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Ultrastructural Study of Calcium-Containing Precipitation in Human Parathyroid Glands

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Summary. Using the potassium pyroantimonate technique for ultrastructural localization of cations and X-ray elemental analysis with both energy dispersive and wave-length dispersive systems, calcium-containing precipitates were found in normal, hyperplastic and adenomatous human parathyroid glands. Differences were observed between oxyphil cells, and suppressed, stimulated and active chief cells in the content and localization of intracellular precipitation. The oxyphil cells and suppressed chief cells possessed precipitates mainly in nuclei and medium-sized and large mitochondria, whereas the stimulated chief cells possessed precipitates in normal-appearing and morphologically altered mitochondria, and in smooth-surfaced vacuoles and cytosol. The active chief cells usually showed a rather sparse precipitation.

Key words: Electron microscopy — Calcium — Parathyroid glands — Chief cells — Oxyphil cells.

Introduction

There is still a limited knowledge, both as to man and other species, about the interrelationship between the different parathyroid parenchymal cell types and about the correlation between structure and function in these cell types. It is usually believed that the various parathyroid cell types are interchangeable and possess different functional activity (Altenähr, 1972; Black *et al.*, 1973; Boquist and Fåhraeus, 1975; Capen, 1971; Roth, 1971). However, opinions differ as to what really characterizes parathyroid cells with low or high endocrinological activity. Because of this, attempts were made in preceding experimental studies on Mongolian gerbils to differentiate structurally between parathyroid cells with a high and low functional activity; distinction was made between atrophic, suppressed, stimulated and active chief cells, differing from each other mainly in cytoplasmic density and content of organelles; a difference, believed to be of functional significance, was also found between these chief cell variants in the content and distribution of calcium-containing precipitates (Boquist and Fåhraeus, 1975; Boquist and Lundgren, 1975). The present study, using the potassium pyroantimonate technique and X-ray elemental analysis for ultrastructural localization and identification of cation precipitation, was undertaken with the aim to see whether calcium-containing precipitation is a feature also of human parathyroid cells and, if so, whether differences exist also between human para-

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thyroid parenchymal cell types as to the content and distribution of the precipitation. A similar study of human parathyroid glands has not previously been reported.

Material and Methods

Parathyroid glands were obtained for examination from 3 female and 5 male patients, aged 18 to 67 years, operated upon because of hyperparathyroidism; two glands were obtained from 4 patients, three glands were obtained from 2 patients, and three and a half glands were obtained from 2 patients. Immediately after removal, the parathyroid glands were fixed by immersion for 2 hours in 3 per cent glutaraldehyde and 2 per cent potassium pyroantimonate adjusted to pH 7.4 with 0.01 N acetic acid with postfixation for 1 hour in 1 per cent osmium tetroxide and 2 per cent potassium pyroantimonate (Gomba *et al.*, 1972; Schäfer, 1973; Schäfer and Klöppel, 1974). Embedding was carried out in Epon 812 and toluidine blue-stained thick sections were used for light microscopic diagnosis and identification of suitable areas for the thin sections. After staining with uranyl acetate and lead citrate the sections were viewed in a Siemens Elmiscope 101 electron microscope.

The specificity of the precipitation was investigated by X-ray analysis performed at the Technical High School, Luleå, Sweden, using unstained 2500 Å pyroantimonate-treated sections analyzed with two different methods: a) an energy-dispersive system (Kevex X-ray Energy Spectrometer Subsystem 5000 A) combined with a JEOL JSEM 200 Scanning-Transmission Electron Microscope, operated in the transmission microscope mode at 80 kV accelerating voltage with a counting time of 180 seconds at each point of analysis; an X-Y recorder (Hewlett-Packard) was used for plotting of the spectra; and b) a wave-length dispersive system (JEOL JXA-50 A) combined with a JEOL JSM 50 A Scanning Microscope, used at 10 to 35 kV accelerating voltage with a counting time of 180 seconds at each point of analysis.

Results

Light microscopic examination of the thick sections disclosed four patients with one adenoma; the other glands examined from everyone of these patients were normal. Diffuse hyperplasia was found in the remaining four patients. The light microscopic examinations also showed the presence both in the normal, hyperplastic and adenomatous glands of light and dark chief cells, and oxyphil cells in varying proportions. No obvious water-clear cells were encountered.

