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Immunohistochemical study of nodular hyperplastic parathyroid glands in patients with secondary hyperparathyroidism

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Summary. Thirty-seven nodular hyperplastic parathyroid glands obtained by subtotal parathyroidectomy from 11 haemodialysed patients with secondary hyperparathyroidism were examined both pathologically and immunohistochemically. Four consecutive sections of the largest section-surface of each gland were subject to 4 different stains (haematoxyline-eosin, Grimelius, and the immunohistochemical stains for parathyroid hormone and chromogranin A) for comparison of each nodule.

It was found that the major part of each nodule consisted of a single cell type with a single pattern of cells. These reacted uniformly to each stain. The mechanism involved in the storage and secretion of the secretory granule appeared to be regulated at the nodule and not at the cell level. The results suggest that the nodules may come from a monoclonal proliferation of a single parathyroid cell.

Our present light microscopic immunohistochemical study, failed to demonstrate completely identical immunoreactive positivity of each nodule or each parathyroid cell to PTH. Chromogranin A or secretory protein-I did not indicate the coexistence of PTH and SP-I in the same secretory granule, which was in good agreement with the electron microscopic immunocytochemical study of Arps using bovine parathyroid glands. Our present study, however, provides good evidence that chromogranin A positivity is demonstrable in the human parathyroid gland outside the adrenal medulla and sympathetic nerves.

Key words: Immunohistochemistry – Parathyroid hormone – Chromogranin A – Secondary hyperparathyroidism – Haemodialysis

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Introduction

It is known that secondary hyperparathyroidism (2 HPT) caused by chronic renal insufficiency has generalised complications such as renal osteodystrophy (ROD), ectopic calcification and pruritus (Slatopolsky and Bricker 1973; Massry et al. 1968). Furthermore, as Massry et al. (1976) described, the parathyroid hormone (PTH) in patients with 2 HPT might play a role as a uraemic toxin.

Since 1979, about one hundred patients with 2 HPT have been treated with aluminium hydroxide (almigel®), calcium agents and activated vitamin D, but due to severe complications they underwent subtotal parathyroidectomy with successful results. The histopathological findings of these resected parathyroid glands may be highly diagnostic of hyperplasia, consisting mainly of chief cells, transitional oxyphil cells or oxyphil cells with nodular or diffuse cellular patterns in hematoxylin-eosin (H&E) stained sections. However, the H&E stain alone cannot reveal how or where PTH is stored and secreted from the hyperplastic parathyroid glands. In order to elucidate PTH related problems, we made an immunohistochemical study of 2 HPT using an anti-PTH and an anti-chromogranin A antibody which cross-reacts with secretroy protein-I (SP-I), one of the major secretory proteins in the parathyroid glands (Cohn et al. 1982). These antibodies were employed in addition to Grimelius stain (1968), which has been the conventional method available to detect the cells from the neural crest.

Materials and methods

Thirty-seven nodular hyperplastic parathyroid glands were studied, obtained by subtotal parathyroidectomy from 11 hae-

