal cells, BrdU with pepsinogen double staining to identify zymogenic cells and BrdU with peptide double staining to identify endocrine cells, may be of help. We assume that BrdU-positive cells superposing with specific glandular cells types will be observed, as revealed by silver grains in ^{3}H-thymidine autoradiography (Karam, 1993; Karam and Leblond, 1993c,d).

BrdU labelling offers the opportunity to perform cell kinetic studies without the use of a radioactive precursor. In recent years, the introduction of microwave

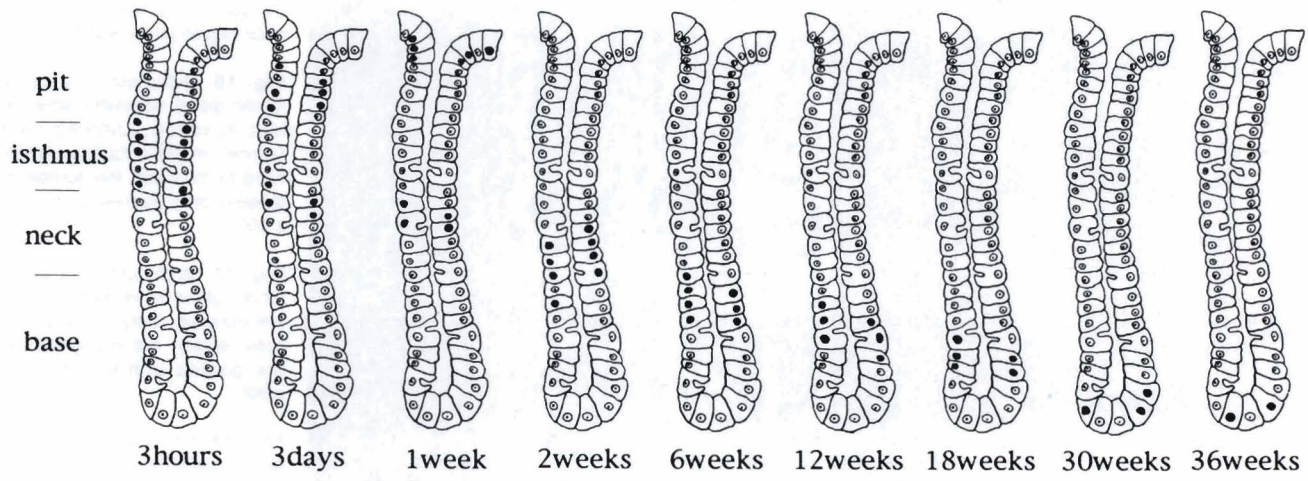

Fig. 19. Schematic diagram summarizing the migration of BrdU-labeled cells in the rat fundic gland. Black nuclei indicate the localization of labeled cells. Migration speed of stem cells to the luminal surface is calculated to be about one week, while that of downward speed to the base is estimated to be 30-36 weeks.

techniques has made BrdU immunostaining more sensitive, less laborious and with lower background staining (Van De Kant et al., 1988; Dover and Patel, 1994), although BrdU used to require *in vivo* administration or *in vitro* incubation of fresh tissue (Yu et al., 1992). In our laboratory, some investigators observed that BrdU combined with skin occlusive dressing method was an excellent alternative to whole body injection. This allowed *in vivo* studies of cell kinetics in a variety of human organs which have a free surface, such as skin, rectum and nasal cavity without serious side effects (unpublished data). We hope our present experiments will extend the range of application of BrdU immunohistochemistry in cell kinetic studies.

Acknowledgements. The authors are grateful to Mr. S. Nonaka and Mr. S. Imamura for their technical assistance and to Mr. M.G. Gore for his careful proof-reading. A part of this work was supported by Grants-in-Aid No. 07670026 from the Ministry of Education, Science and Culture of Japan to F.M. and the Kodama Foundation of Medical Research.

References

Bacchi C.E. and Gown A.M. (1993). Detection of cell proliferation in tissue sections. Braz. J. Med. Biol. Res. 26, 677-687.

Dover R. and Patel K. (1994). Improved methodology for detecting bromodeoxyuridine in cultured cells and tissue sections by immunocytochemistry. Histochemistry 102, 383-387.

Fernández-Suarez A., López J.M., Carbajo S., Alvarez-Uría M. and Carbajo-Pérez E. (1996). New insights into the cytodynamics of the hamster Harderian gland as provided by the bromodeoxyuridine-labelling method. Histol. Histopathol. 11, 351-355.

Gratzner H.G. (1982). Monoclonal antibody to 5-bromo- and 5-iododeoxyuridine: a new reagent for detection of DNA replication. Science 218, 474-475.

Hattori T. (1974). On cell proliferation and differentiation of the fundic mucosa of the golden hamster. Cell Tissue Res. 148, 213-226.

Hattori T. and Fujita S. (1976). Tritiated thymidine autoradiographic study on cellular migration in the gastric gland of the golden hamster. Cell Tissue Res. 172, 171-184.

