Gastric Morphology, Gastrin and Somatostatin in Experimental Gastritis in Mice[*]

Shinya KISHIMOTO, Satoru SHIMIZU, Masahiro YAMAMOTO,
Hassei KOH, Takaji TAMARU, Kohichi SANO, Kohji SUMII,
Goro KAJIYAMA and Akima MIYOSHI*

The 1st Department of Internal Medicine, Hiroshima University School of Medicine
* *Shizuoka General Hospital, Shizuoka 420, Japan*
(Received December 25, 1982)

ABSTRACT

The purpose of this study was to investigate the morphological changes and endocrine profile in the gastric mucosa of mice with atrophic autoimmune gastritis induced by neonatal thymectomy. The gastritis was produced in neonatal mice [BALB/C(+/?)] of both sexes by thymectomy 3 days after birth. Of the thymectomized mice, 27.4% had marked reduction of parietal and chief cells as well as inflammatory cell infiltration, 64.1% of which had positive parietal cell antibody. Hypergastrinemia (332.0±231.9, control: 70.8±41.3 pg/ml) was observed in mice with this type of atrophic gastritis. Mucosal concentration of antral gastrin was also increased (11.0±4.2, control: 7.25± 2.93 microgr/g) and antral G cell hyperplasia was observed in these mice (23.0±7.3, control:12.8±4.7 cells/UA). Mucosal concentration of somatostatin in the body mucosa in mice with gastritis was decreased (0.598±0.391, control:0.840±0.440ng/mg) but in the antrum the concentration was almost the same as in the control(0.529±0.230 control: 0.418±0.340ng/ml). The number of body D cells was also decreased in these mice (4.0±1.8, control: 6.1±0.8 cells/UA), but antral D cells were extremely few both in mice with gastritis and in control. Premature cells and unclassified endocrine cells were increased in the body mucosa at ultrastructural levels and immunocytochemical studies did not revealed any noteworthy changes in endocrine cells such as enteric endocrine cells other than gastrin and somatostatin in mice with gastritis.

INTRODUCTION

Hypergastrinemia and G cell hyperplasia may occur in achlorhydric patients with Addisonian pernicious anemia or atrophic gastritis[4,7,22,26,30]. This may be explained by loss of inhibitory effect of hydrochloric acid on gastrin producing G cells in the antral mucosa. This concept has been well documented. However, the concept of body confined gastric atrophy (body atrophic gastritis) should be reviewed in experimental animals.

A number of observations[1,3,10,12,19] made in recent years have demonstrated that somatostatin influences various hormones and functions in the gastrointestinal tract and pancreas. Gastric somatostatin (D cell) is distributed in all areas of the stomach[1,27] and is known to suppress gastric acid secretion as well as gastric motility[13]. It is therefore assumed that pathological conditions in gastric mucosa are associated with altered gastric somatostatin[5,14,28]. It is therefore suspected that atrophic mucosa in gastritis and pernicious anemia can affect gastric somatostatin.

It has also been assumed that endocrine profile in the stomach may change in atrophic mucosa of chronic atrophic gastritis and pernicious anemia[31].

The aim of this study was to investigate the changes of the gastric mucosal appearance and gastrin and somatostatin levels in the blood as well as their mucosal concentrations, and G and D cells in atrophic mucosa of gastritis induced experimentally by thymectomy in neonatal mice.

MATERIALS AND METHODS

1. Production of gastritis and preparation of tissue sections

175 neonatal mice [BALB/C(+/?)] of both sexes 3 days after birth were thymectomized according to the method of Kojima et al.[11]. The animals were kept in a conventional manner and were sacrificed 2 months after the operation. The blood was drawn for evaluation of gastrin and parietal cell antibodies (PCA). The abdomen was opened and the stomach was resected and opened along the greater curve. A few tissue samples (0.5 × 0.5 cm) were taken from the body and antral mucosa and were then fixed in Bouin's solution (15 ml saturated picric acid, 5 ml neutral formaldehyde, and 1 ml glacial acetic acid) for 3 hours. After fixation the tissue specimens were embedded in paraffin wax and sectioned 3 microns in thickness.

2. Conventional histopathology and histochemistry

Sections were stained with hematoxylin and eosin for histopathologic examination and with PAS–Alcian blue at pH 2.5 for evaluation of intestinalization of the gastric mucosa. Fibrous changes in the stomach were evaluated by Azan stain.

The number of parietal cells was counted per 5 visual fields under microscope (×400 magnification) and the mean number was shown per unit are (one visual field).

3. Radioimmunoassay of gastrin and somatostatin

Gastrin in the blood and antral mucosa were determined by radioimmunoassay[40]. Mucosal concentrations of gastrin were determined as follows, pyloric mucosa removed immediately after sacrifice was heated to boiling points, homogenized, and centrifuged and the supernatant thus obtained was diluted in Beronal buffer. And then, gastrin was determined in the diluent by radioimmunoassay. For somatostatin determination, the pylorus and body removed immediately after sacrifice were weighed and homogenized in 1 ml of 2M acetic acid. The acid extract was heated to boiling points and centrifuged, and the supernatant was lyophilised and dissolved in buffer. Somatostatin was determined in an aliquot of the extract by radioimmunoassay[2].