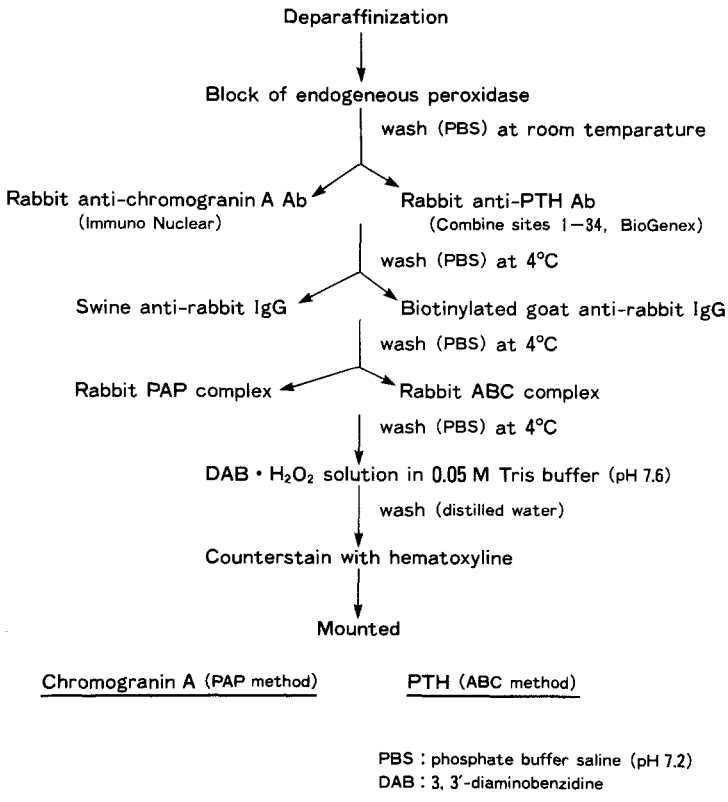


Fig. 1. Immunohistochemical stainings of PTH and chromogranin A

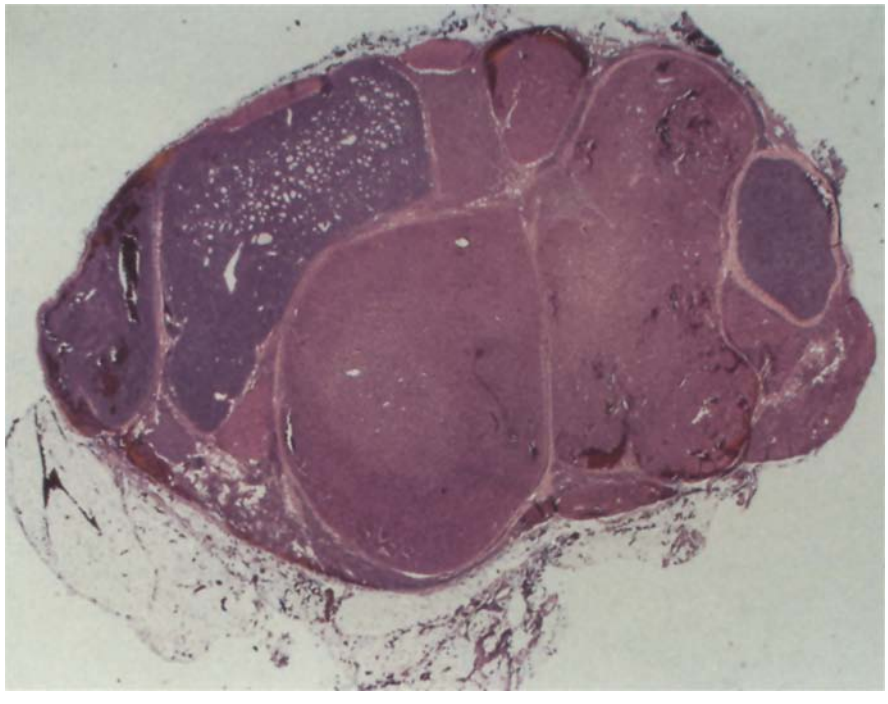


Fig. 2. Section of a certain gland with H&E stain shows that the major part of each nodule consists of a single cell with a single cellular pattern (×12)

modialysed patients with 2 HPT. These patients consisted of 4 males and 7 females with a mean age of 53.4 years, ranging from 40 to 72 years. At the time of operation, the mean duration of haemodialysis was 97.2 months, with a range of 21 to 163 months and the mean value of serum C-terminal PTH was 32.6 ng/ml with a range of 6.02 to 91.0 ng/ml. In spite of conservative therapy all the patients had severe complications caused by 2 HPT such as ROD, ectopic calcification involving the walls of the abdominal aorta and the soft tissues, red eyes and pruritus. The mean weight of 37 resected parathyroid glands

