Virchows Archiv A Pathol Anat (1989) 414:331–337

Virchows Archiv A
Pathological Anatomy and Histopathology

Different responses between the upper and the lower parathyroid gland in a state of secondary hyperfunction

A study on chronic renal failure by morphometry and nuclear DNA analysis

Hiroshi Matsushita

Department of Pathology, Toranomon Hospital, Okinaka Memorial Institute for Medical Research, Toranomon 2-2-2, Minato-ku, Tokyo 105, Japan

Summary. The size of the parathyroid gland and the size, the numerical density and nuclear DNA-content of the parathyroid gland cells were evaluated in chronic renal failure (CRF) and revealed a difference between the upper and the lower glands in the manner of adaptation to a state of long-term hyperfunction, secondary to CRF. The parathyroid gland enlarged as a whole in CRF, an effect more marked in the lower gland, whereas individual parathyroid gland cell enlargement in CRF was mainly seen in the upper gland cells. The numerical density of the lower parathyroid gland cells was higher than that of the upper gland. Nuclear DNA-content of the parathyroid gland cells were increased in CRF and the lower gland tended to show hyperdiploid aneuploidy. These findings are probably related to the fact that parathyroid adenomas occur most often in the lower gland. The higher proliferative activity of the lower parathyroid gland in long-term hyperfunction may explain the higher risk for the lower gland in the occurrence of adenomas.

Key words: Parathyroid gland – Chronic renal failure – Hyperplasia – Morphometry – DNA content

Introduction

Serum parathyroid hormone (PTH) levels increase in chronic renal failure (CRF) to maintain normal plasma calcium levels (Feinfeld and Sherwood 1988). It is well known that the parathyroid glands enlarge in CRF (Pappenheimer and Willens 1935; Gilmour and Martin 1937; Wiliams 1974; Mori 1978; Grimelius et al. 1981; Matsushita et al. 1984). The degree of the enlargement of the parathyroid gland correlates well with serum PTH levels in CRF (Malmaeus et al. 1984), and the enlargement of the parathyroid gland in CRF is thought to be an adaptation to the state of long-term hyperfunction of the parathyroid glands (Wiliams 1974; Kay 1976).

It is also known that there is a difference in size between the upper and the lower parathyroid glands. The lower parathyroid gland is larger than the upper gland, not only in the normal state (Pappenheimer and Willens 1935; Gilmour and Martin 1937; Grimelius et al. 1981; Matsushita et al. 1984) but also in CRF (Pappenheimer and Willens 1935; Wiliams 1974; Matsushita et al. 1984). When the functional level of the parathyroid gland is suppressed however, the lower parathyroid gland shows a decrease in size, in contrast to the upper gland (Matsushita et al. 1984). It is therefore speculated that there is a difference between the upper and the lower parathyroid glands in their role in regulating the serum calcium level (Matsushita et al. 1984).

The parathyroid glands in CRF were examined in this study in an attempt to demonstrate the difference in cytological changes between the upper and the lower parathyroid glands in a state of long-term hyperfunction secondary to CRF.

Materials and methods

Materials

Parathyroid glands were obtained from autopsy cases. Autopsy was performed within 4 h after death in every case. The parathyroid glands were fixed in 10% formalin at autopsy.

The control group consisted of 8 cases (12 upper parathyroid glands and 12 lower parathyroid glands) with normal renal function and normal serum calcium level (Table 1). The value for serum calcium level was corrected after the calculation by Krane and Potts (1980) using values of serum albumin (mg/dl) and serum globulin (mg/dl) to exclude protein-bound calcium, as shown below;

Table 1. Clinical and laboratory informations

Case	Location of parathyroid glands[a]	Serum calcium concentration[b] (mEq/l)	Serum creatinie concentration[c] (mg/l)	Duration of CRF (years)	Causes of CRF[d]
Control					
1	RU, LL	2.57	1.0		
2	LU, RU*, LL*, RL*	2.75	1.6		
3	RU, RL	2.80	0.9		
4	LU*	2.40	0.8		
5	LU*, RU, LL, RL	2.77	1.1		
6	LU, RU, LL, RL	2.66	0.9		
7	LU, RU, LL, RL	2.65	0.9		
8	LU, LL, RL	2.60	1.1		
CRF					
9	LU*, RU, RL*	2.50	5.3	2.0	DN
10	LU*, RU, LL	2.77	3.8	0.5	DN
11	RU, RL	2.80	5.6	1.5	AN
12	LU, LL	2.80	13.2	10.0	CGN
13	LU*, RU*, LL*, RL	2.70	3.2	2.5	CGN
14	LU, RU, LL, RL	2.50	4.9	3.0	DN
15	LU*, RU*, LL*, RL*	2.50	9.2	3.0	AN
16	LL, RL	2.50	11.5	4.5	DN