4. Identification of gastric endocrine cells

Prior to immunostaining, paraffin sections were deparaffinized by immersion in xylene and petroleum ether. All sections were stained with antisera at dilutions as follows, gastrin 1 : 200 (immunofluorescense: IF), 1 : 2000 (Peroxidase antiperoxidase: PAP), and somatostatin 1 : 100 (IF), 1 : 1000 (PAP).

5. Indirect immunofluorescent method[6]

The primary antisera were applied for 12–18 hours at 4°C. The labeled second layer antisera were applied for 1 hour at room temperature. FITC conjugated goat antirabbit globulin (MBL) was used at solution ratio of 1 : 100.

6. Unlabeled antibody enzyme (peroxidase anti-peroxidase) method[35]

Prior to application of primary antisera, the sections were first treated with hydrogen peroxide to exhaust the endogenous peroxidase activity and then were incubated with normal goat serum to block non-specific background staining. The specific antisera were applied for 12–18 hours at 4°C. The conjugated antiglobulins were used at room temperature. The following peroxidase anti-peroxidase complexes were then applied: horseradish peroxidase rabbit anti-horse radish peroxidase (Dakopatt A/S) at 1 : 300 at room temperature. The peroxidase activity was revealed by incubation for 2–3 minutes an a freshly prepared solution of 0.05 % of 3, 3–diaminobenzidine tetrahydrochloride in phosphate buffered saline, pH 7.2, containing 0.01% hydrogen peroxide.

The number of immunoreactive G and D cells was counted per unit area (cm^2) under microscope (×400 magnification).

7. Control for immunocytochemistry

In order to demonstrate that the immunocytochemical reactions were specific, the following tests were performed. 1) Prior to immunostaining, the diluted antisera were absorbed with samples of synthetic peptides (gastrin and somatostatin), 2) Normal rabbit serum was used instead of the primary antibody as the first layer, 3) The FITC second layer was applied alone in the immunofluorescent method, 4) The PAP complex was applied alone and developed by the unlabeled antibody enzyme technique.

8. Electron microscopic study

The specimens from the body and antrum were fixed for 2 hours at 4°C in 3% glutaraldehyde at pH 7.4 and with 1% osmium tetroxide in the same buffer at 4°C for 1 hour, which

were dehydrated in graded alcohols and embedded in Epon. Each one micron ultrathin section in thickness was double-stained with uranyl acetate and lead citrate and individual cells were examined a JEM 100S electron microscope and photographed.

9. Parietal cell antibodies (PCA)

Gastric parietal cell antibodies were detected by indirect immunofluorescent method[37].

10. pH in gastric juice

Gastric pH was determined by immersing test paper in the gastric juice.

11. Control in this study

Sham-operated animals were used for the control (30 mice).

RESULTS

1. Gastric morphology

The incidence of gastritis was 27.4% of neonatally thymectomised mice. In remaining 72.6% of the mice, pathological changes were slightly seen in the gastric mucosa. The histological findings in the gastric mucosa are summarized in Table 1. The stomach of mice with gastritis was enlarged and the gastric mucosa was markedly thickened (Fig. 1).

