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Serum Calcium and Phosphate Concentrations and Parathyroid Morphology in Rats Treated with Vitamin D Metabolites

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Summary. The serum concentrations of calcium and phosphate and parathyroid morphology were studied in rats treated with vitamin D metabolites. Twenty hours after a single injection of 1.25-dihydroxycholecalciferol (1.25-DHCC) or 25-hydroxycholecalciferol (25-HCC) the serum calcium and phosphate concentrations were not significantly altered in any group, but the 1.25-DHCC treated rats exhibited an increased number of dark chief cells and occurrence of a few atrophic chief cells. Four to eight weeks after daily injections of the vitamin D metabolites the 1.25-DHCC treated rats exhibited significantly increased serum calcium concentrations and parathyroid glands composed of atrophic and dark chief cells in solid and follicular arrangement, whereas the rats treated with 25-HCC showed unaffected serum calcium concentrations and parathyroid glands composed of solid sheets of light chief cells, often with vacuolated cytoplasm, a few dark chief cells, but no atrophic cells. The findings suggest a direct or indirect suppressive influence of 1.25-DHCC on parathyroid activity in rats.

Key words: Parathyroids, morphology, vitamin D, rat, calcium, inorganic phosphate.

INTRODUCTION

In a preceding study (16) of patients with primary hyperparathyroidism, 1.25-dihydroxycholecalciferol (1.25-DHCC) treatment seemed, at least temporarily, to suppress the activity of the parathyroid glands. Animal studies have suggested a direct action of vitamin D metabolites on the parathyroid glands (9); 1.25-DHCC may stimulate (10) or inhibit (12) the release of parathormone (PTH), and is selectively accumulated by chick parathyroid glands (14), which possess cytoplasmic and nuclear receptors for this vitamin D metabolite (8). Moreover, the weight of the parathyroid glands has been reported to be reduced in chicken given high doses of 1.25-DHCC (15). The present study was undertaken with the aim to see whether short-term and long-term injection of vitamin D metabolites in adult rats has any structurally detectable effect upon the parathyroid glands and on the serum concentrations of calcium and phosphate.

MATERIAL AND METHODS

Animals and Treatment

The animals used were Sprague-Dawley rats (obtained from Anticimex Co., Stockholm, Sweden); 6 months old for the short term experiments and 11 1/2 to 12 1/2 months old for the long term experiments. Their initial body weights were 480 to 550 grams. The animals were divided randomly into 2 control groups (Groups 1a and b) and 8 experimental groups differing from each other with respect to kind and duration of treatment (Table 1). The rats in subgroups b were the same as used in a parallel study directed to skeletal changes after administration of vitamin D metabolites (17). 25-HCC and 1.25-DHCC were dissolved in 95% ethanol and injected intraperitoneally at the doses given in Table 1. The control animals received intraperitoneal injections of 25% ethanol alone.

All animals were given a normal laboratory ration (Ewos Co., Södertälje, Sweden) containing 1.0 per cent Ca and 0.75 per cent P with a Ca:P ratio of 1:0.75. The contents of vitamin D_3 were approximately 150 I.U. per 100 g diet. Diet and deionized water were given ad libitum. The animals were housed in large cages without access to direct sunlight. At the end of the experimental

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Table 1. Groups of rats differing from each other with respect to kind and duration of treatment

Group	n	Treatment		
		Substance	Number of injections	Duration
I a	8	100 µl 95% ethanol	One	20 hours[a]
I b	9	-"-	Daily[c]	8 weeks
II a	8	30 ng 1.25-DHCC	One	20 hours[a]
II b	9	-"-	Daily[c]	8 weeks
III a	8	120 ng 1.25-DHCC	One	20 hours[a]
III b	13	120 - 60 ng 1.25-DHCC	Daily[c]	4 weeks[b]
IV a	8	30 ng 25-HCC	One	20 hours[a]
IV b	9	-"-	Daily[c]	8 weeks
V a	8	120 ng 25-HCC	One	20 hours[a]
V b	9	-"-	Daily[c]	8 weeks

[a] Animals killed 20 hours after single injection

[b] 120 ng for 2 weeks followed by 60 ng for another 2 weeks

[c] Daily 5 days a week

periods the animals were sacrificed by exsanguination from the femoral artery under ether anaesthesia.