[a] LU, left upper; RU, right upper; LL, left lower; RL, right lower. Parathyroid glands with asterisks were examined by the nuclear DNA analysis

[b] Serum calcium concentrations are corrected by the values of serum albumin and serum globulin [normal range: 2.3–2.8 mEq/l] (Krane and Potts 1980)

[c] Serum creatinine levels are represented by the mean value during the last three months before death

[d] DN, diabetic nephrosclerosis; AN, arteriosclerotic nephrosclerosis; CGN, chronic glomerulonephritis

$$\text{corrected calcium concentration} = \frac{\{100-[\text{albumin}\times 8+\text{globulin}\times 2+3]\}\times \text{serum calcium (mEq/l)}}{100}$$

[normal range: 2.3–2.8 mEq/l].

The mean age of this group was 66.0 years.

The study group with CRF consisted of 8 cases (12 upper parathyroid glands and 12 lower parathyroid glands). These cases showed a continuous increase in serum creatinine level, higher than 3.0 mg/l, for more than 6 months before death (Table 1). The mean age of this group was 64.8 years. There was no case which showed hypercalcemia in CRF.

Methods

For morphometry, the parathyroid glands were embedded in paraffin, cut at 3 μm thick and stained with haematoxylin and eosin.

The size of the parathyroid gland was estimated by the value of the parenchymal area of the central plane of the gland including both the hilum and the longest axis as described previously (Matsushita et al. 1984).

Areas of 250 profiles of parathyroid cell nuclei in total were measured on two different sections per parathyroid gland, because the mean nuclear profile diameter and its standard deviation of the parathyroid gland had been demonstrated to become constant when 250 nuclei were measured (Banerjee et al. 1983). The system was composed of a microscope (Olympus BH, manification ×1000 in oil immersion) equipped with a drawing tube (Olympus BH-DA-LB, magnification ×5) and the apparatus for morphometry (Kontron, MOP/AMO 3). The mean nuclear area thus obtained is different from the true equator area of the nucleus because of the loss of small profiles (Weibel 1979b). This defect was corrected by the graphical method of Giger and Riedwyl (1970) and then the mean nuclear profile area (an) was determined. The mean nuclear profile diameter (dn) was calculated on each parathyroid gland as follows;

$$dn = 2\times\sqrt{(an/\pi)}.$$

The mean nuclear diameter (Dn) was then calculated by correcting the effect of section thickness (t) by the following equation (Weibel 1979b);

$$Dn = 4/\pi \times dn - t/2.$$

The nuclear volume (Vn) was then obtained by

$$Vn = \pi/6 \times Dn^3.$$

The cytoplasmic volume (Vc) was calculated by the value of the nuclear volume (Vn) and the ratio of the volume density of the nucleus to the cytoplasm which was determined by the point counting method using an eye-piece graticule, 10 mm square, divided into 100 squares. At the magnification ×40, one small square of the graticule was 25 μm across. Point counting was performed on four areas in each parathyroid gland. The proportion of multi-nucleated parathyroid gland cells in each parathyroid gland was first estimated. There was no multi-nucleated parathyroid gland cell in 36 parathyroid glands. There were 12 parathyroid glands with multi-nucleated parathyroid gland cells, and the highest rate of the multi-nucleated

Table 2. Morphometrical results

	Parenchymal area (mm^2)		Nuclear volume (μ^3)		Cytoplasmic volume (μ^3)		Numerical density ($\times 10^6/mm^3$)	
	upper	lower	upper	lower	upper	lower	upper	lower
Control	3.1 ± 1.3	4.0 ± 0.9	49.0 ± 13.0	48.5 ± 11.1	276.0 ± 124.8	196.8 ± 72.8	1.44 ± 0.26	1.83 ± 0.29
							$P<0.01$	
CRF	5.8 ± 2.7	12.8 ± 5.4**	65.8 ± 11.6**	65.9 ± 20.1*	528.2 ± 254.2**	328.7 ± 104.2	1.11 ± 0.18**	1.43 ± 0.25**
	$P<0.01$				$P<0.01$		$P<0.01$	