The *ultrastructural examinations* were mainly directed to the occurrence of electron dense precipitation in the different parathyroid parenchymal cell types, among which the chief cells ultrastructurally were differentiated into suppressed, stimulated and active variants, based upon the density of the cytoplasm and the content and appearance of organelles (Boquist and Fåhraeus, 1975; Boquist and Lundgren, 1975).

In all glands, electron dense precipitates were found at a frequency which varied in different patients and different glands. Some variations were also recorded in the content of electron dense precipitates between different cells of the same variant, localized to one and the same gland. Despite of these variations there were clear differences between the parenchymal cell types in the content and distribution of intracellular precipitation.

The oxyphil cells showed a rather rich precipitation in many, but not all, mitochondria and sometimes also in the nuclei. The mitochondrial deposits were situated in the inner compartment, occasionally in electron lucent areas, without any clear association to cristae or membranes (Fig. 1). These mitochondria were

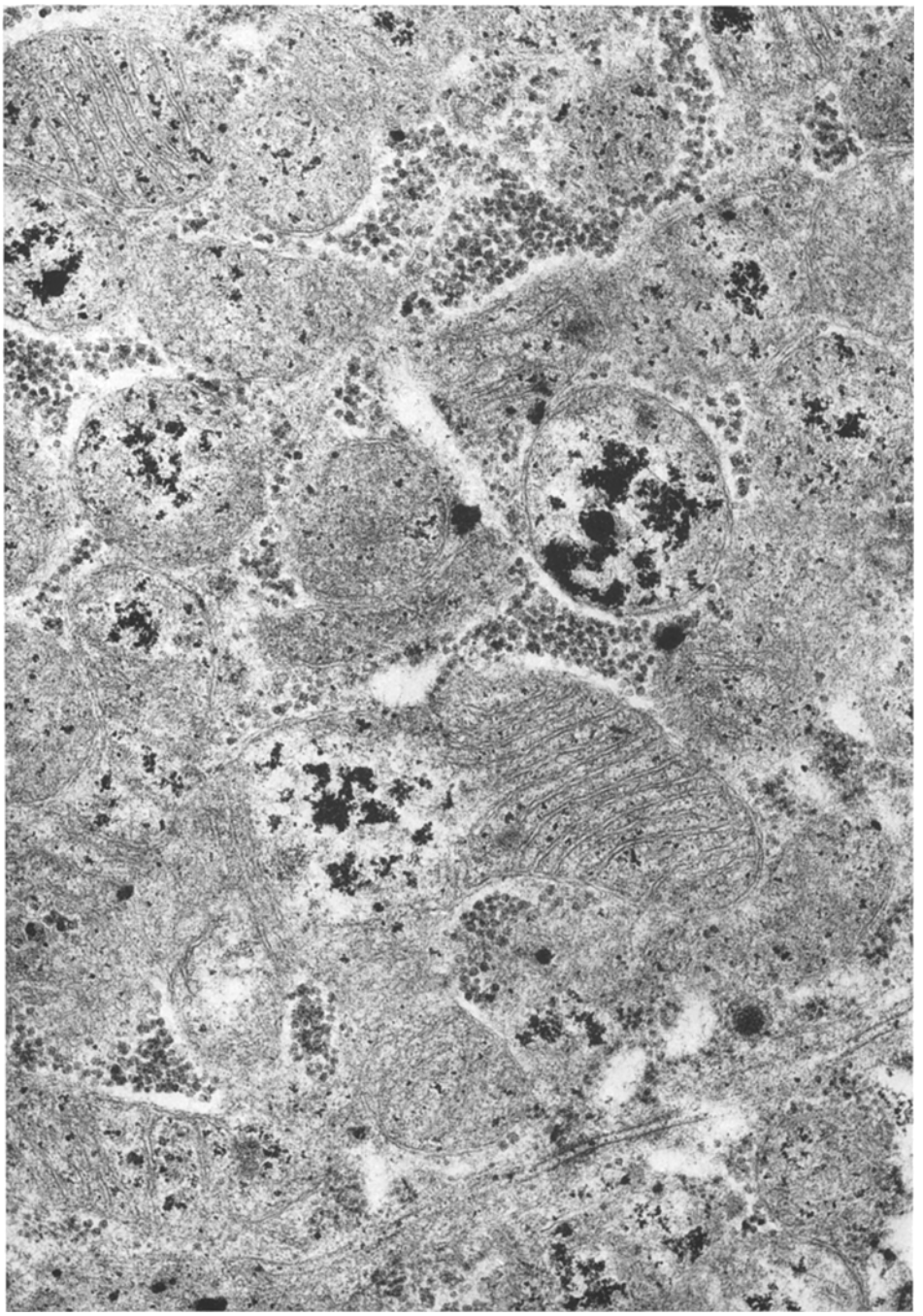
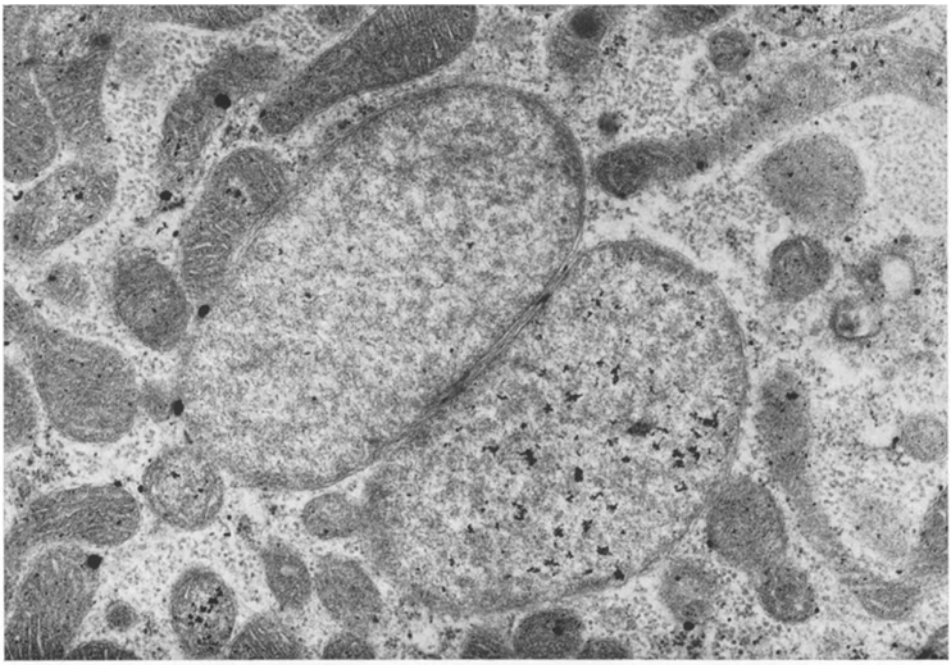