Table 1. Relationship among cell type, cellular pattern and three stainings

Cell type	Cellular pattern	Grimelius (%)			Chromogranin A (%)			PTH (%)			Total No. of nodules
		neg.	+	++	neg.	+	++	neg.	+	++	
Chief	Solid	41.7	40.6	17.7	55.2	28.1	16.7	20.8	38.5	40.6	96
	A–T	29.4	70.6	0	52.9	35.3	11.8	17.6	41.2	41.2	17
Transitional	Solid	38.5	34.6	26.9	26.9	40.3	30.8	12.5	38.5	53.8	26
	A–T	23.8	47.6	28.6	47.6	19.0	33.3	0	28.6	71.4	21
Oxyphil	Solid	50.0	16.7	33.3	66.7	16.7	16.7	16.7	25.0	58.3	12
	A–T	57.1	28.6	14.3	57.1	42.9	0	28.6	0	71.4	7
Overall	Solid	41.8	37.3	20.9	50.7	29.9	19.4	17.9	37.3	44.8	134
	A–T	31.1	53.3	15.6	51.1	28.9	20.0	11.1	28.9	60.0	45

Negative (neg.); nil or sparse stain-positive cells ++; diffuse and dense stain-positive cells

A–T; Acinar-Tubular

was 918 mg, ranging from 15 to 6,770 mg. Each resected parathyroid gland was fixed in Zamboni's solution (Zamboni and Demartino 1967) at 4° C for 24 to 48 h immediately after weighing and measuring, and then embedded in paraffin. Four consecutive sections of the largest section-surface were subjected to 4 different stains, that is, H&E, Grimelius, and the immunohistochemical stains of PTH and chromogranin A. The stains of PTH and chromogranin A were employed according to the methods described by Hsu et al. (1981) and Sternberger et al. (1970), respectively. As primary antibodies in the immunohistochemical stains of PTH and chromogranin A, we used, rabbit immunized anti-human PTH antibody with combine sites of 1 to 34 amino acid residues purchased from BioGenex Lab. and rabbit immunized anti-bovine chromogranin A antibody purchased from Immuno Nuclear, respectively. The procedures for the stains of PTH and chromogranin A are schematically represented in Fig. 1.

The antisera pre-absorbed by human PTH were used for the PTH control slide.

Results

Each section of the 37 resected nodular hyperplastic parathyroid glands had 1 to 16 nodules of various sizes in a total of 179 nodules. H&E staining revealed that the major part of each nodule was occupied by a single cell type (chief, transitional oxyphil or oxyphil cell) and a single pattern of cells (sheet, trabecular, acinar or tubular) (Fig. 2). These 179 nodules were grouped into 6 classes formed from the combinations of 3 cell types, that is, chief, transitional oxyphil and oxyphil cells, and 2 patterns of cell type were recognized: solid, showing a sheet or trabecular pattern, and acinar-tubular type. The 179 nodules were composed of 113 nodules of the chief cell type with 96 of the solid and 17 of the acinar-tubular type, 47 nodules of the transitional oxyphil cell type with 26 of the solid and 21 of the acinar-tubular type, and 19 nodules of the oxyphil cell type with 12 of the solid and 7 of the acinar-tubular type. Although it is very difficult to make a sharp distinction between transitional oxyphil cells and oxyphil cells, the former could be distinguished from the latter by less intense cytoplasmic eosinophilia, having rather ambiguous cell membranes (Castleman and Roth 1978). All of the 37 nodular hyperplastic parathyroid glands had at least one nodule of the chief cell type.

The results of the intensity of immunohistochemical stains in each group are shown in Table 1. With all stains, the stain positive nodules were most abundant in the transitional oxyphil cell type group, while those in the oxyphil cell type group were the fewest. Although all nodules of the transitional oxyphil cell type with an acinar-tubular pattern proved to be immuno-reactively PTH-positive, there seemed to be no remarkable differences, as a whole, between two types of pattern of cells. An investigation of the relationship to neighboring nodules showed that some neighborning nodules displayed a reciprocal relation to certain stains (Fig. 3).

The results of the three separate stainings (Grimelius, PTH and chromogranin A) for each nodule are shown in Table 2 for comparison. In general the Grimelius and chromogranin A stains showed the highest similarity, with similarity rate of 60.4% in the solid type and 71.1% in the acinar-tubular type; in fact, in the group of oxyphil cells with an acinar-tubular pattern the stains were completely identical. As for the stain positivity, however, the Grimelius and PTH stains showed the highest similarity, having a rate of 49.3% in the solid type and 60.0% in the acinar-tubular type. Next came the chromogranin A and PTH stain (44.8% in the solid type and 44.4% in the acinar-tubular type),

