

—Original—

Morphological and Histochemical Characteristics of Mast Cells and the Content of In-tissue Histamine in Various Pathological Parathyroids: Do Mast Cells Participate in Hormone Secretion in Human Parathyroids?

Taro Iwamura, Kazuo Shimizu and Shigeo Tanaka

Department of Surgery II, Nippon Medical School

Abstract

The possibility of the participation of mast cells in human parathyroid hormone secretion was studied with regard to the frequency, distribution, and sub-types of mast cells and the content of in-tissue histamine, a chemical mediator in mast-cell granules, in human parathyroids with various pathological conditions. The above factors were compared between those of a 'normal' parathyroid group and those of 'pathological' parathyroids associated with adenoma and hyperplasia.

Specimens were scanned for the mean value of the mast cell number per field of microscopic view and for the ratio of the mast cell number in glandular parenchymal tissue to that in interstitial tissue. The activated state of the mast cells was examined through classifying the mast cells into two sub-types, mucosal mast cells and connective-tissue mast cells. The high-performance liquid chromatography (HPLC) method was used for assay of in-tissue histamine. The frequency of mast cells showed no difference between the groups, whereas the distribution of mast cells, showed a distinct difference. The occurrence rate of mast cells in glandular parenchymal tissue in the 'pathological' group presented an increase as compared with that in the 'normal' group. Furthermore, the occurrence rate of mucosal mast cells in an activated state also showed an increase. This suggests that mast cells are likely to participate in parathyroid hormone secretion. The histamine-content in the 'normal' group was significantly larger than that in the 'pathological' group, which was a different outcome from that observed in mast cells from the results of light microscopy. This may require taking into consideration the difference in the histamine content of the mast cells themselves between that of mucosal mast cells and connective-tissue mast cells. (J Nippon Med Sch 2002; 69: 347–354)

Key words: mast cell, histamine, PTH secretion

Introduction

Mast cells include abundant granules which are chemical mediators, such as histamine which is associated with inflammatory reactions, and plays

a major role in type-I allergic reactions. There is recognition, however, that these cells exist not only in the conditions of inflammation and allergic reactions but also in all organs. Mast cells are also known to change their granular morphology and the developing pattern of their chemical mediators depending

Correspondence to Taro Iwamura, MD, Department of Surgery II, Nippon Medical School Second Hospital, 1-396 Kosugicho, Nakahara-ku, Kawasaki-shi, Kanagawa 211-8533, Japan

E-mail: iwatmr@nms.ac.jp

Journal Website (<http://www.nms.ac.jp/jnms/>)

on the environment. This is evident from the fact that mast cells can be divided into sub-types, mucosal mast cells (MMC) and connective-tissue mast cells (CTMC). Hence, these cells are believed to play various other physiological roles.

With rat thyroids, chemical mediators released by the degranulation of mast cells are reported to increase thyroid hormone secretion while stimulating the thyroid follicular epithelium¹. Human parathyroid cells are known to react *in vitro* to histamine, promoting parathyroid hormone (PTH) secretion by increasing intracellular cyclic AMP²⁻⁵. With human endocrine organs as well, mast cells are most likely to participate in hormone secretion.

In addition to the frequency and distribution of mast cells and in-tissue histamine content in various pathological conditions of parathyroids, we discuss the activated condition of mast cells through their classification into sub-types in order to clarify the role of mast cells in PTH secretion.

Materials and Methods

(1) Observation of mast cells in human parathyroids

We examined 10 cases of normal parathyroid glands that were removed in conjunction with surgery for a thyroid malignant tumor from the part adjacent to the lesion from women aged 24 to 76 years (mean age 42.0 years). The breakdown of the primary diseases is 8-cases with papillary carcinoma and 2-cases with follicular carcinoma. Examined were pathological parathyroid glands that had been removed from one man with adenoma and 15 women (13 cases with adenoma and 2 cases with hyperplasia) ranging from age 21 to 75 years old (mean age: 56.2 years) according to the diagnosis of primary hyperparathyroidism. The mast cells in the resected samples were identified by alkaline toluidine-blue staining in addition to HE staining.