Fig. 1. Portion of oxyphil cell in human parathyroid adenoma showing numerous rather large mitochondria with electron dense precipitation in the inner compartment. Glycogen particles are seen outside the mitochondria. ×20000



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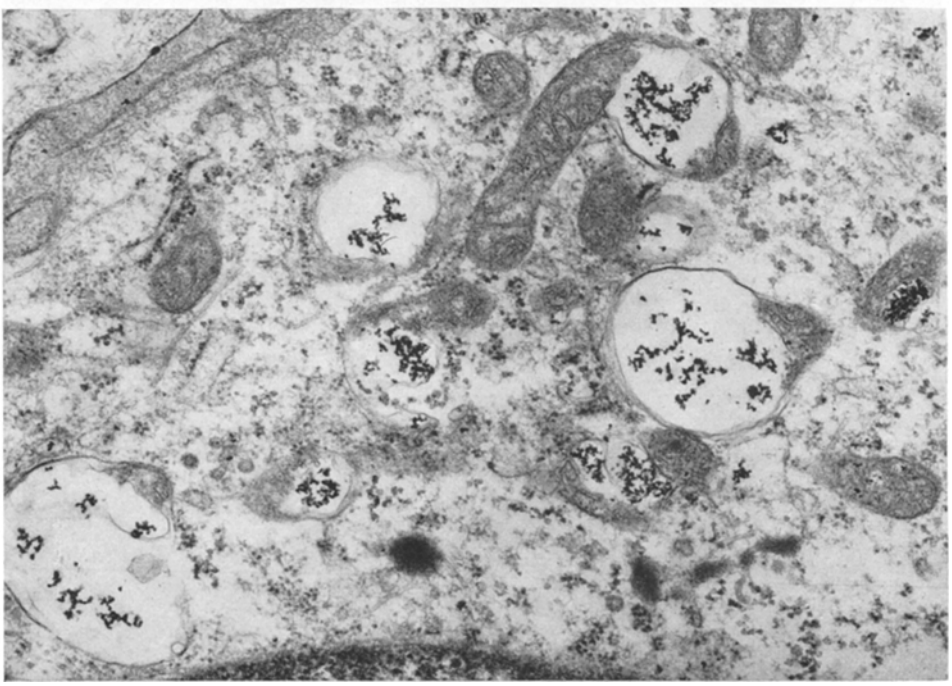