Giant rugae were found in the body mucosa. In the tissue specimens from the body, the inflammatory infiltration consisted of lymphocytes, plasma cells, and occasional eosinophils in the lamina propria. These cells were observed principally at the base of the mucosa and adjacent to the muscularis mucosae although these were also found subepithelially around the foveoli. The normal arrangement of the epithelial cells composing the branched grounds was disrupted and the normal architecture of the mucosa was no longer found. A marked reduction of parietal cells were observed and these were replaced with inflammtory cells and occasionally with pseudopyloric cells (Table 1 and Fig. 2). The antropyloric mucosa was also hypertrophied and thickened (Fig. 3). However, the mucosal architecture was kept relatively intact when compared to the body mucosa. Intestinalization in the antropyloric mucosa was less marked than in the body. The degree of infiltration was more severe in the body than in the
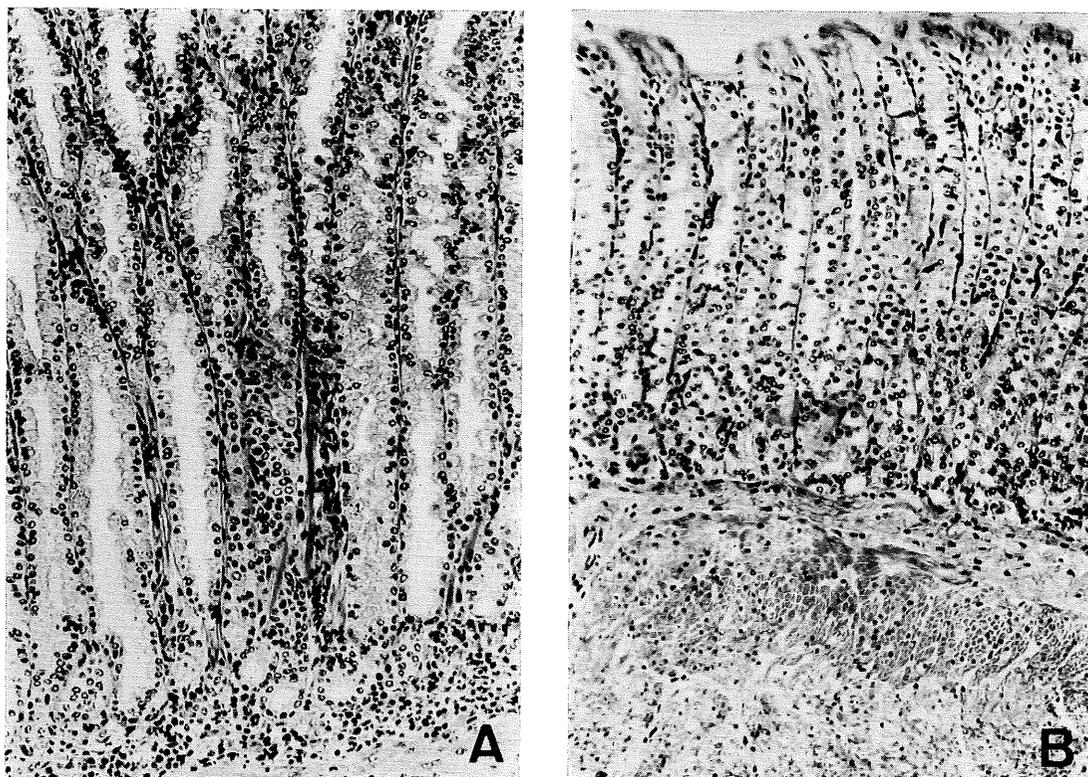
Fig. 1. The stomach of mice with gastritis was markedly enlarged and thickened (Hematoxylin and eosin stain, ×100, A). Control(B).

antropyloric mucosa (Fig. 4). Lymphoid fol-
licles were seen above the muscularis mucosae
both in the body and antropylorus. Fibrous
tissue shown by Azan staining was more
marked in the body than in the antropylorus
(Fig. 5). Generally, these pathological changes
were less involved in the antropyloric mucosa

than in the body mucosa. Intestinal metaplasia
was hardly observed in gastric body and antrum
of mice with gastritis.

Electron microscopic studies showed that cel-
lular changes in both parietal and chief cells
were less remarkable although these cells were
so few, while many immatured cells similar

Table 1. Summaries in morphological findings.