Values are mean $\pm$ SD

* Significantly different from control group, $P<0.05$
** Significantly different from control group, $P<0.01$

cell in a parathyroid gland was 2.0%. Since the rate of the multi-nucleated cell was very low in the parathyroid gland in this study, the mean cytoplasmic volume of the parathyroid gland cell (Vc) could be calculated by the following equation on each parathyroid gland (Hecker and Burri 1979);

$$Vc = Vn \times (Pn/Pc),$$

where Pn was the fraction of the test points falling on the nucleus of the parathyroid gland cell and Pc was those on the cytoplasm of the parathyroid gland cell.

Numerical density of the parathyroid gland cell was represented by the numerical density of the nucleus of the parathyroid gland cell in a unit volume, because multi-nucleated parathyroid gland cells were seldom found in the parathyroid glands in this study. The number of the nuclei in a unit area was counted with a microscope at 400× magnification. The size of the test area used was 100×100 (μ^2) at this magnification and four areas were tested in each parathyroid gland. The numerical density (Nv) of the nucleus was obtained by

$$Nv = Na/(Dn + t),$$

where Na was observed profile number per unit section area (1 mm^2), Dn was the nuclear diameter and t was the section thickness (Weibel 1979a).

Nuclear DNA-content was measured on 5 parathyroid glands from the control and on 10 parathyroid glands from CRF by DNA flow cytometry. According to the method of Hedley et al. (1983), single cell suspensions of the parathyroid gland for flow cytometry were prepared from formalin-fixed and paraffin-embedded autopsy materials. It has been demonstrated that the nuclear DNA-content is not altered in the immediate postmortem period (Raber et al. 1984). The sections (50 μm thick) were deparaffinized and suspended in 0.5% pepsin (Sigma, p-6887), in 0.9% NaCl, adjusted to PH 1.5 with 2 N HCl, and incubated at 37° C for 1 h. Supernatants of the free cell suspensions were washed twice in physiological saline solution. After treatment with 0.1% ribonuclease (Sigma, Type Ia, R-5503) in phosphate buffered saline, for 30 min at 40° C, the free cell suspensions were washed twice in 1.0% sodium citrate at 4° C. The nuclei of the parathyroid gland cell were then stained with a solution of 2.5 μg/ml propidium iodide (Sigma, P-5264) in 1.0% sodium citrate, containing 0.2% Nonidet P-40 (Nonidet, Kebo AB), for 30 min at 4° C, adapting the method of Krishan (1975). Nuclear DNA-contents were measured on 5000–10000 cells from each gland using the flow cytometer (EPICS C, Coulter) with an excitation of 488 nm by the argon ion laser. DNA ploidy was quantified by the DNA index (Barlogie et al. 1983) which represented the relative DNA content of the parathyroid stem cell peak (G0/1) to the reference peak of control lymphocytes obtained from the normal lymphnode fixed in formalin and embedded in paraffin.

Results are given as means $\pm$ SD. Statistical comparisons of morphometrical data were made by two way analysis of variance (ANOVA) for repeated measures as a fixed-effects model. When F tests indicated $P<0.05$, the differences between and within groups were examined with Duncan's multiple range test (Duncan 1955). Significance was judged by a P value of <0.05.

Results

A strong interaction ($P<0.005$) was found in the size of the parathyroid gland by two way ANOVA for repeated measures. The form of the interaction was interpreted to show a multiplicative effect (Table 2). The parenchymal area of the lower parathyroid gland was significantly larger than that of the upper gland ($P<0.001$) and the parenchymal area of the parathyroid gland in CRF was significantly larger than that of the parathyroid gland in controls ($P<0.001$) using the F-test. The parenchymal area of the lower parathyroid gland in CRF was significantly enlarged compared both with that of the lower gland in the control ($P<0.01$) and with that of the upper gland in CRF ($P<0.01$) by Duncan's multiple range test. The parenchymal area of the upper parathyroid gland in CRF tended to be larger than that of the upper gland in the control ($P<0.1$). However, there was no significant difference in the parenchymal area between the left and the right parathyroid gland on F-test.