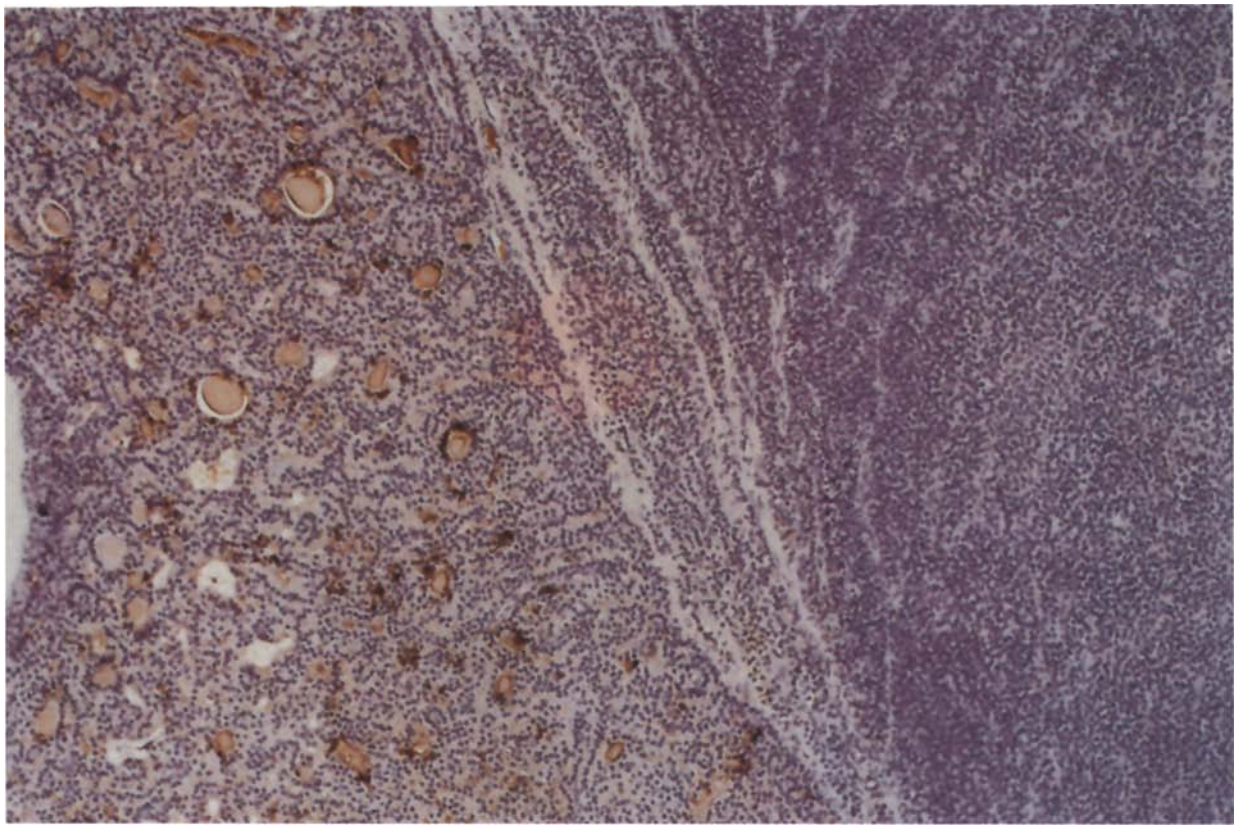


Fig. 3. Immunohistochemical stain of PTH. The left nodule has much immunoreactive PTH, the right nodule only a little (× 50)