	Histo-pathology	Control	Gastritis PCA(+)	PCA(−)	Gastritis (−)
BODY	Infiltration	−	⧺	⧺	+
	Fibrosis	−	⧺	⧺	±
	Reduction of Parietal cells	−	⧻	⧺	−
	Chief cells	−	⧺	⧺	−
	Lymph follicles	−	+	+	−
	Intestinal Metaplasia	−	−	−	−
	Pseudopyloric Metaplasia	−	+	±	−
ANT.	Infiltration	−	+	+	−
	Lymph follicles	−	−	−	−
	Intestinal Metaplasia	−	−	−	−
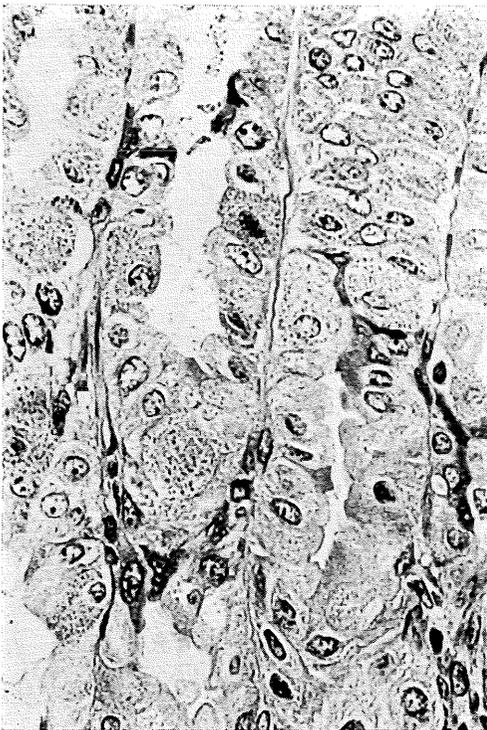
Fig. 2. A marked reduction of parietal cells and
chief cells was noted in the body mucosa of mice
with gastritis, being replaced with inflammatory
cells (Hematoxylin and eosin, ×400).
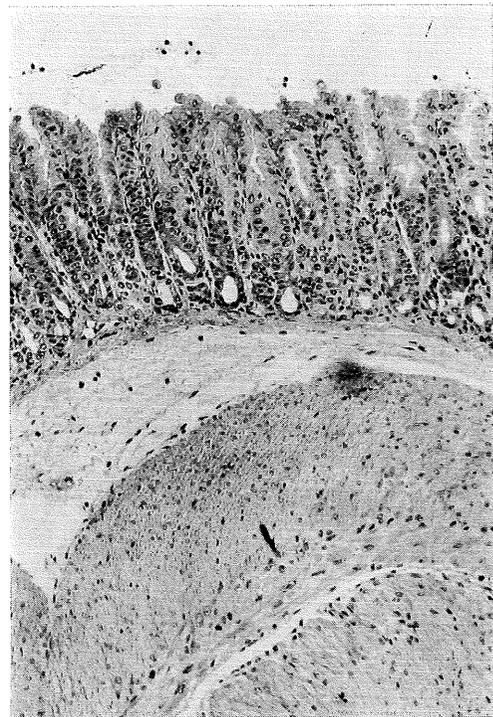
Fig. 3. Antropyloric mucosa was also enlarged
and thickened but sparingly (Hematoxylin and
eosin, ×100).
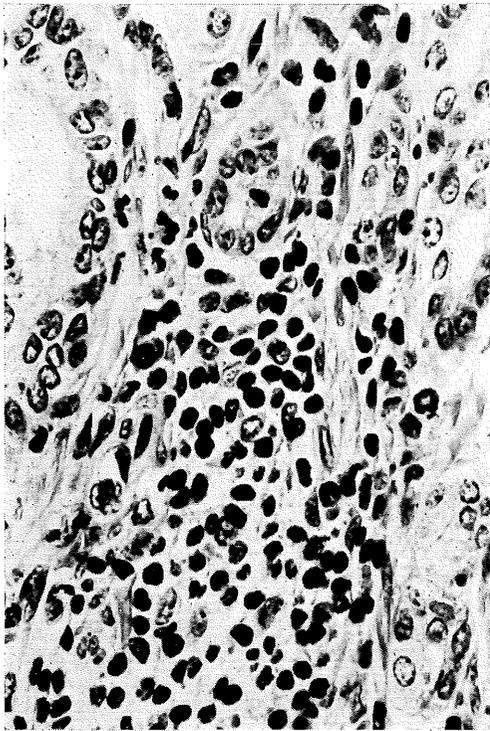
Fig. 4. There was a marked infiltration of inflammatory cells composed of lymphocytes and plasma cells in the body mucosa (Hematoxylin and eosin, ×400).
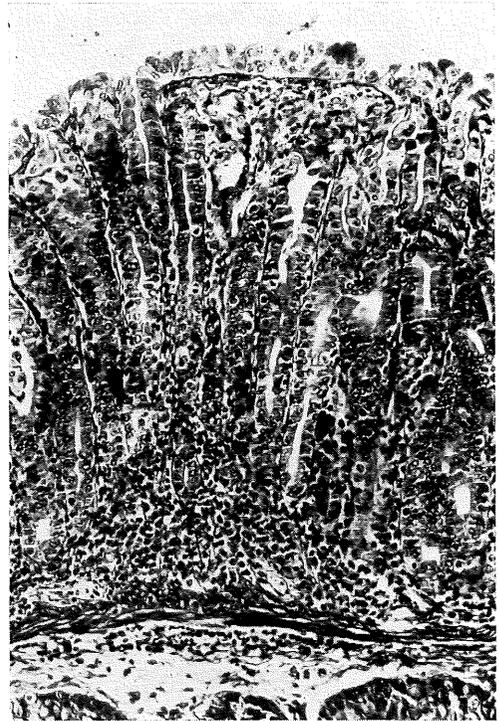
Fig. 5. Fibrous tissue shown by Azan staining was markedly observed in the body mucosa of mice with gastritis (×100).

to which were often observed usually in the neck regions were found scattered between parietal and chief cells in the whole body mucosa. Many enterochromaffin-like cells and unclassified endocrine cells were observed in the body mucosa (Fig. 6).

A marked reduction in the number of parietal cells per visual field (×400) was seen in mice with gastritis (25. 3±13. 9 in gastritis with positive PCA, 32. 3±17. 4 in gastritis without positive PCA, 110. 0±9. 9 in non-gastritis, and 124. 1±24. 5 in the control, Table 2, Fig. 7). Gastric juice pH was increased 7 to 8 in mice with gastritis (Table 2).

2. The incidence of parietal cell antibodies (PCA)

Seropositive PCA was seen in 64.1% of mice with gastritis, while 10% was in mice without gastritis. No mice with seropositive PCA were seen among the control animals.