Hunt T.E. and Hunt E.A. (1962). Radioautographic study of proliferation in the stomach of the rat using thymidine-H^3 and compound 48/80. Anat. Rec. 142, 507-517.

Ihida K., Suganuma T., Tsuyama S. and Murata F. (1988). Glycoconjugate histochemistry of the rat fundic gland using *Griffonia simplicifolia* agglutinin-II during the development. Am. J. Anat. 182, 250-256.

Karam S.M. (1993). Dynamics of epithelial cells in the corpus of the mouse stomach. IV. Bidirectional migration of parietal cells ending in their gradual degeneration and loss. Anat. Rec. 236, 314-332.

Karam S.M. and Leblond C.P. (1992). Identifying and counting epithelial cells in the corpus of the mouse stomach. Anat. Rec. 232, 231-246.

Karam S.M. and Leblond C.P. (1993a). Dynamics of epithelial cells in the corpus of the mouse stomach. I. Identification of proliferative cell types and pinpointing of the stem cell. Anat. Rec. 236, 259-279.

Karam S.M. and Leblond C.P. (1993b). Dynamics of epithelial cells in the corpus of the mouse stomach. II. Outward migration of pit cells. Anat. Rec. 236, 286-296.

Karam S.M. and Leblond C.P. (1993c). Dynamics of epithelial cells in the corpus of the mouse stomach. III. Inward migration of neck cells followed by progressive transformation into zymogenic cells. Anat. Rec. 236, 297-313.

Karam S.M. and Leblond C.P. (1993d). Dynamics of epithelial cells in the corpus of the mouse stomach. V. Behavior of entero-endocrine and caveolated cells: general conclusion on cell kinetics in the oxyntic epithelium. Anat. Rec. 236, 333-340.

Kataoka K. (1970). Electron microscopic observations on cell proliferation and differentiation in the gastric mucosa of the mouse. Arch. Histol. Jpn. 32, 251-273.

Lacy E.R., Kuwayama H., Cowart K.S., King J.S., Deutz A.H. and Sistrunk S. (1991). A rapid, accurate immunohistochemical method to label proliferating cells in the digestive tract. Gastroenterology 100, 259-262.

Lehy T. and Willems G. (1976). Population kinectis of antral gastrin cells in the mouse. Gastroenterology 71, 614-619.

Madrid J.F., Ballesta J., Castells M.T. and Hernández F. (1990). Glycoconjugate distribution in the human fundic mucosa revealed by lectin- and glycoprotein-gold cytochemistry. Histochemistry 95, 179-187.

Messier B. and Leblond C.P. (1960). Cell proliferation and migration as revealed by radioautography after injection of thymidine-H^3 into male rats and mice. Am. J. Anat. 106, 247-285.

Murata F. (1991). Stem cell in the digestive tract: immunocytochemical approach. Acta. Anat. Nippon 66, 80 (In Japanese).

Murata F., Suganuma T., Tsuyama S. and Ihida K. (1988). Mucous neck cells: proposal that mucous neck cells are the precursor cells of the chief cells-studied based on glycoconjugates cytochemistry. Acta. Anat. Nippon. 63, 12-19.

Sato A. and Spicer S.S. (1980). Ultrastructural cytochemistry of complex carbohydrates of gastric epithelium in the guinea pig. Am. J. Anat. 159, 307-329.

Suzuki S., Tsuyama S. and Murata F. (1983). Cells intermediate between mucous neck cells and chief cells in rat stomach. Cell Tissue Res. 233, 475-484.

Thompson E.M., Price Y.E. and Wright N.A. (1990). Kinetics of enteroendocrine cells with implications for their origin: a study of the cholecystokinin and gastrin subpopulations combining tritiated thymidine labelling with immunocytochemistry in the mouse. Gut 31, 406-411.

Tsuyama S., Yang D.H., Kanae T., Kasamo H. and Murata F. (1994). Immunoelectron microscopic labeling of digestive tract stem cells by means of bromodeoxyuridine antibody. Acta Histochem. Cytochem. 27, 339-345.

Van de Kant H.J.G., Boon M.E. and De Rooij D.G. (1988). Microwave-added technique to detect bromodeoxyuridine in S-phase cells using immunogold-silver staining and plastic-embedded sections. Histochem. J. 20, 335-340.

Yang D-H., Kasamo H., Miyauchi M., Tsuyama S. and Murata F. (1996). Ontogeny of sulphated glycoconjugate-producing cells in the rat fundic gland. Histochem. J. 28, 33-43.

Yeomans N.D. and Trier J.S. (1976). Epithelial cell proliferation and migration in the developing rat gastric mucosa. Dev. Biol. 53, 206-216.

Yu CC-W., Woods A.L. and Levison D.A. (1992). The assessment of cellular proliferation by immunohistochemistry: a review of currently available methods and their applications. Histochem. J. 24, 121-131.

Accepted January 28, 1997