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Fig. 2. Portion of suppressed chief cell in hyperplastic parathyroid gland showing electron dense precipitation in the inner compartment of two mitochondria with vastly augmented matrix, and in other mitochondria with a normal appearance. A few precipitates are seen at the periphery of the mitochondria. ×16000

Fig. 3. Cytoplasm of stimulated chief cell in parathyroid adenoma showing electron dense precipitation in electron lucent smooth-surfaced vacuoles with a more or less evident association to mitochondria. ×18000

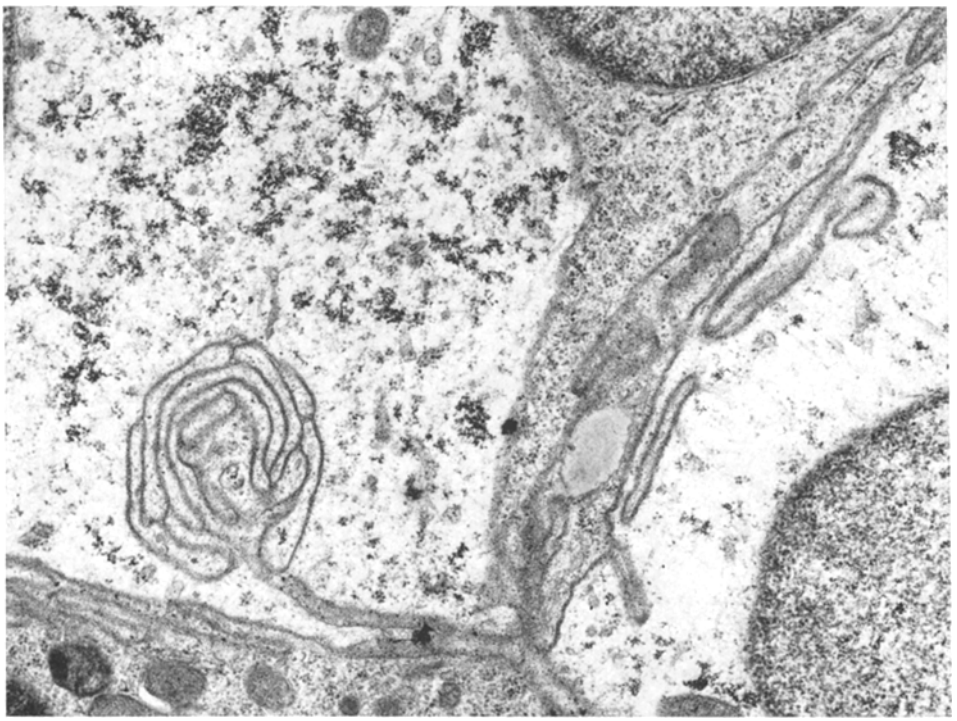


Fig. 4. Area of normal parathyroid gland showing portion of two active chief cells with a moderate cytoplasmic density and two stimulated chief cells with a low cytoplasmic density and few organelles. The latter cells possess electron dense precipitates, often in small groups, diffusely in the cytosol. Interdigitating membranes are also seen. ×12000

either medium-sized or enlarged. The nuclear precipitates were situated in the euchromatin. The suppressed chief cells also showed a rather rich precipitation in medium-sized or enlarged mitochondria (Fig. 2) and nuclei, and occasionally also in rough-surfaced cytoplasmic vacuoles.