3. Immunoreactive gastrin and somatostatin

The fasting levels of gastrin were significantly higher in mice with gastritis than those in control animals (Table 2). However, mice with positive PCA–gastritis tended to show increased levels of fasting gastrin when compared to mice with negative PCA–gastritis, although the difference was not statistically singificant (Table 2). The gastrin levels in blood varied between 75 and 914 pg/ml in mice with positive PCA–gastritis.
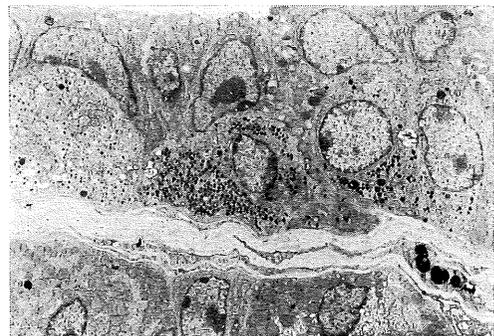
Fig. 6. Unclassified endocrine cells and enterochromaffin-like cells were observed in the body mucosa of mice with gastritis (×2400).

The Numbers of Parietal
Cell per Unit Area

$(Cells/cm^2)$

*** $p < 0.001$
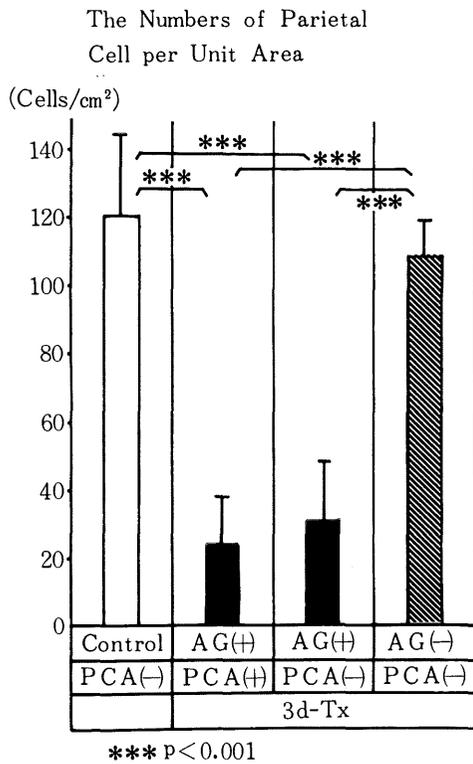
Fig. 7. A significant reduction in the number of parietal cells per visual field ($\times 400$) was noted in mice with gastritis.

Gastrin concentrations of the antral mucosa also varied between 7.34 and 16.5 $\mu g/g$ in mice with gastritis associated with or without PCA, although it was significantly increased when compared to those in control animals (Table 2).

A significant increase in the number of antral G cells per unit area was seen in mice with gastritis associated with or without PCA when compared to that in control animals (Table 2, Fig. 8).

Mucosal concentrations of somatostatin in the body mucosa were decreased in mice with gastritis, but this decrease was not significantly different between gastritis and control animals (Table 2, Fig. 9). There was, however, no difference in somatostatin content in the antral mucosa between gastritis and control animals (Table 2).

A significant decrease in the number of somatostatin D cells in the body mucosa was observed in mice with gastritis (Table 2). However, antral somatostatin D cells were very

Table 2. Gastric parietal cells, gastric pH, gastrin, G cells, somatostatin and D cells.

	P cells	Gastric pH	Gastrin Blood (pg/ml)	Gastrin Mucosa (micg/mg)	G cells (/UA)	Somatostatin (ng/ng) Body	Somatostatin (ng/ng) Antrum	Body D cells (/UA)	Antrum
Gastritis PCA (+)	25.3±13.9	7/8	332.0±231.9	11.0±4.2	23.0±7.3	0.598±0.391	0.529±0.230	4.0±1.8	NT
Gastritis PCA (−)	32.2±17.4	6/8	252.4±139.8		23.8±0.7			4.4±1.5	
Non-Gastritis	110.0±9.9	3	161.2±134.4	NT	17.5±7.8	NT	NT	NT	NT
Control	124.1±24.9	2	70.8±41.3	7.25±2.93	12.8±4.7	0.840±0.440	0.418±0.340	6.1±0.8	NT

P: Parietal, UA: Unit Area, NT: Not treated.
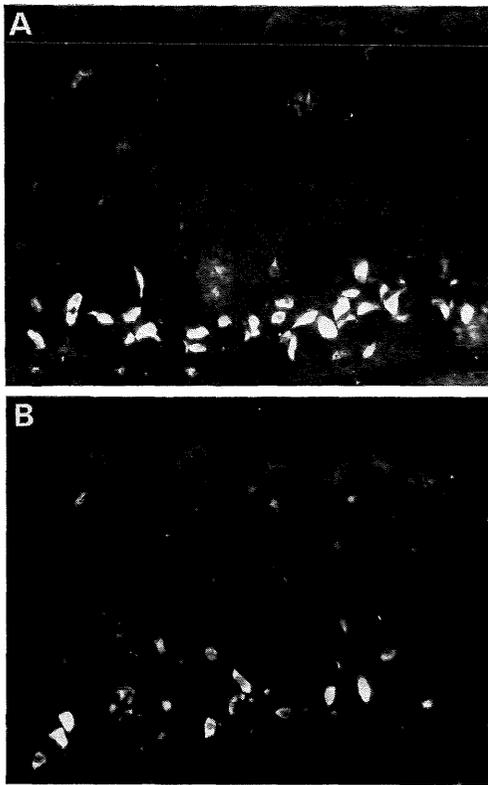
Fig. 8. A, B. Marked G cell hyperplasia in the antrum was seen in mice with gastritis and seropositive PCA (A), when compared to the control (B).

few in number and no difference could be observed between gastritis and control animals (Table 2).