(2) Observation of the mast-cell frequency and distribution

The frequency of the mast cell occurrence was determined by taking a mean value of the mast cell numbers per field ($\times 20$ object lens) obtained from all sections of each specimen for comparison

between the normal parathyroid group (NP group) and parathyroids of the hyperparathyroidism group (HP group) (adenomas and hyperplasia). The fields rich in fat spaces in the NP group were adopted into the mast cell counting, while those fields in which normal parathyroid rims were observed were excluded.

The distribution of mast cells was compared between the groups in regard to the ratio of the number of mast cells existing in glandular parenchymal tissue to that of mast cells existing in comparatively wide interstitial tissue in proximity to capillaries and fat spaces.

(3) Classification of mast cells into sub-types

Mast cells were classified into the sub-types, mucosal mast cells (MMC) and connective tissue mast cells (CTMC), according to the difference between the reactivity to antitryptase antibody and that to antichymase antibody. Of the resected samples referred to earlier, serial sections were prepared, including 4 cases of normal parathyroids and 11 cases of parathyroids with hyperparathyroidism (10 adenomas and 1 hyperplasia). Following the Carnot-fixation of the serial sections, the ABC immune peroxidase method was applied to the anti-human tryptase monoclonal antibody (DAKO-Mast Cell AA 1) and the anti-human chymase monoclonal antibody (CHEMI CON, MAB 1254 B). With the antibodies diluted to concentrations of 1:100 and 1:600, coloring was made with diaminobenzidine for reactivity comparisons.

(4) Assay of histamine

Samples for assay for in-tissue histamine were taken from 5 cases of normal parathyroids and 23 cases with hyperparathyroidism (18 cases with primary hyperparathyroidism and 5 cases with secondary hyperparathyroidism). The specimens (0.1~0.2 g) obtained were homogenized with 3 ml of 0.5 N perchloric acid, followed by centrifugation. The 800 μ l supernatant of the centrifuged liquid was taken for alkalization to a pH of 5~6 with 30% KOH, and then frozen, remelted, and centrifuged. Its supernatant was diluted, and a specimen of 100 μ g diluent was poured into a high-performance liquid chromatography (HPLC), a HITACHI L-6200 type

liquid chromatograph. The content of histamine was calculated with a HITACHI F-1000 type fluorescent spectrofluorometer set at an excitation wavelength of 360 nm and emission wavelength of 450 nm.

The differences between groups were statistically analyzed by the Student's t-test, where $p < 0.05$ was regarded as statistically significant.

The samples used in this study were generously donated by the Department of Surgery II, Nippon Medical School, Ito Hospital in Tokyo, and Kuma Hospital in Kobe.

Results

1. Mast cells in human parathyroids

(1) Light-microscopic observation

The cytoplasmic granules in the mast cells were clearly stained pale-violet by toluidine blue staining. In the normal parathyroids, it was observed that glandular cells were forming into cell bundles and cellulae, connective tissue and capillaries existed between the glandular cell bundles, and there were many fat cells mediating in various proportions. Mast cells were mostly observed in the interstitial tissue space often in proximity to capillaries and adjacent to fat spaces, and there were extremely few in the glandular parenchyma tissue (**Photo 1 a, b**).

In adenomas, massive proliferation of sheet-form, cordlike chief cells were observed. The nuclei of the adenoma cells were large-sized and exhibited polymorphism, with multinucleate cells noted as well. However, mediation of fat cells was hardly observed. In hyperplasia, glandular-cavity-like proliferation of chief cells was seen together with a small number of fat cells. In adenomas and hyperplasia, many mast cells were also detected in the narrow interstices of proliferated glandular tissue (**Photo 2 a, b**).

(2) Evaluation of mast-cell frequency and distribution

The mean frequency of mast cells per field was 6.93 ± 4.77 pcs in the NP group and 6.35 ± 3.48 pcs in the HP group, with no statistically significant difference in the frequency acknowledged between the groups (**Fig. 1**).