Laboratory Analyses

Determination of serum calcium was made on samples collected at sacrifice. Atomic absorption spectrophotometry was used after precipitation of proteins with 20 per cent trichloro-acetic acid and addition of $SrCl_2$ for depression of the phosphorus interference according to Willis (21). The absorption was read in a Unicam SP 90 spectrophotometer (Unicam Instr. Ltd., London, England). The coefficient of variation for these analyses was less than 1 per cent. Serum inorganic phosphate was determined with the aid of a Technicon autoanalyzer (Technicon Instr. Corp., Ardsley, N.Y., USA). The coefficient of variation for these analyses was less than 1.5 per cent. Student's test was used for the statistical treatment of data.

Light Microscopy

The thyroid lobes were removed immediately after death and were then fixed in 10 per cent formalin and stained with hematoxylin-eosin, van Gieson's stain and periodic acid-Schiff (P.A.S.).

Table 2. Serum concentrations of calcium and inorganic phosphate in the same group of rats as in Table 1

Group	Serum concentration (mean: nmol/l S.E.)	
	Calcium	Phosphate
I a	2.56 ± 0.04	2.36 ± 0.26
I b	2.48 ± 0.25	2.08 ± 0.21
II a	2.53 ± 0.24	2.63 ± 0.28
II b	2.89 ± 0.32	2.44 ± 0.39
III a	2.55 ± 0.20	2.54 ± 0.17
III b	3.67 ± 0.31	2.29 ± 0.16
IV a	2.47 ± 0.09	2.24 ± 0.05
IV b	2.52 ± 0.11	1.83 ± 0.10
V a	2.35 ± 0.16	2.15 ± 0.15
V b	2.53 ± 0.10	2.01 ± 0.10

RESULTS

Body Weight

Weight determinations were only carried out in the long-term experiments (subgroups b). The control animals (group 1b) showed a tendency to reduced body weight during the experimental period, but the difference was not significant. In group IIIb an average loss of body weight of 40 per cent was observed ($p < 0.001$), whereas group IIb