There was no significant interaction in the nuclear volume on two way ANOVA for repeated measures. The nuclear volume of both the upper and the lower parathyroid glands in CRF were significantly larger than those of the upper and the lower parathyroid glands in the control ($P<$

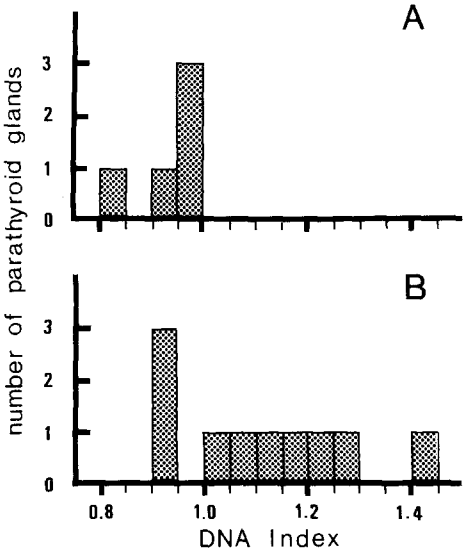

Fig. 1. Frequency distribution of ploidy abnormality by the values of DNA Index. (**A**) The parathyroid glands in the control group; (**B**) The parathyroid glands in CRF group. Note there are hyperdiploid abnormalities in CRF group

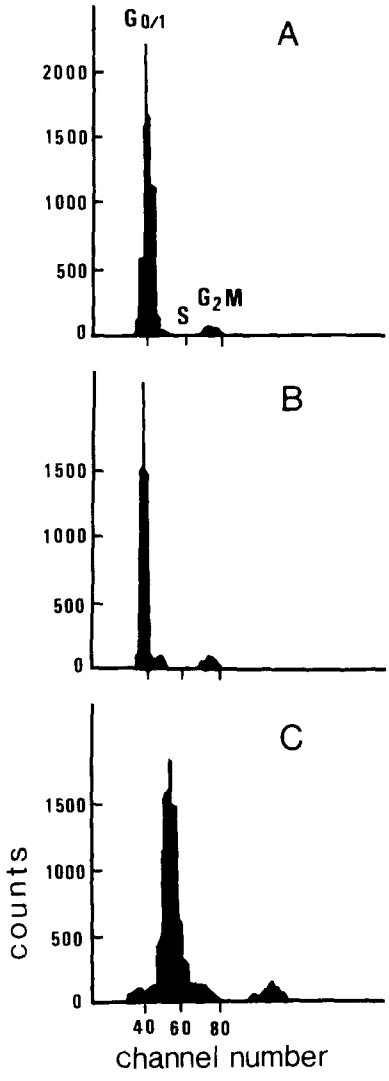

Fig. 2. DNA histograms obtained by flow cytometry. (**A**) DNA distribution for control lymphocytes. (**B**) DNA distribution for the parathyroid gland in the control group. (**C**) DNA distribution for the enlarged lower parathyroid gland in CRF with hyperdiploid aneuploidy. DNA index of the stem cell line in this gland is increased (DI = 1.42). DNA Index represents the ratio of channel numbers for the examined parathyroid glands versus normal lymphocytes G0/1 population

0.01 for the upper gland and $P<0.05$ for the lower gland) by Duncan's multiple range test (Table 2). There was no difference in the nuclear volume between the upper and the lower parathyroid gland both in the control and in CRF. There was no significant difference between the left and the right parathyroid gland cell on *F*-test.

There was no significant interaction in the cytoplasmic volume on two way ANOVA for repeated measures. The cytoplasmic volume of the upper parathyroid gland cell in CRF was significantly enlarged compared with that of the upper parathyroid gland cell in the control ($P<0.01$) and with that of the lower parathyroid gland cell in CRF ($P<0.01$) by Duncan's multiple range test (Table 2). The cytoplasmic volume of the lower parathyroid gland cell in CRF tended to be larger than that of the lower parathyroid gland cell in the control ($P<0.1$). No significant difference was found in the cytoplasmic volume between the upper and the lower parathyroid gland cell in the control on Duncan's multiple range test. There was no significant difference in the cytoplasmic volume between the left and the right parathyroid gland cell on *F*-test.

There was no significant interaction in the numerical density on two way ANOVA for repeated measures. The numerical density in the lower parathyroid gland was significantly higher than that in the upper parathyroid gland both in the control and in CRF ($P<0.01$, respectively) by Duncan's multiple range test (Table 2). The numerical densities in both the upper and the lower parathyroid gland in the control were significantly higher than those in the upper and the lower parathyroid gland in CRF ($P<0.01$, respectively) by Duncan's multiple range test. There was no significant difference in the numerical density between the left and the right parathyroid gland on *F*-test.