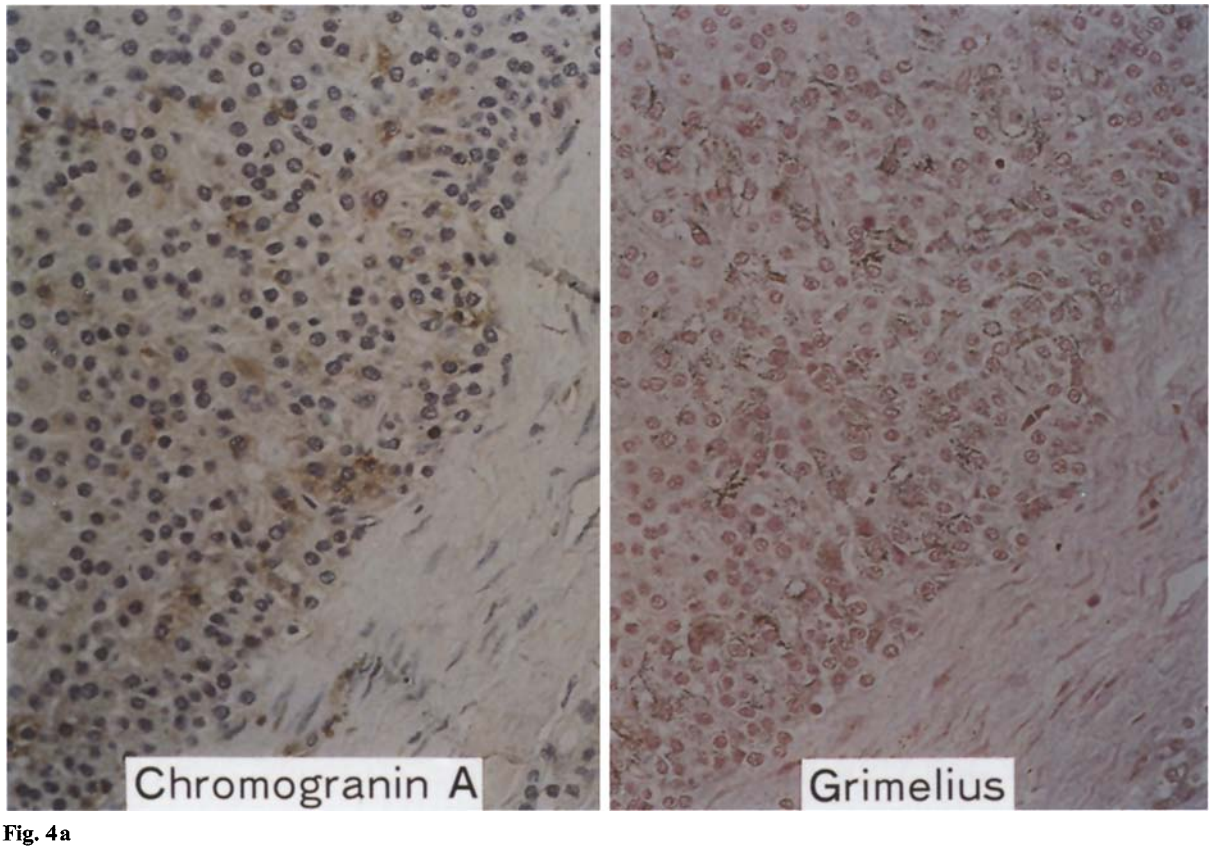
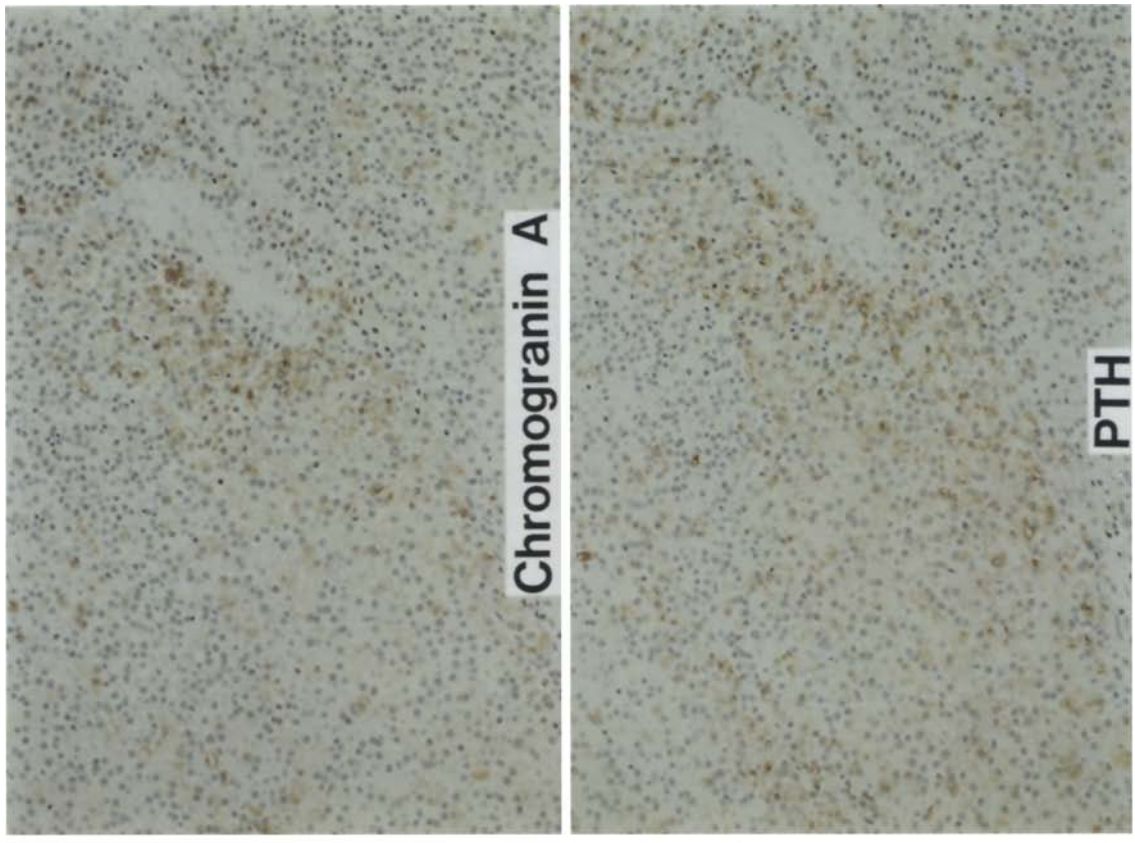