When viewed from the distribution of mast cells, a difference seemed to exist between the groups with regard to the rate of mast cells involved in glandular

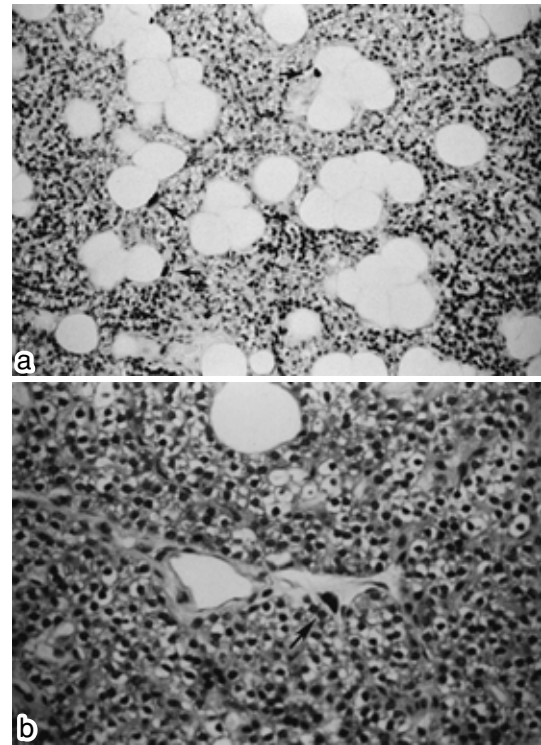


Photo. 1a, b Distribution of mast cells in the 'normal' group
Mast cells were mostly observed in the interstitial tissue spaces, often in close proximity to arterioles and venules, and also adjacent to fat spaces (toluidine blue stain, a, b, $\times 200$).

parenchymal tissue. For study of between-group comparisons, the ratio of the number of mast cells in the glandular parenchymal tissue to that in the interstitial tissue, adjacent to fat spaces and capillary tissue, was used for the NP group, while the ratio of the mast cells in the neoplastic proliferation region of the adenocyte to that in the interstitial tissue (**Fig. 2**), was used for the HP group. The mean ratio was 0.36 in the NP group, while it was 5.37 in the HP group, indicating abundant mast cells appearing in the proliferation site of tumors in the HP group with a statistically significant difference existing between groups.

2. Classification of mast cells into sub-types

The granules of a mast cell contain various types of proteases. The exploration of the differences of mast cells involved in various human tissues presently uses a method wherein mast cells are classified by examination of the distribution of these proteases

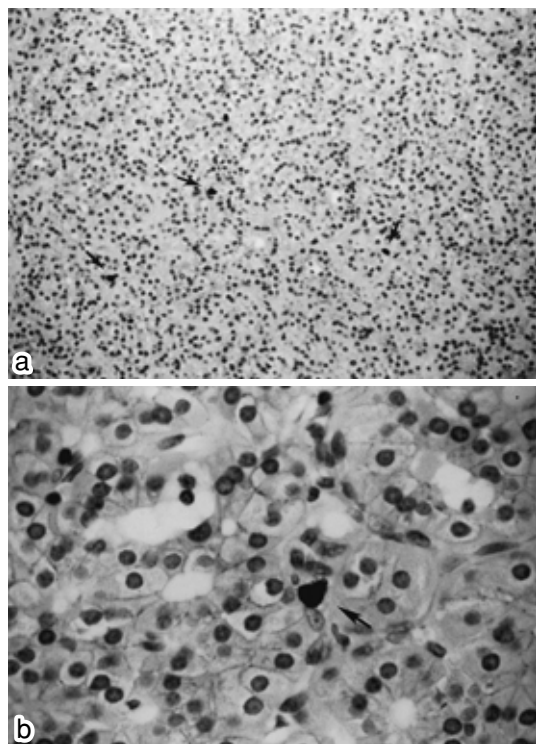


Photo. 2a, b Distribution of mast cells in the 'hyperparathyroidism' group
Mast cells were observed in large numbers even in the proliferating glandular tissue of the 'hyperparathyroidism' group (adenomas, toluidine blue stain, a, $\times 200$, b, $\times 400$).