DNA ploidy quantitated by DNA index (DI) was diploid or near diploid in the control ($DI = 0.92 \pm 0.07$), whereas the value of DI in CRF was increased ($DI = 1.10 \pm 0.17$) (Fig. 1). There was no parathyroid gland with DI value above 1.0 in the

control, whereas DI values in 7 out of 10 parathyroid glands in CRF were higher than 1.0 (Fig. 1). There were 3 lower parathyroid glands in CRF with DI values higher than 1.2 and one lower parathyroid gland showed an ploidy abnormality to near-triploidy (DI = 1.42) (Fig. 2). On the other hand, DI values of the upper parathyroid glands in CRF were lower than 1.2.

Discussion

Both the nucleus and the cytoplasm of the parathyroid gland cell enlarged in CRF. The nuclear size of the upper parathyroid gland cell was almost equal to that of the lower parathyroid gland cell both in the control group and in CRF group, whereas the cytoplasmic size of the upper parathyroid gland cell was larger than that of the lower parathyroid gland cell in both groups. Consequently, it is evident that both the upper and the lower parathyroid gland cells as a whole enlarged in CRF with size increase most marked in the upper parathyroid gland cell. On the other hand, both the upper and the lower parathyroid gland enlarged as a whole in CRF, with the lower gland increasing in size by a greater amount.

The results on the numerical density of the parathyroid gland cell also support the interpretation of a greater increase in all numbers of the cell in the lower gland in CRF. The cell density in the lower parathyroid gland cell was higher than that of the upper parathyroid gland cell, not only in the controls but also in CRF. Since the lower parathyroid gland is larger than the upper gland in CRF, it is evident that the lower gland is composed of larger number of parenchymal cells than the upper in CRF. The numerical densities of both the upper and the lower parathyroid gland in CRF were lower than those of the upper and the lower parathyroid gland in the controls probably because of cell enlargement. There were some discrepancies between the cell volume as calculated from the nuclear and the cytoplasmic volume and the cell volume as deduced from the numerical density. It seems to be the effect of correction by section thickness that causes this discrepancy, because the ratio of the section thickness to the nuclear diameter was relatively high.

Thus, it is concluded that the parathyroid gland cells enlarge their volume and increase their number in reaction to chronic renal failure. In this reaction, the upper parathyroid gland exceeds the lower gland in the degree of the enlargement of the size of the parenchymal cells, whereas the lower parathyroid gland exceeds the upper gland in the degree of the increase in the number of the parenchymal cells. As the result of this reaction, in addition, the lower parathyroid gland becomes significantly larger than the upper gland in CRF.

In studies on regulation of PTH secretion by the dispersed parathyroid gland cells in vitro, it has been demonstrated that there was frequently an increase in the set-point for calcium (the calcium concentration causing half-maximal inhibition of PTH secretion) in parathyroid hyperplasia due to chronic renal failure without a change in the maximal secretory rate per cell (Brown et al. 1982; Cantley et al. 1985). PTH hypersecretion in secondary parathyroid hyperplasia is thought to result from a change in the set-point for calcium as well as from an increase in the total number of cells (Brown et al. 1982). It is therefore suspected that the lower parathyroid gland plays a more important role than the upper in increasing PTH secretion rate in CRF, because the lower parathyroid gland exceeds the upper gland in the degree of the increase in the number of the parenchymal cells. Nevertheless, it may be possible that the upper parathyroid gland exceeds the lower gland in the secretory ability of PTH at the level of the individual cell, because the individual parathyroid gland cell of the upper gland is larger than that of the lower gland in CRF. However, no supporting finding has been reported on this possibility in studies on the regulation of PTH secretion of parathyroid gland cells (Brown et al. 1982).