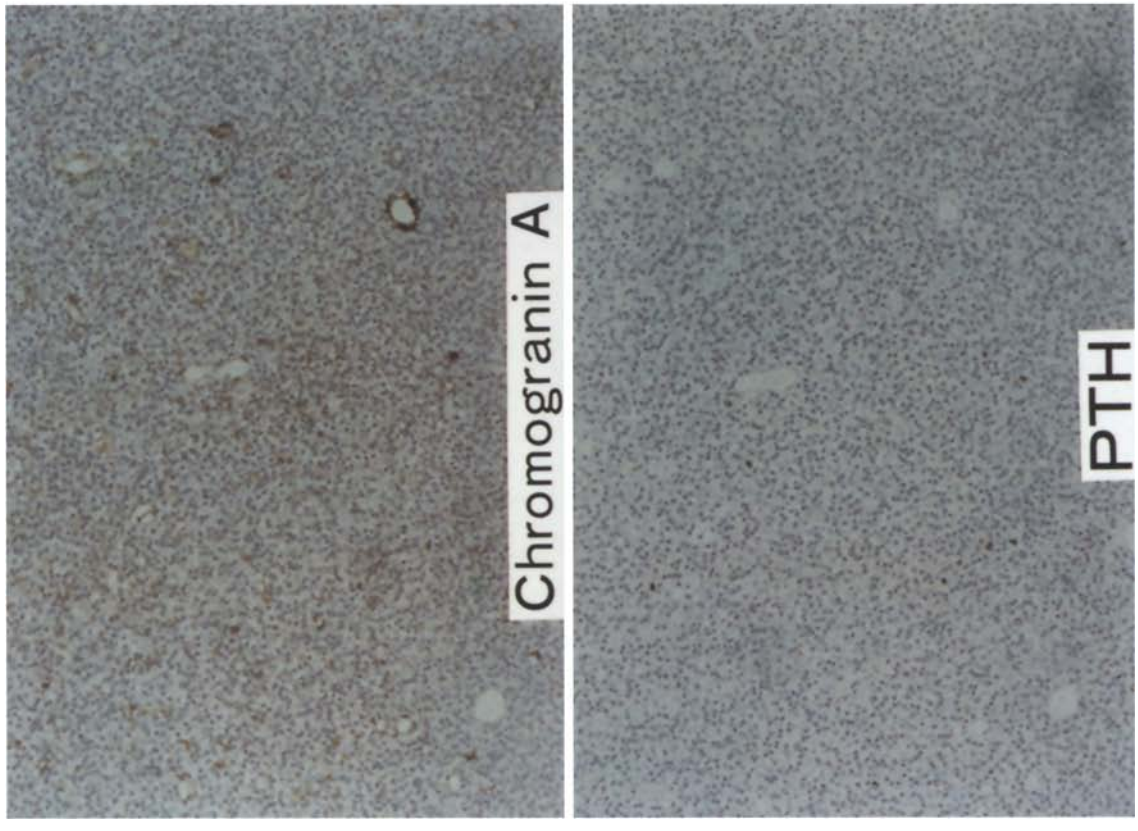