with the antibodies of proteases as a marker. In this study, we attempted to classify mast cells into the sub-types, mucosal mast cells (MMC) and connective tissue mast cells (CTMC), based on the difference between the reactivity of the mast cells to tryptase and chymase. The occurrence site of the stained mast cells almost coincided with that of the mast cells stained with toluidine blue (**Photo 3 a, b**). The application of this classification to human mast cells revealed that MMC represents a reactivity of tryptase-positive and chymase-negative, while CTMC represents a reactivity positive both to tryptase and chymase. An attempt was made to compare the reactivity of antitryptase antibody and that of antichymase antibody in the serial sections respectively; however, such a comparison of two different reactivities in the same cell proved to be impossible. So, the ratio of the number of tryptase positive cells to that of chymase positive cells was used for comparison. Such ratios were 0.73 in the NP group and 3.16 in

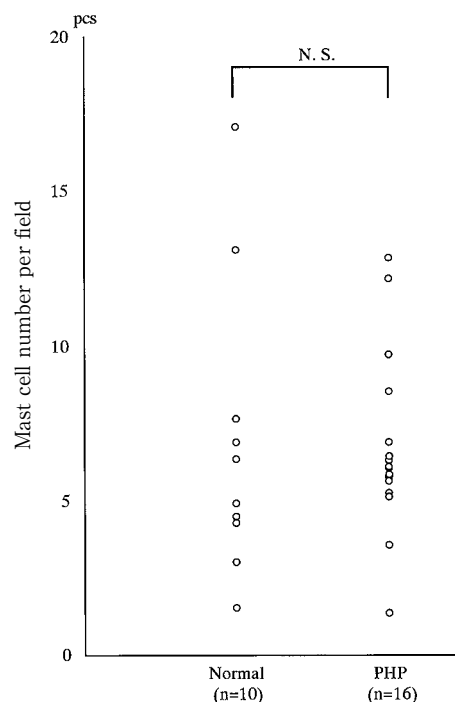


Fig. 1 Frequency of the occurrence of mast cells
Average values of the number of mast cells in all fields scanned in each group. No significant difference was observed between the two groups.
PHP: primary hyperparathyroidism group

the HP group (**Fig. 3**), indicating a higher ratio of MMC in parathyroids with hyperparathyroidism than in normal glands.

3. Assay of histamine

Histamine is highly concentrated in the basophilic granule of mast cells. The content of in-tissue histamine appears to reflect that of histamine, a chemical mediator in the granules of mast cells included in a three-dimensional structure. The average measurements of in-tissue histamine in the NP, primary hyperparathyroidism, and secondary hyperparathyroidism groups were $6.27 \pm 3.39 \mu\text{g/g}$, $2.66 \pm 2.39 \mu\text{g/g}$, $1.42 \pm 1.37 \mu\text{g/g}$, respectively. The value in the NP group was significantly higher compared with that in the primary and secondary hyperparathyroidism groups (**Fig. 4**).

Discussion

There is a report that mast cells are related to endocrinological function. Concerning rat thyroids,

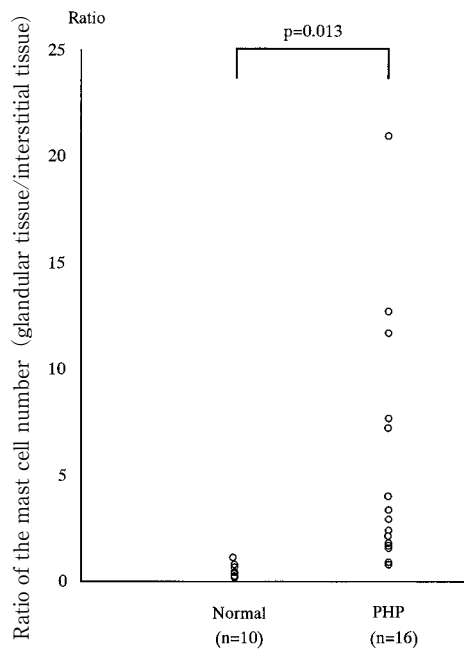


Fig. 2 Distribution of mast cells
 Comparison of the occurrence site of mast cells between the 'normal' group and 'hyperparathyroidism' group (glandular tissue/interstitial tissue)
 Average values of the ratio of the number of mast cells existing in glandular parenchyma tissue per field to that in interstitial tissue in each group. Mast cells were observed in large numbers in glandular parenchyma in the 'hyperparathyroidism' group compared with that in the 'normal' group, with a significant difference acknowledged between the two groups.
 Abbreviations are the same as in Fig. 1.