DISCUSSION

Atrophic gastritis is essentially a result of reduction in the number of parietal cells and chief cells in the mucosa of the body and fundus of the stomach[24]. Atrophic gastritis, therefore, was defined in the present study to have a reduction of parietal cells essentially, in addition, associated with cellular infiltration and fibrosis in lamina propria mucosae. The present study showed that thymectomy of 3 dayed neonatal mice resulted in development of atrophic gastritis filled the above definition. The pathological findings of this gastritis observed mainly in oxyntic gland mucosa consisted of a significant reduction in the number of parietal cells in oxyntic gland mucosa, inflammatory cell infiltration, lymphfolicles and fibrosis in lamina propria mucosae. These lesions were
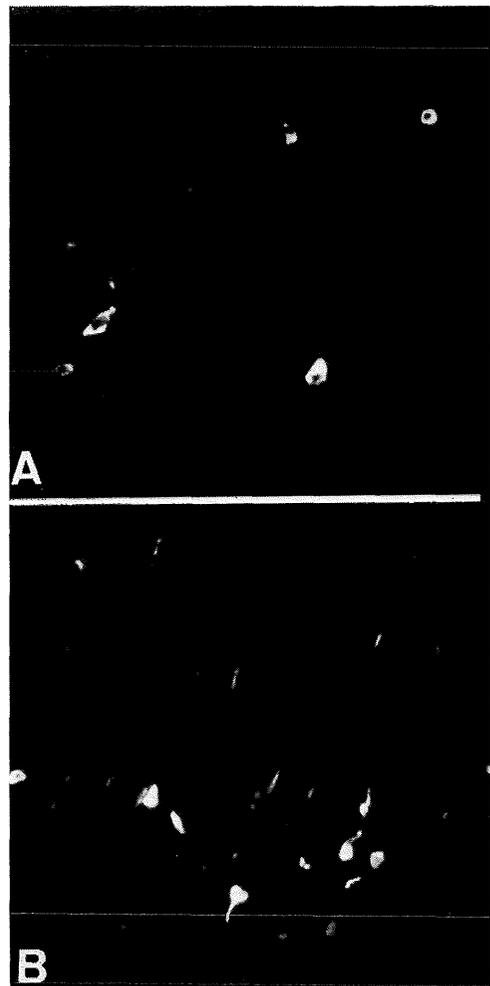
Fig. 9. A, B. Marked decrease of body somatostatin containing D cells was seen in mice with gastritis (A), (B) The controls.

very similar to the pathological mucosa of autoimmune gastritis in patients with pernicious anemia, in which the lesions were localised in the body and fundus (oxyintic gland mucosa) of the stomach according to the early descriptions of gastric atrophy[21,36] although separation may not always be well defined[18,34].

The mechanism involved in the production of postothymectomy gastritis remains proof. However, according to Kojima et al.[11], who defined this method, it is likely important that peripherised T-cells may initiate the autoimmune reactions in response to endogenous stimuli and the reaction, once they occur, may not be controlled in such neonatally thymectomised mice. Furthermore, a certain kind of lymph-

ocytes (probably non tolerant immunocompetent T cells) may be involved in the mechanism inducing autoimmune gastritis in mice. In addition, high frequency of occurrence of positive parietal cell antibodies in the blood of mice with atrophic gastritis was not phenomena secondarily resulted in atrophic changes of gastric mucosa[20] but the antibodies have some effects on parietal cells[9,23]. Peripherised T cells may probably suppress or inhibit growth of prematured cells to parietal cells and chief cells. Many prematured cells which resemble mucous neck cells in appearance were observed instead of reduced number of parietal and chief cells, but the mucosa was hyperplastic. Although it can not be explained in the present study why the mucosa was hyperplastic but atrophic in this type of autoimmune atrophic gastritis in mice, the mucosa is possibly a hyperplastic stage which proceeds the atrophic stage in the course of atrophic change. Since the authors have observed that the thickness of gastric mucosa was less in mice with gastritis 15 months after thymectomy than that in mice with atrophic gastritis 3 months after thymectomy[33].

Hypergastrinemia observed in mice with atrophic gastritis is attributed to the presence of achlorhydria (reduction or disappearance of parietal cells and increased gastric pH) and hence to the lack of normal inhibition of gastrin release from antral G cells by acid[8,22,39] since the authors have observed that serum gastrin levels fell to the normal levels after instillation 1.0 ml of acid solution (pH 2.0) into the stomach of mice with atrophic gastritis[38]. The mice associated with hypergastrinemia had antral G cell hyerplasia as well as increased concentration of mucosal gastrin. The cause of hypergastrinemia may explain on the presence of antral G cell hyperplasia. Some mice associated with hypergastrinemia, however, did not have antral G cell hyperplasia. This suggests that hypergastrinemia is not always due to antral G cell hyperplasia but due to exaggerated G cell function[16] in an alkaline environment of the antrum. This also suggests that the cause of G cell hyperplasia was simply not due to an alkaline change of environment in the antrum for a long period but unknown other factors must involve in the cause.