The stimulated chief cells possessed a slight precipitation in normal-appearing mitochondria, and a more rich precipitation in electron lucent areas of the inner compartment of structurally altered mitochondria. Precipitates were also seen in smooth-surfaced vacuoles with (Fig. 3) or without a connection with mitochondria. A finely granular precipitation was also found diffusely in the cytosol (Fig. 4). Rather large smooth-surfaced vacuoles without connection with mitochondria were also seen peripherally close to the cell membranes both in the stimulated cells and in cells interpreted to be in a transition from the stimulated to the active stage (Fig. 5). A few precipitates could occasionally be observed in cisterns of rough endoplasmic reticulum, in Golgi cisterns, and in secretory granules.

The active chief cells showed only a sparse precipitation (Fig. 6). A precipitation of varying severity was also found intercellularly (Fig. 7) and pericapillary. No clear differences in precipitation were recorded between parenchymal cells from the normal, hyperplastic and adenomatous glands.

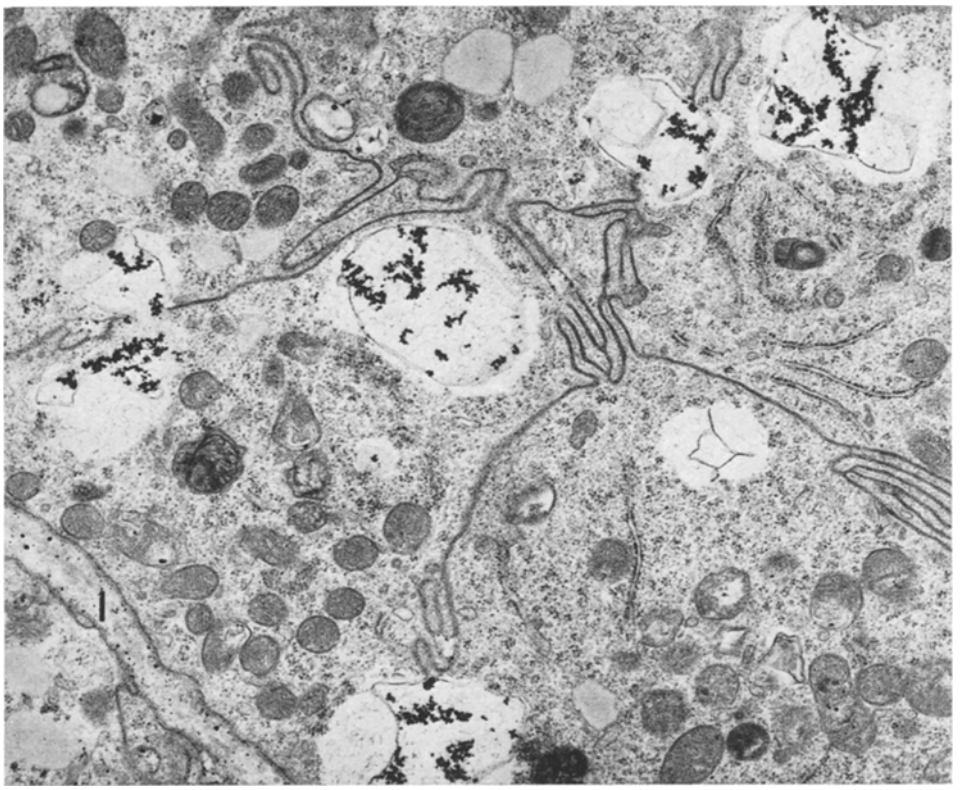
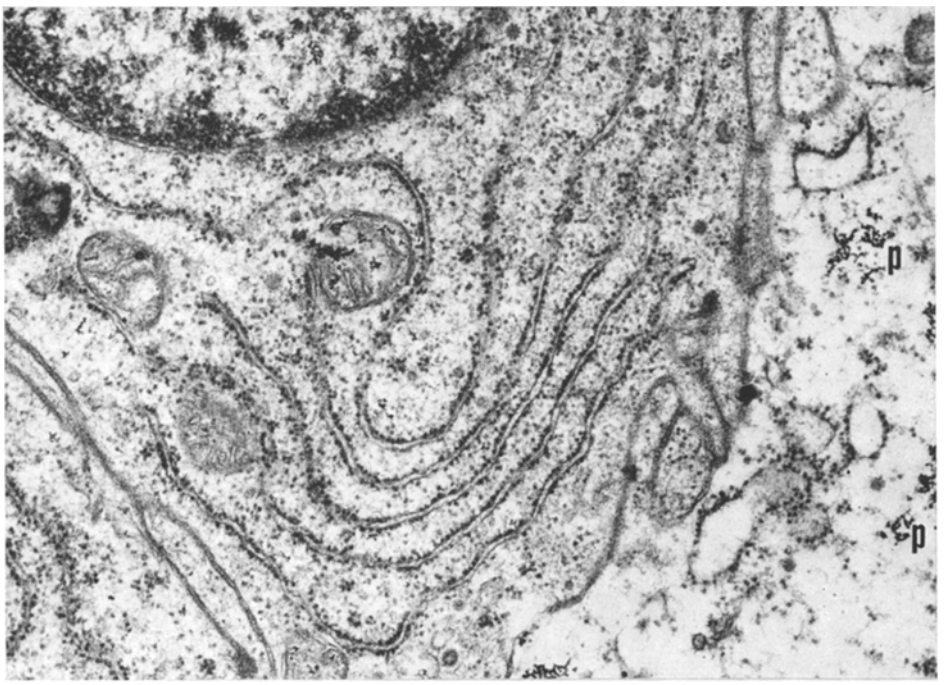