it is recognized that the stimulation of thyroid stimulating hormone (TSH) first degranulates mast cells existing in proximity to peripheral nerves via the substance-P that has been discharged from the peripheral nerves. Thus, the released chemical mediators, such as histamine and serotonin promote the hormone production of follicular epithelial cells⁶. Meanwhile, the studies of human parathyroids in vitro have suggested the existence of histaminereceptors in parathyroid cells^{2-5,7-9}. There are reports that the administration of cimetidine, an H2 receptor antagonist, to patients with hyperparathyroidism decreased PTH blood concentration in vivo though by a small amount¹⁰⁻¹⁷. As well known, histamine is the material that is reserved in mast-cell granules. Because histamine, which is also found in basophilic granulocytes in vivo, exists chiefly in the

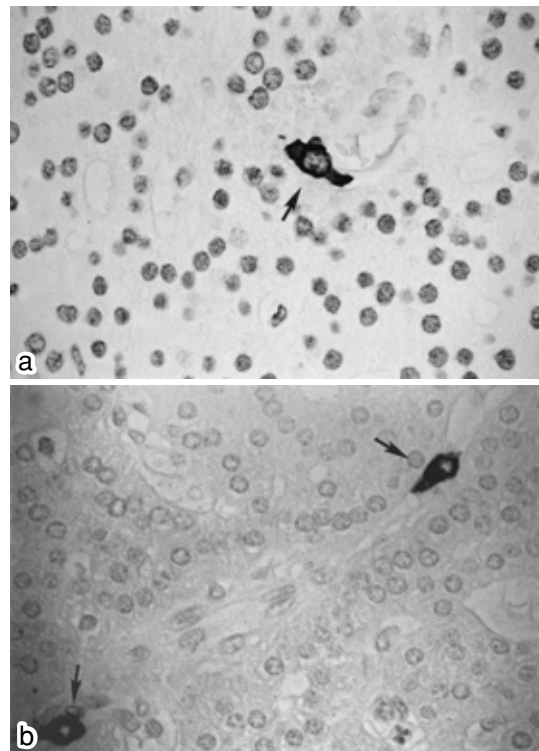


Photo. 3 a, b Immunohistochemical reaction of mast cells to tryptase (a) and chymase (b) in a serial section.

blood stream, mast cells are thought to play a major role in the supply source of histamine in the condition of PTH secretion. This raises the possibility that in human parathyroids, mast cells are associated with PTH secretion.

Since the report made by Erdheim in 1903 on the existence of mast cells in human normal parathyroid glands, there have been studies on this connection by Morgan¹⁸, Gilmour¹⁹, and Anderson et al.²⁰. With normal parathyroids, the mast cells in glandular parenchymal tissue are considered to be small in number and mainly existing in pericapillary interstitial tissue and in proximity to fat spaces. This tendency is also true with parathyroids with adenomas and hyperplasia in a hyperactive condition. Anderson et al.²⁰ concluded that no difference was noted in the occurrence site of mast cells, while the frequency of mast-cells in normal parathyroids was large in comparison to that in glands with adenomas and hyperplasia. Even according to a report on human thyroids, which are the same type of endocrines, mast cells were localized on their interlobular interstitial

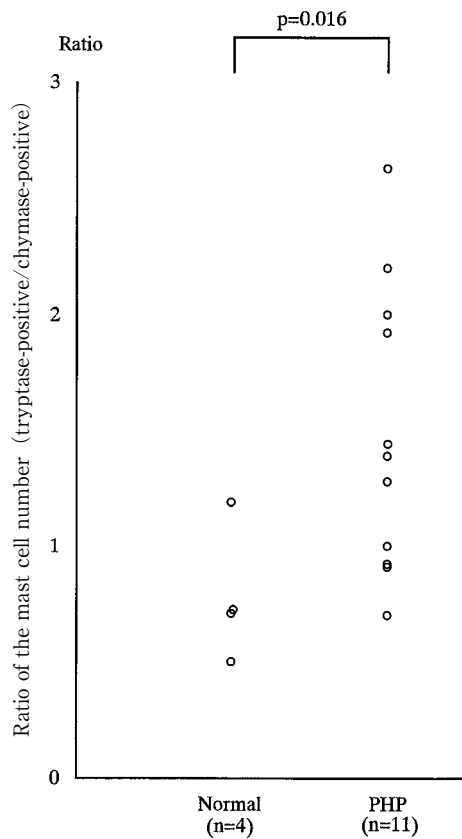


Fig. 3 Ratio of the number of tryptase-positive mast cells to chymase-positive mast cells (tryptase positive/chymase positive). The ratio of the average values of the number of tryptase positive cells in a serial section per field to that of the number of chymase positive cells was compared between the two groups.