A reduction of somatostatin concentration

(D cells) may be a cause of hypergastrinemia in cases with atrophic gastritis[29], since one of actions of somatostatin suppress release of gastrin[15]. Somatostatin D celum are widely distributed in the gastrointestinal tract and pancreas, especially in the stomach. In mice, of this study, gastric D cells and somatostatin concentration both in the antral and body mucosa were determined. Neither D cells nor somatostatin concentration in the antrum were different from those in control animals. These suggest that antral somatostatin does not play a major role in the pathogenesis of hypergastrinemia. The authors considered parietal cell antibodies as another possibility of causes for hypergastrinemia, because serum gastrin levels of patients with pernicious anemia associated with positive parietal cell antibodies significantly higher than those in those patients without parietal cell antibodies[17]. Parietal cell antidobies, therefore, are considered another possible cause for hypergastrinemia. However, the hypothesis is not always supported since hypergastrinemia exists in patients without positive parietal cell antibodies. In the present study of mice, gastrin levels in the serum were not significantly different between mice with and without parietal cell antibodies. This suggests that a significant relationship does not exist between hypergastrinemia and positive parietal cell antibodies.

In the present study, argyrophil endocrine cells have been observed in the body mucosa by Grimelius method. Many enterochromaffin like cells and some unclassified endocrine cells have been found in the body mucosa at the ultrastructural levels although it was not obvious whether these cells contain gastrin or not as pointed out by Pearse et al.[25] and Rubin[31]. These data suggest that atrophic and metastatic mucosa have a potential for developing gastrin and other enteric hormone producing cells.

REFERENCES

1. **Arimura, A., Sato, H., Dupont, A., Nishi, N. and Schally, A. V.** 1975. Somatostain abundance of immunoreactive hormone in rat stomach and pancreas. Science **189** : 1007.

2. **Arimura, A., Sato, H., Coy, D. H. and Schally, A. V.** 1975. Radioimmunoassay for GH-release inhibiting hormone. Proc. Soc. Exptl. Biol. Med. **148** : 784.

3. **Arnold, R. and Lankisch, P. G.** 1980. Somato-

statin and the gastrointestinal tract. Clin. Gastroent. **9** : 733.

4. **Brandsborg, M., Elsborg, L., Andersen, D., Brandsborg, O. and Bastrup-Madsen, P.** 1977. Gastrin concentrations in serum and gastric mucosa in patients with pernicious anemia. Scand J. Gastroent. **12** : 537.

5. **Chayvialle, J. A. P., Descas, F., Bernard, C., Martin, A., Barbe, C. and Partensky, C.** 1978. Somatostatin in mucosa of stomach and duodenum in gastrointestinal disease. Gastroent. **75** : 13.

6. **Coons, A. H., Leduc, E. H. and Connolly, J. M.** 1955. Studies on antibody production. I. A method for the histochemical demonstration of specific antibody and its application to a study of the hyperimmune rabbit. J. Exp. Med. **102** : 49.

7. **Creutzfeldt, W., Arnold, R., Creutzfeldt, C., Feurle, G. and Ketterer, H.** 1971. Gastrin and G cells in the antral mucosa of patients with pernicious anemia, acromegaly and hyperparathyroidism, and in a Zollinger-Ellison tumour of the pancreas. Europ. J. Clin. Invest. **1** : 461.

8. **Canali, P. C., Cullen, D. R. and Irvine, W. J.** 1971. Radioimmunoassay of plasma gastrin in pernicious anemia, achlorhydria without pernicious anemia, hypochlorhydria, and in controls. Lancet **1** : 155.

9. **Jeffries, G. H.** 1965. Recovery of gastric mucosal structure and function in pernicious anemia during predonisolone therapy. Gastroent. **48** : 371.

10. **Koerker, D. J., Ruch, W., Chideckel, E., Palmer, J., Goodner, C. J., Ensinck, J. and Gale, C. C.** 1974. Somatostatin: Hypothalamic inhibition of the endocrine pancreas. Science **184** : 482.

11. **Kojima, A., Taguchi, O. and Nishizuka, Y.** 1980. Experimental production of possible autoimmune gastritis followed by macrocytic anemia in athymic nude mice. Lab. Invest. **42** : 387.

12. **Konturek, S. J., Tasler, J., Obtulowicz, W., Coy, D. H. and Schalley, A. V.** 1976. Effect of growth hormone release-inhibiting factor on hormones stimulating exocrine pancreatic secretion. J. Clin. Invest. **58** : 1.

13. **Konturek, S. J.** 1977. Somatostatin and the digestive system. Gastroent. Clin. Biol. **1** : 849.