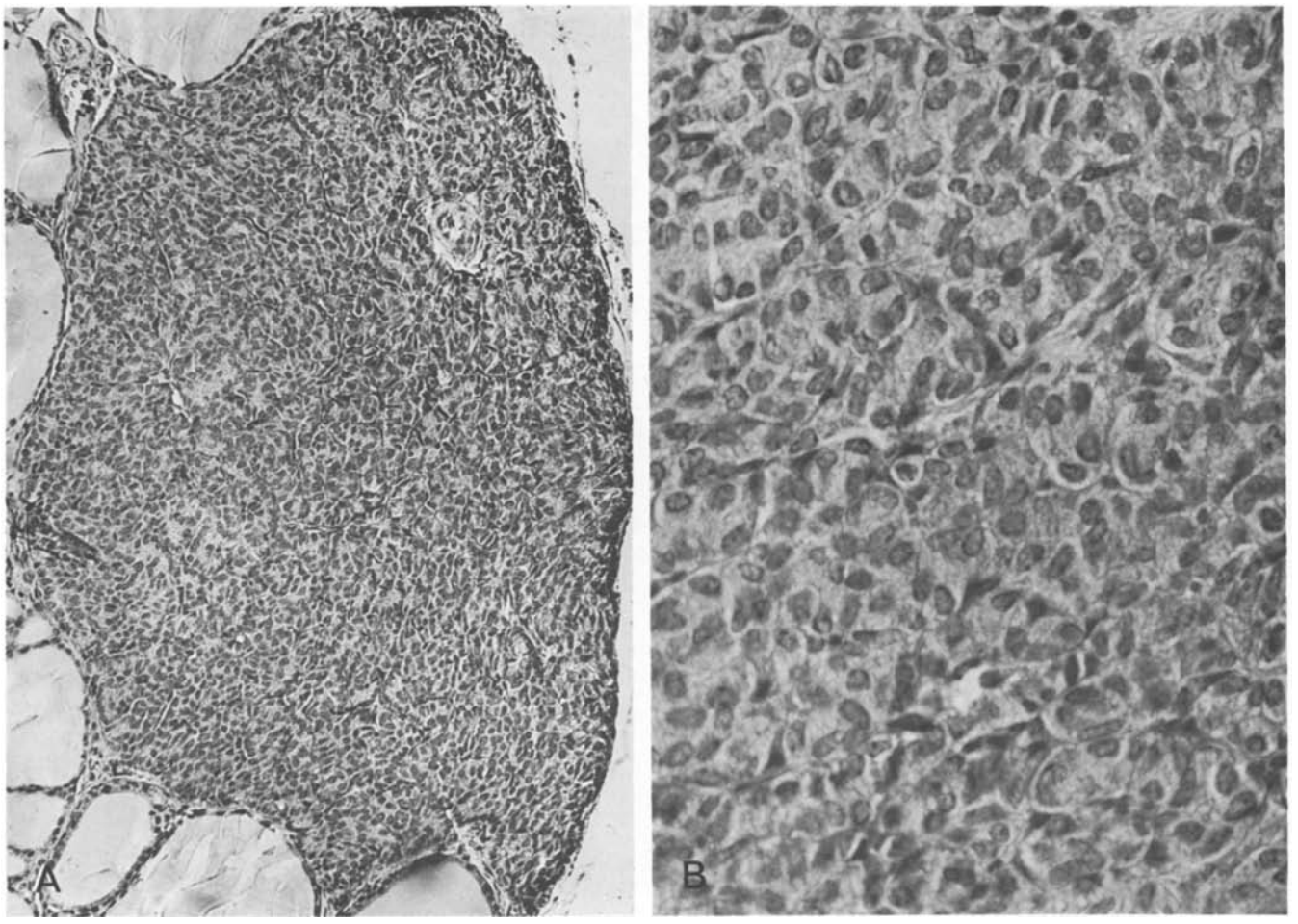

Fig. 1. A Parathyroid gland from control rat showing solid sheets of chief cells with a varying staining affinity of the cytoplasm. Hematoxylin-eosin x 60. B Higher magnification of parathyroid gland from control rat demonstrating solid sheets of chief cells exhibiting moderately stained cytoplasm. A few chief cells with light staining cytoplasm are also observed. The nuclei are medium-sized and possess a moderate chromatin density. Hematoxylin-eosin x 320

exhibited body weight reductions which averaged 21 per cent ($p < 0.001$). Groups IVb and Vb showed only a tendency to reduced body weight without significance at the end of the experimental period.

Serum Calcium and Inorganic Phosphate

In the short-term studies no significant alteration in the serum concentration of calcium or phosphate was observed in any of the groups (Table 2).

The serum calcium concentration was, however, significantly increased ($p < 0.001$) in the two groups of rats subjected to long-term treatment with 1.25-DHCC (groups IIb and IIIb), while no significant difference was seen in those given 25-HCC. The serum calcium level in group IIIb was significantly higher ($p < 0.001$) than that observed in group IIb.

The serum inorganic phosphate concentration was almost significantly higher ($0.01 < p < 0.05$) in group IIb than in group Ib. No other significant changes were found in phosphate concentration in the long-term studies.

Light Microscopy

The parathyroid glands in group Ia and Ib (controls) were composed of solid sheets of chief cells with a varying staining affinity of the cytoplasm and medium-sized, rather light staining nuclei (Figs. 1 A and B). No water-clear or oxyphil cells were encountered. Nor were any follicles seen. Mast cells were not observed.

The parathyroid glands from group IIa were composed of solid sheets of dark chief cells and an occasional atrophic cell. No water-clear or oxyphil cells were seen. Follicles were not found. Only a few mast cells could be identified.

In group IIb a prominent feature was the occurrence of follicles of varying size (Fig. 2 A). They were usually slightly irregular in outline, and