Abbreviations are the same as in Fig. 1.

tissue, and were in a considerably estranged relationship with the follicular epithelium. The report also described that no differences were apparent in the distribution of mast cells even in glands under hyperactive conditions such as seen in Graves' disease²¹. Most of the papers report that mast cells in human endocrine organs are, thus, most likely to play the role of providing an architectural support for tissue from the viewpoint of the frequency and distribution of mast cells, and that the participation of mast cells in hormone secretion is ambiguous.

Fibroblasts are reported to have been deeply associated with the maturity and differentiation of mast cells²². It is quite justifiable to say that mast cells exist in large numbers in interstitial tissue where collagenous fibers constituted by fibroblasts develop

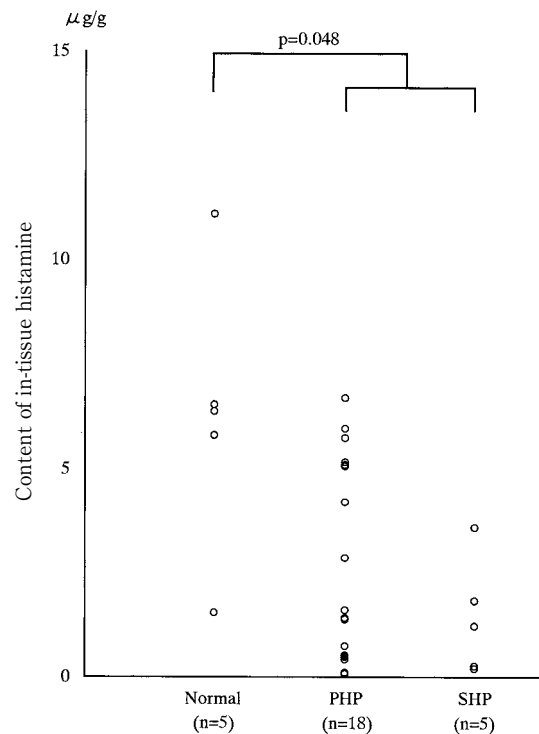


Fig. 4 Content of in-tissue histamine in the NP group and HP group

The content of in-tissue histamine in the 'normal' group (NP) was significantly higher compared to that in the 'hyperparathyroidism' group (HP).

Abbreviations are the same as in Fig. 1.

PHP: primary hyperparathyroidism

SHP: secondary hyperparathyroidism

and that they are abundant around the periphery of fat spaces having the same origin as that of fibroblasts. Our study confirmed, in the case of normal parathyroids, the existence of almost all mast cells in comparatively large interstitial tissue and in the proximity of fat spaces. In the case of adenomas and hyperplasia, in contrast, the study observed large numbers of mast cells in proliferating glandular parenchymal tissue rather than in interstitial tissue. The comparison of the frequency of mast cells did not reveal any significant difference between normal parathyroids and pathological parathyroids (adenomas and hyperplasia), which appears to contradict the results of the study of Anderson et al.²⁰. The point worthy of attention is the difference in the nature of samples as normal parathyroids. We selected for study, normal parathyroids excised at the same time of surgery as that for thyroid malignancy, whereas Anderson

Table 1 Comparison of characteristics of mast cell between NP group and HP group

	NP group	HP group	
		PHP	SHP
Frequency of the occurrence of mast cells (pcs)	6.93 ± 4.77 (n = 10)	6.35 ± 3.48 (n = 16)	
Distribution of mast cells (ratio)*	0.36 (n = 10)	5.37 (n = 16)	
Tryptase positive/chymase positive (ratio)*	0.76 (n = 4)	3.16 (n = 11)	
Content of in-tissue histamine (μg/g)*	6.27 ± 3.39 (n = 5)	2.66 ± 2.39 (n = 18)	1.42 ± 1.37 (n = 5)