Fig. 4a



4b



4c

Fig. 4. **a** and **b**, show that each nodule reacts the same way to two stainings, while **c**, shows that this nodule has a completely different reaction of PTH and chromogranin A (**a**: ×248, **b**: ×168 and **c**: ×50)

Table 2. Similarity of intensity of stain for the three stainings

Combination of Staining		Chief cell (%)		Transitional (%)		Oxyphil cell (%)		Overall (%)	
		Solid	A–T	Solid	A–T	Solid	A–T	Solid	A–T
Grimelius	pos.	31.3	41.2	46.2	47.6	33.3	42.9	34.3	44.4
|	total	58.3	64.7	57.7	66.7	83.3	100	60.4	71.1
Chromogranin A	neg.	27.1	23.5	11.5	19.0	50.0	57.1	26.1	26.7
Grimelius	pos.	49.0	52.9	53.8	76.2	41.7	28.6	49.3	60.0
|	total	58.3	52.9	53.8	76.2	50.0	42.9	56.7	62.2
PTH	neg.	9.4	0	0	0	8.3	14.3	7.5	2.2
Chromogranin A	pos.	39.6	41.2	73.1	52.4	25.0	28.6	44.8	44.4
|	total	54.2	52.9	80.8	52.4	33.3	42.9	57.5	51.1
PTH	neg.	14.6	11.8	7.7	0	8.3	14.3	12.7	6.7
Grimelius									
|	pos.	28.1	35.3	46.2	47.6	25.0	28.6	31.3	40.0
Chromogranin A	total	35.4	35.3	46.2	47.6	33.3	42.9	37.3	42.2
|	neg.	7.3	0	0	0	8.3	14.3	6.0	2.2
PTH									
Total No. of nodules		96	17	26	21	12	7	134	45

pos.: positive, neg.: negative, A–T: Acinar-Tubular

followed by the Grimelius and chromogranin A one (34.3% in the solid type and 44.4% in the acinar-tubular type). In particular, the group of transitional oxyphil cell type with acinar-tubular pattern of cells to the Grimelius and PTH stain (76.2%) and the group of the transitional oxyphil cell type with solid pattern of cells to the chromogranin A and PTH stain (73.1%) prove to have the most similar positivity. Figure 4a and b show that the respective reactions to Grimelius and chromogranin A stains, and those to chromogranin A and PTH match almost perfectly. On the other hand, some nodules shown in Fig. 4c reacted in completely different manner to the three stainings.

The PTH control slide was totally negative.

Discussion

There are many pathogenic factors of hyperparathyroidism to be considered in renal failure. It is generally assumed that the depression of serum ionized calcium concentration in renal failure, resulting from phosphate retention due to the decrease of glomerular filtration rate (GFR), produces reciprocal lowering of serum ionized calcium. There is also decreased conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D (the active form vitamin D promoting gastrointestinal absorption of dietary calcium). These factors will stimulate the increased secretion of PTH in the parathyroid glands (Castleman and Mallory 1937; Nilsson 1984). Overproduction of PTH in the parathyroid glands stimulates bone resorption, which may bring about the complications such as ROD and ectopic calcification in the walls of large vessels and soft tissues (Dalinka and Melchior 1980; Heaf et al. 1983). In order to prevent these complications, drugs such as alminium hydroxide (almigel®), calcium agents and active vitamin D are indicated for patients with chronic renal failure (Nilsson 1984). However, despite such therapy, many haemodialysed patients still suffer from severe complications associated with 2HPT. The only available treatment for these patients is neck surgery. We have performed subtotal parathyroidectomy for about one hundred haemodialysed patients since 1979 and good results have been obtained. Although histopathological diagnosis of resected parathyroid glands is usually classified as hyperplasia with conventional H&E staining, this does not indicate how and where PTH is stored and secreted in the hyperplastic parathyroid glands.