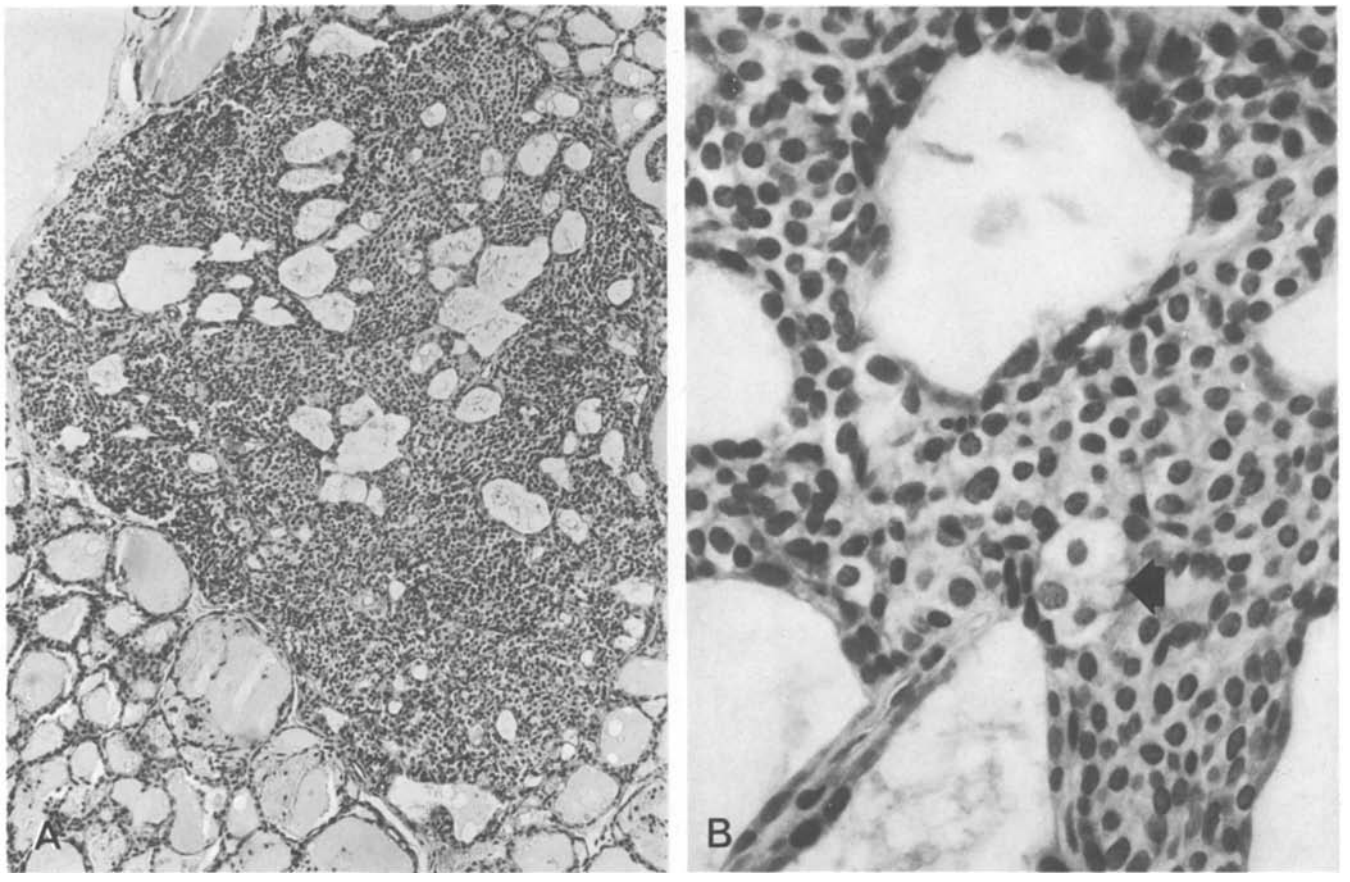

Fig. 2. A Parathyroid gland localized at periphery of thyroid lobe from rat treated with 30 ng 1.25-DHCC daily for 8 weeks. Numerous follicles of varying size are seen. Hematoxylin-eosin x 85. B Portion of parathyroid gland from rat treated in the same way as in Fig. 2 A showing slightly irregular follicles containing some flocculent and threadlike material of low density. Follicles are lined by flattened or cuboidal dark chief cells. Between the follicles there are strands of dark chief cells exhibiting varying size, shape and staining affinity of the nuclei. A small group of light chief cells are also observed (arrow). Hematoxylin-eosin x 320

were either empty or contained some flocculent or thread-like material (Fig. 2 B). Cellular debris was observed in a few follicle lumina. The follicles were lined by cuboidal, rounded or irregular chief cells possessing a moderately dark cytoplasm, and some atrophic chief cells. Other parts of the glands were composed of solid sheets of dark chief cells and atrophic chief cells. The nuclei of the atrophic cells were small, dark and occasionally pyknotic. Single light chief cells, or small groups of such cells were infrequently seen. There was no obvious acinar or trabecular arrangement. A rather rich number of mast cells was seen. Some transitional oxyphil cells and a few oxyphil cells were also observed.