14. **Konturek, S. J., Swierczek, J., Kwiecien, N. and Olebksy, J.** Effect of somatostain on meal-induced gastric secretion in duodenal ulcer patients. Gastroent. **72** : 818.

15. **Konturek, S. J., Talser, J., Cieszkowski, M., Coy, D. H. and Schally, A. V.** 1976. Effect of growth hormone release-inhibiting hormone on gastric secretion, mucosal blood flow and serum gastrin. Gastroent. **70** : 737.

16. **Korman, M. G., Strickland, R. G. and Hansky, J.** 1972. The functional G cell mass in atrophic gastritis. Gut **13** : 349.

17. **Korman, M. G., Strickland, R. G. and Hansky, J.** 1971. Serum gastrin in chronic gastritis. Brit. Med. J. **2** : 16.

18. **Lewin, K. J., Dowling, F., Wright, J. P. and Taylor, K. B.** 1976. Gastric morphology and serum gastrin levels in pernicious anemia. Gut. **17** : 551.

19. **Larsson, L. I., Golfermann, N., De Magistris, L., Rehfeld, J. F. and Schwartz, T. W.** 1979. Somatostain cell processes as pathways for paracrine secretion. Science **205** : 1393.

20. **Mackay, I. R.** 1964. Autoimmune serological studies in chronic gastritis and pernicious anemia. Gut **5** : 23.

21. **Magnus, H. A. and Ungley, C. C.** 1938. The gastric lesion in pernicious anemia. Lancet **1** : 420.

22. **McGuigan, J. E. and Trudeau, W. L.** 1970. Serum gastrin concentrations in pernicious anemia. New Engl. J. Med. **282** : 358.

23. **Miyoshi, A.** 1967. Chronic gastritis and autoimmunity. Gendai Naikagaku Taikei (*Suppl.) pp. 68.

24. **Morson, B. C. and Dawson, I. M. P.** 1979. Gastrointestinal pathology. pp. 91, 2nd Ed. Blackwell Scientific Pub. London 1979.

25. **Pearse, A. G. E., Busolati, G. and Polak, J. M.** 1972. Immunofluorescent studies on the G cells in normal and abnormal mucosa. Acta Hepatogastroent. **19** : 291.

26. **Polak, J. M., Hoffbrand, A. V., Reed, P. I., Bloom, S. R. and Pearse, A. G. E.** 1973. Qualitative and quantitative studies of antral and fundic G cells in pernicious anemia. Scand. J. Gastroent. **8** : 361.

27. **Polak, J. M., Bloom, S. R., Sullivan, S. N. and Arimura, A.** 1975. Growth hormone release inhibiting hormone (GH-RIH) in gastrointestinal and pancreatic D cells. Lancet **i** : 1220.

28. **Polak, J. M., Grimelius, L., Pearse, A. G. E., Timson, C. M. and Arimura, A.** 1976. Studies in gastric D cell pathology. Gut **17** : 400.

29. **Polak, J. M., Bloom, S. R., Bishop, A. E. and McCrossan, M. V.** 1978. D cell pathology in duodenal ulcers and achlorhydria. Metab. **27,** (Suppl.) **1** : 1239.

30. **Reinhardt, J. D., McCloy, R. M. and Blackwell, C.** 1976. Autoimmune atrophic gastritis with hypergastrinemia. South Med. J. **69** : 155.

31. **Rubin, W.** 1969. Proliferation of endocrine-like (enterochromaffin) cells in atrophic gastric mucosa. Gastroent. **57** : 641.

32. **Saffouri, B., Weir, G., Bitar, K. and Makhlouf, G.** 1979. Stimulation of gastrin secretion from the perfused rat stomach by somatostatin antiserum. Life Sci. **25** : 1749.

33. **Sano, K.** 1982. Experimental studies on atrophic gastritis in the mouse. Hiroshima Daigaku Igakuzasshi **30** : 187.

34. **Seifert, E. and Knoll, H.**   1968. Bioptische Ergebnisse bei gleichzeitiger Entnahme von Fundus-und Antrumschleimhaut des Magens. Med. Welt **1** : 1219.

35. **Sternberger, L. A. and Cuclis, J. J.**   1967. Method for enzymatic intensifications of the immunocytochemical reaction without use of labeled antibodies. J. Histochem. Cytochem. **17** : 190.

36. **Strickland, R. G., Bhathal, P. S., Korman, M. G. and Hamsky, J.**   1971. Serum gastrin on the antral mucosa in atrophic gastritis. Brit. Med. J.

4 : 451.

37. **Taylor, K. B., Roitt, M., Domach, D. et al.** 1962. Autoimmune phenomena in pernicious anemia gastric antibodies. Brit. Med. J. **2** : 1347.

38. **Unpublished data.**

39. **Yalow, K. S. and Berson, S. A.**   1970. Radioimmunoassay of gastrin. Gastroent. **58** : 1.

40. **Yalow, S. R. and Berson, S. A.**   1973. Methods in radioimmunoassay of peptide hormones. Ed.R. S. Yalow. North-Holland Co. pp. 196.