There have been a few studies on the analysis of nuclear DNA-content of the parathyroid gland cell (Bengtsson et al. 1977; Bowlby et al. 1987) in which no hyperplastic parathyroid gland in chronic renal failure showed abnormal DNA ploidy. However, hyperdiploid aneuploidy was found in enlarged parathyroid glands in CRF in the present study. It is well known that ploidy abnormalities are frequently associated with neoplasms, especially with malignant tumors (Barlogie et al. 1983). Ploidy abnormalities have been described in either benign or premalignant neoplastic lesions in various organs including the parathyroid glands (Bengtsson 1977; Irvin and Bagwell 1979; Anniko et al. 1984; Doseva et al. 1984; Bondeson et al. 1986; Bowlby et al. 1987). Several studies report an occurrence of adenoma against the background of hyperplasia of the parathyroid gland in CRF (Chodack et al. 1962; Nichols and Roth 1963; Golden et al. 1965; Kay 1976; Berland et al. 1982) or secondary parathyroid hyperplasia with autonomous hyperfunction (McPhaul et al. 1964). It is also speculated that chronic intracellular calcium depletion may be a common mechanism which sti-

mulates the growth of parathyroid cells both in secondary parathyroid hyperplasia and in parathyroid adenoma (Dietel et al. 1987). Some authers suggest that a number of cases of primary hyperparathyroidism may be a consequence of long-term stimulation of the parathyroid gland (Reiss and Canterbury 1971). It is therefore supposed that there may be an occurrence of possible neoplastic cell line in enlarged parathyroid glands in CRF which showed DNA ploidy abnormality in this study. The occurrence of neoplastic cell lines against the background of secondary parathyroid hyperplasia seems to be one of main causes of tertiary hyperparathyroidism.

It should be emphasized that the nuclear DNA-content of the lower parathyroid gland tended to be higher than that of the upper gland in CRF and that hyperdiploid aneuploidy was found in the lower parathyroid glands in CRF. This result seems to correspond well with the fact that parathyroid adenomas occur more frequently in the lower parathyroid glands than in the upper glands (Norris 1947; Kay 1976). According to Norris (1947), 83.8% of parathyroid adenomas occur in the lower parathyroid glands. This fact is not surprising in view of the response of the lower parathyroid gland in increasing the number of parenchymal cells in long-term hyperfunction. One of the important factors in experimental carcinogenesis is an increase in the number of cells or the rate of DNA and RNA synthesis in exposed tissues (Diamond et al. 1980). Thus, the higher proliferative activity of the lower parathyroid gland seems to explain the higher risk of the lower parathyroid gland for the development of adenomas.

Acknowledgements. The author is grateful to Dr. M. Hara (Department of Pathology, Toranomon Hospital), Dr. Y. Endo (Department of Immunology, Toranomon Hospital) and Dr. I. Kai (Department of Public Health, Teikyo University, School of Medicine) for supports in accomplishing the study.

References

Anniko M, Tribulait B, Wersäll J (1984) DNA ploidy and cell phase in human pituitary tumors. Cancer 53:1708–1713

Banerjee SS, Faragher B, Hasleton PS (1983) Nuclear diameter in parathyroid disease. J Clin Pathol 36:143–148

Barlogie B, Raber MN, Schumann J, Johnson TS, Drewinko B, Swartzendruber DE, Göhnde W, Andreeff M, Freireich EJ (1983) Flow cytometry in clinical cancer research. Cancer Res 43:3982–3997

Bengtsson A, Grimelius L, Johansson H, Pontén J (1977) Nuclear DNA-content of parathyroid cells in adenomas, hyperplastic and normal glands. Acta Pathol Microbiol Scand (A) 85:455–460

Berland Y, Olmer M, Lebreuil G, Grisoli J (1982) Parathyroid carcinoma, adenoma and hyperplasia in a case of chronic renal insufficiency on dialysis. Clin Nephrol 18:154–158

Bondeson L, Azavedo E, Bondeson AG, Caspersson T, Ljungberg O (1986) Nuclear DNA content and behavior of oxyphil thyroid tumors. Cancer 58:672–675

Bowlby LS, DeBault LE, Abraham SR (1987) Flow cytometric DNA analysis of parathyroid glands. Relationship between nuclear DNA and pathologic classifications. Am J Pathol 128:338–344

Brown EM, Wilson RE, Eastman RC, Pallotta J, Marynick SP (1982) Abnormal regulation of parathyroid hormone release by calcium in secondary hyperparathyroidism due to chronic renal failure. J Clin Endocrinol Metab 54:172–179

Cantley LK, Ontjes DA, Cooper CW, Thomas CG, Leight GS, Wells SA (1985) Parathyroid hormone secretion from dispersed human hyperparathyroid cells: Increased secretion in cells from hyperplastic glands versus adenomas. J Clin Endocrinol Metab 60:1032–1037

Chodack P, Derman H, Kantrowitz AR (1962) Primary hyperparathyroidism and coexistent parathyroid hyperplasia secondary to independent renal leision. NY State J Med 15:3615–3627