Group IIIa exhibited glands composed of dark chief cells and a few atrophic cells in solid arrangement. In group IIIb similar findings were made as in group IIb. However, there were more atrophic cells, follicles, mast cells, transitional oxyphil cells, and oxyphil cells in group IIIb than in group IIb.

The parathyroid glands from group IVa exhibited solid sheets of dark, and some light chief cells. In group IVb the glands were composed of light chief cells, often with vacuolated cytoplasm, in acinar and trabecular arrangement (Fig. 3 A). The nuclei of these cells were large or medium-sized and exhibited moderate chromatin density. Some cells possessed peripherally localized nuclei which often were semilunar or irregular and rather dark-staining (Fig. 3 B). No follicles, oxyphil cells or mast cells were seen.

Group Va exhibited glands consisting of dark chief cells and a few light chief cells in solid arrangement. The parathyroid glands from group Vb showed similar, but somewhat more marked structural changes than observed in group IVb.

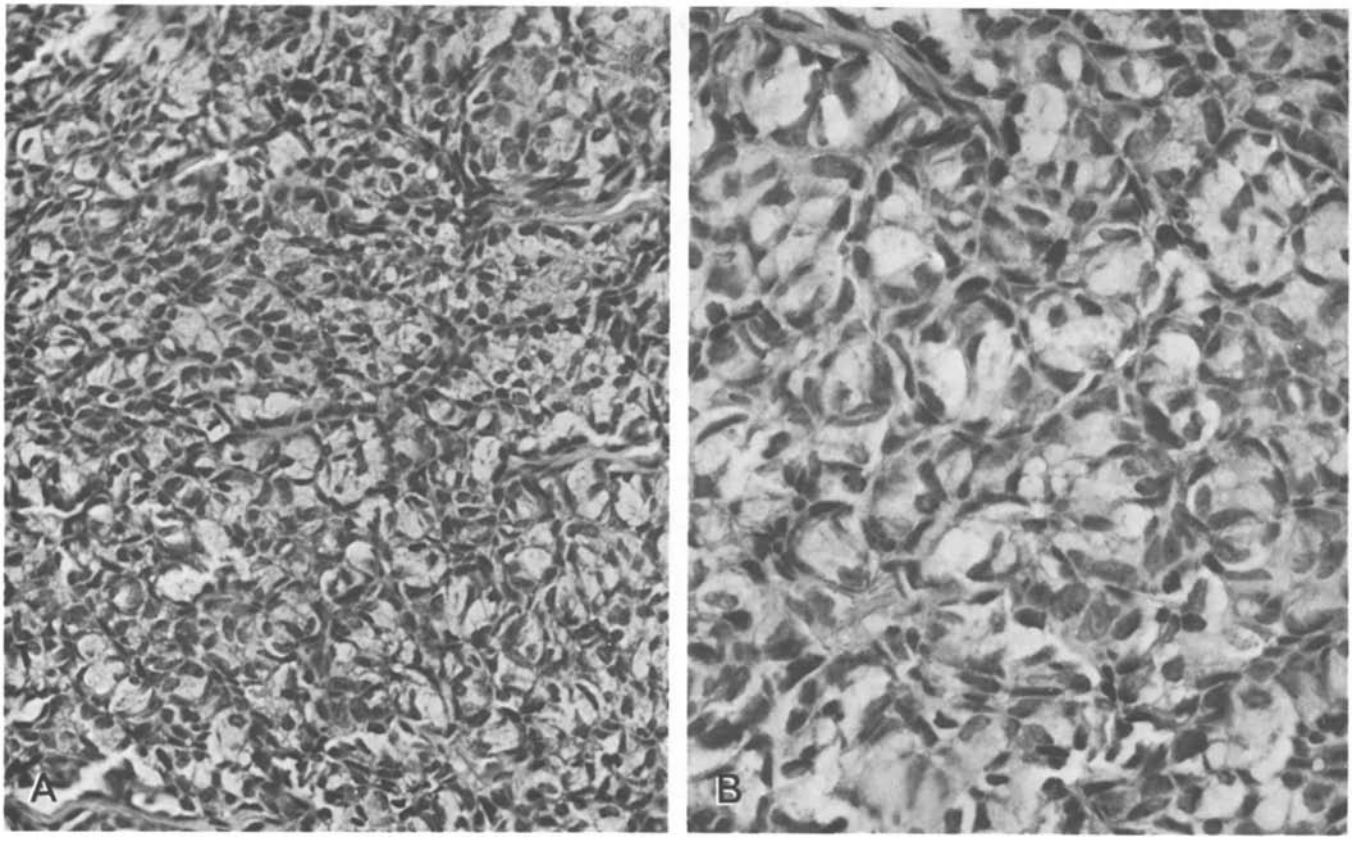

Fig. 3. A Portion of parathyroid gland from rat treated with 30 ng 25-HCC daily for 8 weeks demonstrating light chief cells in acinar and trabecular arrangement. Hematoxylin-eosin x 140. B Portion of parathyroid gland from rat treated in the same way as in Fig. 3 A showing groups of light chief cells possessing peripherally localized semilunar, oval or irregular nuclei. Vacuoles are seen in the cytoplasm of some of these cells. Hematoxylin-eosin x 320

DISCUSSION

The reduction in body weight in groups IIb and IIIb was probably due to dehydration (17). Unpublished morphological data have shown that this does not affect the structure of the parathyroid glands of rats. The hypercalcaemia observed in groups IIb and IIIb may be associated with inhibition of PTH secretion, directly or indirectly caused by the administered 1.25-DHCC. This would be consistent with the decreased serum iPTH concentration observed in patients with primary hyperparathyroidism (16) or hyperparathyroidism secondary to renal failure (11, 13, 20) treated with 1.25-DHCC or the synthetic analogue 1α -hydroxycholecalciferol (1α -HCC), and with the report that 1.25-DHCC inhibits PTH release in bovine parathyroid slices and decreases the serum PTH concentration in intact rats (12).