* p < 0.05

NP group: normal parathyroids

HP group: hyperparathyroids (adenoma and hyperplasia)

et al.²⁰ used other glands, excised simultaneously with parathyroids with adenomas, and normal rims on the periphery of the adenoma²⁰. The complication of an adenoma is thought to contract the glandular tissue of the targeted normal gland, eliciting an increase in the amount of interstitial tissue or an increase in fat tissue. This seems finally to lead to the observation of the increased number of mast cells. When these differences and problems are taken into consideration, our study results on mast cell frequency may not in a unifying fashion be compared with those of Anderson et al²⁰.

Our study did not acknowledge any differences in the frequency of mast cells in human parathyroids between the NP group and the HP group; however, it revealed a clear difference in the distribution of the mast cells. The comparison of the ratio of the number of mast cells in glandular parenchymal tissue to that in interstitial tissue (glandular parenchymal tissue/interstitial tissue) resulted in a ratio of 0.36 in the NP group and 5.37 in the HP group (**Table 1**).

This means that mast cells in the HP group were found in glandular parenchymal tissue by about 15 times that in the NP group. Those mast cells exist within extremely narrow connective tissue between proliferating glandular cell cords, which may justifiably be called the place of hormone secretion. In rat thyroids, where mast cells are reported to be associated with hormone secretion, the mast cells are chiefly distributed in narrow interfollicular connective tissue, and are thought to fulfill a role in the thyroid hormone secretion²¹. The mast cell site thus seems

to bear an important meaning in the role they play. Moreover, the review of the sub-types of mast cells recognized a difference in the ratio of the number of MMCs to that of CTMCs between the groups (**Table 1**), i.e. a greater number of MMCs occurring in the HP group. The MMC, which is smaller than the CTMC in cell diameter and in number of granules and takes on a variety of sizes, can be called a mast cell in an activated condition releasing the contents of the granules. This, in other words, indicates that the number of mast cells in an activated condition is large in hyperactive parathyroids. As mentioned above, the differences between the groups in regard to the distribution and activated condition of mast cells appear to suggest the relation of mast cells to PTH production and secretion.

Meanwhile, the content of in-tissue histamine in the NP group was significantly large compared with that in the HP group (**Table 1**). The observation at the level of two-dimensional light-microscopy found no differences between the groups with regard to the frequency of mast cells, seemingly producing a contradictory outcome. The explanation of this discrepancy requires taking into consideration the difference in the histamine content in the mast cells themselves. The CTMC existing in large numbers in the NP group is a steady-state mast cell having a high histamine content. In contrast, the MMC existing in large numbers in the HP group, is a mast cell in an activated condition, releasing granular contents and having low histamine content. The half-life of histamine is thought to be short, within one-or

two-minutes, and it disappears promptly from inside of the tissue, though it is released by degranulation. In other words, it is presumed that the difference in the activated condition of mast cells between the groups develops a difference in the content of the intissue histamine. Accordingly, the content of intissue histamine does not necessarily reflect the number of mast cells existing in a three-dimensional structure, without contradiction to the observation results at the level of two-dimensional light-microscopy.

Our experiments demonstrated that the number of mast cells in glandular parenchyma of human pathological parathyroids showed an increase compared with that of normal parathyroids. The difference in the activated condition of mast cells between the groups led us to the conclusion that mast cells participate in some way or another in PTH production and secretion.

Acknowledgement: We deeply appreciate the supply of samples from Ito Hospital and Dr. Akira Kobayashi in Kuma Hospital, and the technical cooperation afforded by the staff in the Division of Pathology in Ito Hospital.