Diamond L, O'Breien TG, Baird WM (1980) Tumor promoters and the mechanism of tumor promotion. Adv Cancer Res 32:1–74

Dietel M, Niendorf A, Schafer HJ, Hölzel F (1987) Abnormal calcium distribution in human parathyroid adenomas as possible cause of primary hyperparathyroidism. Horm Metabol Res 19:177–181

Doseva D, Christov K, Kristeva K (1984) DNA content in reactive hyperplasia, precancerosis and carcinomas of the oral cavity, a cytophotometric study. Acta Histochem 75:113–119

Duncan AJ (1955) Multiple range and multiple *F*-tests. Biometrics 11:1–42

Feinfeld DA, Sherwood LM (1988) Parathyroid hormone and $1,25(OH_2)D_3$ in chronic renal failure. Kidney Int 33:1049–1058

Giger H, Riedwyl H (1970) Bestimmung der Größenverteilung von Kugeln aus Schnittkreisradien. Biometr Zeitschr 12:156–165

Gilmour JR, Martin WJ (1937) The weight of the parathyroid glands. J Pathol Bacteriol 44:431–462

Golden A, Canary JJ, Kerwin DM (1965) Concurrence of hyperplasia and neoplasia of the parathyroid glands. Am J Med 38:562–578

Grimelius L, Åkerström G, Johansson H, Bergström R (1981) Anatomy and histopathology of human parathyroid glands. Pathol Annu 16:1–24

Hecker H, Burri PH (1979) Sampling procedure and morphometric evaluation. In: Weibel ER (ed) Stereological Methods Vol. 1. Academic Press Inc. London, pp 297–301

Hedley DW, Friedlander ML, Taylor IW, Rugg CA, Musgrove EA (1983) Method for analysis of cellular DNA content of paraffin-embedded pathological material using flow cytometry. J Histochem Cytochem 31:1333–1335

Irvin GL, Bagwell CG (1979) Identification of histologically undetectable parathyroid hyperplasia by flow cytometry. Am J Surg 138:567–571

Kay S (1976) The abnormal parathyroid. Hum Pathol 7:127–138

Krane SM, Potts Jr JT (1980) Disorders of bone and bone mineral metabolism. In: Isselbacher et al. (eds) Principles of Internal Medicine (9th ed). McGraw-Hill Kogakusha, Tokyo, p 1826

Krishan A (1975) Rapid cytofluorometric analysis of mammalian cell cycle by propidium iodide staining. J Cell Biol 66:188–193

Malmaeus J, Grimelius L, Johansson H, Åkerström G, Ljunghall S (1984) Parathyroid pathology in hyperparathyroidism secondary to chronic renal failure. Scand J Urol Nephrol 18:157–166

Matsushita H, Hara M, Shishiba Y, Nakazawa H (1984) An evaluation of the size of the parathyroid glands. Endocrinol Jpn 31:127–131

McPhaul JJ, McIntosh DA, Hammond WS, Park OK (1964) Autonomous secondary (renal) parathyroid hyperplasia. N Engl J Med 271:1342–1345

Mori S (1978) Parenchymal weight of the parathyroid gland and the kidney in chronic glomerulonephritis. Tohoku J exp Med 125:85–91

Nichols G, Roth SI (1963) Chronic renal disease and hypercalcemia. N Engl J Med 268:943–953

Norris EH (1947) The parathyroid adenoma, a study of 322 cases. Int Abstr Surg 84:1–41

Pappenheimer AM, Willens SL (1935) Enlargement of the parathyroid glands in renal disease. Am J Pathol 11:83–91

Raber MN, Barlogie B, Luna M (1984) Flow-cytometric analysis of DNA content in postmortem tissue. Cancer 53:1705–1707

Reiss E, Canterbury JM (1971) Genesis of hyperparathyroidism. Am J Med 50:679–685

Weibel ER (1979a) Numerical density of particles. In: Weibel ER (ed) Stereological methods, vol. 1. Academic Press Inc, London, pp 40–48

Weibel ER (1979b) Particle size and shape. In: Weibel ER (ed) Stereological methods, vol. 1. Academic Press Inc, London pp 162–203

Wiliams ED (1974) Pathology of the parathyroid glands. Clin Endocrinol Metabol 3:285–303

Received September 26, 1988/Accepted December 16, 1988