The structural findings of suppressed parathyroid activity in groups IIb and IIIb, according to criteria based upon our experience from experimental studies of parathyroid glands subjected to different calcium concentration (2, 3, 5, 6) lend further support to our opinion that 1.25-DHCC, either directly or by increasing serum calcium, inhibits parathyroid activity. Furthermore, the increased bone mass found in the parallel study (17) in the 1.25-DHCC treated rats may, at least in part, be due to reduced bone resorption, which in turn may be the result of suppressed parathyroid activity.

Since it is very well known that high ambient calcium concentration inhibits PTH secretion, the structural changes in groups IIb and IIIb, might be secondary to the hypercalcaemia recorded in these groups, which in turn may be the result of the administration of 1.25-DHCC. Another possibility is that 1.25-DHCC per se inhibits parathyroid activity, with or without concomitant hypercalcaemia. A direct effect of 1.25-DHCC upon the parathyroid glands has been suggested in patients with secondary hyperparathyroidism (7, 18), and in patients with osteomalacia both serum calcium and vitamin D metabolites have been ascribed an inhibitory influence on PTH release (19). Although the results of the present study indicate that 1.25-DHCC inhibits parathyroid function, the available data do not allow any interpretation of whether this effect is a direct one, or is mediated

via an increase in the serum calcium concentration caused by 1.25-DHCC.

25-HCC administration did not alter the serum concentrations of calcium or phosphate, but the parathyroid glands exhibited structural changes which according to our criteria suggest stimulation. Whether this is due to an effect on the parathyroid glands of 25-HHC or not, is, so far, unknown.

Numerous follicles were found in groups IIb and IIIb, but not at all in the other experimental groups or in the controls. This is consistent with the results of an experimental study of parathyroid follicles, suggesting that follicle formation is associated with functional suppression (4).

In a study specially directed to mast cells in parathyroid glands of human hyperparathyroidism, differences were observed between pathological and non-pathological tissue, although no conclusions could be drawn from this with regard to the significance of these cells (Andersson, 1974). The significance of the occurrence of mast cells in some of the groups in the present study is not known. However, these cells were most frequent in suppressed glands.

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