References

- Melander A, Sundler F: Significance of thyroid mast cells in hormone secretion. *Endocrinology* 1972; 90: 802-807.
- Brown EM, Gardner DC, Windeck RA, Hurwitz S, Brennan MF, Aurbach GD: β -Adrenergically stimulated adenosine 3' 5' -monophosphate accumulation in and parathyroid hormone release from dispersed human parathyroid cells. *J Clin Endocrinol Metab* 1979; 48: 618-626.
- Williams GA, Longley RS, Hargis GK, Bowser EN, Kukreja SC, Johnson PA, Jackson BL, Kawahara WJ: Effect of histamine on parathyroid hormone. *Clin Res* 1979; 27: 704 (Abstract).
- Abboud HE, Zimmerman D, Edis AJ, Dousa TP: Histamine and Parathyroid adenoma: effect on cyclic AMP accumulation. *Clin Res* 1980; 28: 515 (Abstract).
- Brown EM: Histamine receptors in parathyroid adenomas. *Clin Res* 1980; 28: 388 A (Abstract).
- Clayton JA, Masuoka DT: TSH-induced mobilization of serotonin from perivasucular mast cells in the rat thyroids. *Endocrinology* 1968; 83: 267-271.
- Brown EM: Histamine Receptors on Dispersed Parathyroid Cells from Pathological Human Parathyroid Tissue. *J Clin Endocrinol Metab* 1980; 51: 1325-1329.
- Abboud HA, Zimmerman D, Edis AJ, Heath HIII, Dousa TP: Histamine and human parathyroid adenoma. Effect on adenosine 3' 5' monophosphate in vitro. *J Clin Endocr Metab* 1981; 53: 276-281.
- Sicard GA, Melzer JS, Dickemper DR, Martin KJ, Etherege EE, Anderson CB: Histamine stimulation and inhibition of human cryopreserved parathyroid tissue. *Surgery* 1981; 90: 396-400.
- Beehler D, Becker J, Rosenquist R, Shnkel S: Effect of cimetidine on parathyroid hormone (PTH) in chronically dialysed patients. *Kidney int* 1979; 16: 951 (Abstract).
- Bourgoignie JJ, Jacob AT, Lanier DJr, Gavellas G, Lautenburg JM: Cimetidine (C) inhibition of i PTH in chronic renal disease. *Kidney int* 1979; 16: 918 (Abstract).
- Jacob AI, Lanier D Jr, Canterbury J, Bourgoignie JJ: Reduction by cimetidine of serum parathyroid hormone levels in uremic patients. *New Engl J Med* 1980; 302: 671-674.
- Lanier D Jr, Favre H, Jacob AI, Bourgoignie JJ: Cimetidine therapy for severe hypercalcemia in two chronic hemodialysis patients. *Ann intern Med* 1980; 93: 573-574.
- Sherwood JK, Ackroyd FW, Garcia M: Effect of cimetidine on circulating parathyroid hormone in primary hyperparathyroidism. *Lancet* 1980; I: 616-620.
- Sherwood JK, Reinhard D, Garcia M: Does cimetidine inhibit parathyroid hormone secretion? *New Engl J Med* 1979; 300: 200-201.
- Williams GA: Parathyroid Hormone Secretion in Normal Men and in Primary Hyperparathyroidism: Role of Histamine H₂ Receptors. *Journal of Clinical Endocrinology and metabolism* 1981; 52: 122-127.
- Wagner PK, Krause U, Scharfe Th, Beyer J, Rothmund M: Effect of cimetidine on Basal and Histamine-induced Secretion of Parathyroid Hormone in vitro. *Nephron* 1984; 36: 89-93.
- Morgan JRE: The parathyroid glands. *Arch Path* 1936; 21: 10-26.
- Gilmour JR: The normal histology of the parathyroid glands. *J Path Bact* 1939; 48: 187-222.
- Anderson TJ: Mast cells in parathyroid glands of hyperparathyroidism. *J Clin Path* 1974; 27: 31-34.
- Akasu H, Kawanami O: Morphological characteristics of human mast cells in normal and pathological thyroid glands; Functional aspect of human mast cells in comparison with rat mast cells. *J Nippon Med Sch* 1994; 61: 53 (Abstract).
- Fujita J, Nakayama H, Onoue H, Kanakura Y, Nakao T, Asai H, Takeda S, Honjo T, Kitamura Y: Fibroblast-dependent growth of mouse mast cells in vitro; Duplication of mast cell depletion in mutant mice of W/W genotype. *J Cell Physiol* 1988; 134: 78-84.

(Received, December 3, 2001)

(Accepted, February 8, 2002)