Only a few papers dealing with this problem have been so far published (Draper and Nissenson 1982; Dietel and Holzel 1983; Arps 1987). Castleman and Roth (1978) have shown that the cycle of each chief cell, undergoing a cyclic process of PTH synthesis and secretion, is independent of that of its neighbour and that in the normal parathy-

roid gland only 20–30% of the chief cells are in the active phases of the cycle at any time. However, our results demonstrate that the major part of each nodule is composed of a single cell type with a single pattern of cells and reacts almost consistently to individual stainings with Grimelius, PTH and chromogranin A. It appears that the storage and secretion of secretory materials, such as PTH, in nodular hyperplastic parathyroid glands might be regulated not at the cell level but at the nodule level. As a consequence, the formation of each nodule in nodular hyperplastic parathyroid glands may come from monoclonal proliferation.

Chromogranin A is a major soluble protein, accounting for about 40 percent of the soluble proteins in the catecholamine storage vesicles of the adrenal medullas and sympathetic nerves and has been a useful index of exocytosis during sympathoadrenal neurosecretion (O'Connor et al. 1983; Lloyd et al. 1984). O'Connor et al. (1984) described that human chromogranin A is a 68000 dalton monomeric protein with an unusual amino acid composition (31.53% of its weight is glutamic acid), having an acidic, microheterogeneous isoelectric point (4.57–4.68), a characteristic tryptic digest peptide map and marked dissimilarity from dopamine hydroxylase. Recently, immunoreactive chromogranin A has also been found in neuroendocrine cells outside the adrenal medullas and sympathetic nerves (O'Connor et al. 1983). Our present study demonstrates that a majority of nodules contain immunoreactive chromogranin A positivity cells and provides the evidence of immunoreactive chromogranin A positivity in human parathyroid glands. However, it is known that chromogranin A is chemically, physically and immunologically very similar to secretory protein-I (SP-I), which is known to be one of the major secretory proteins in the parathyroid glands (Cohn and Elting 1983) and named by Morrissey and Cohn (1978). Cohn and Elting (1983) showed that SP-I is composed of two main cellular and secreted species of about 64000 and 72000 daltons in pig and 70000 and 72000 daltons in bovine parathyroid and that this protein coexists with PTH in the same secretory granules. They also demonstrated that anti-chromogranin A antibody cross-reacts with SP-I. Unfortunately, however, we failed to identify PTH and chromogranin A (or SP-I) positivity in this light microscopic immunohistochemical study, although Arps et al. (1987) demonstrated that PTH and SP-I were co-localized in the same secretory granules of the bovine parathyroid cell, using the colloidal gold-double immunocytochemical technique at the ultrastructural level. Our immunohistochemical study on the human parathyroid glands will be made in future under electron microscopy.

Wang (1985) indicated that in hyperparathyroidism the number of lipid droplets within the chief cells of the gland was noticeably diminished, often to the extent of virtual absence. We also examined several of the 37 glands with Oil-red O stain, but no intracellular lipid could be found. However, Rodoriguez et al. (1983) have indicated that the numbers of intracellular secretory granules do not necessarily reflect the synthesis and secretory activity of hormones. Dietel and Holzel (1983) suggested that the ionized calcium sensitivity of parathyroid adenoma cells might be changed in vitro and parathyroid adenomas may contain lower amounts of PTH than normal glands, corresponding to a lower storage capacity with higher rates of hormone release. Furthermore, 4 sections of the specimen are a tenuous basis for a discussion of these activities in the whole parathyroid gland.

We believe that conservative therapy is best for the patients with 2 HPT, but as the formation of each nodule in nodular hyperplastic parathyroid glands may be the consequence of the monoclonal proliferation of a single parathyroid cell, immunological examination using monoclonal antibodies, such as MAB (4F2) identified by Posillico et al. (1987) may be valuable.

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