Fig. 5. Chief cells interpreted to be in a transition from the stimulated to the active stage in parathyroid adenoma showing a moderate cytoplasmic density, lamellae of rough-surfaced endoplasmic reticulum, rather small mitochondria, interdigitating membranes and rather large smooth-surfaced, electron lucent vacuoles with electron dense precipitation. These vacuoles are localized close to and in contact with the cell membranes. Precipitates are also seen in the interstitial area (I) and a few precipitates are observed in the mitochondria. ×12000

The *X-ray analysis* gave significant peaks over precipitates localized to nuclei, mitochondria, rough-surfaced and smooth-surfaced vacuoles, cytosol, as well as intercellular and pericapillary spaces. However, small single precipitates (<0.1 μ) could not be analyzed. Using the energy dispersive system, a peak was found for calcium and antimony (Fig. 8); the spectrum for calcium (Ca_{Ka}=3.69 KeV) could not clearly be differentiated from that of antimony (Sb_{La}=3.60 KeV; $Sb_{L\beta}$ =3.84 KeV). With the wavelength dispersive system it was possible to resolve the peaks for calcium and antimony, and significant peaks for calcium were obtained over precipitates with the localizations described above (Fig. 9). No significant peaks were obtained over the precipitates for other cations with any of the dispersive systems, apart from those associated with the techniques used for preparation and mounting of the specimens, *e.g.* copper (Cu) and rhodium (Rh) in the grids.



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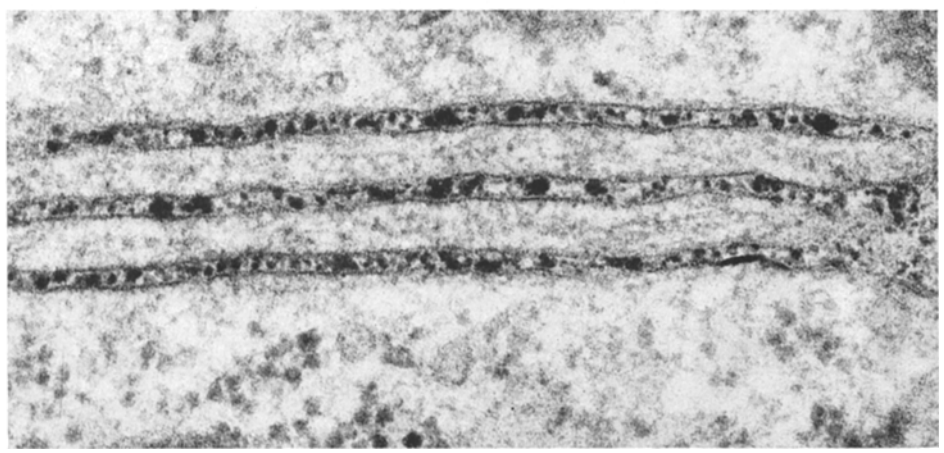

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Fig. 6. Portion of active chief cells (to the left) in hyperplastic parathyroid gland showing a moderate cytoplasmic density, prominent lamellar rough-surfaced endoplasmic reticulum, and sparse mitochondrial electron dense precipitation (arrow). Portion of a stimulated chief cell (to the right) with electron dense precipitation (*p*) in the cytosol is also seen. ×17000

Fig. 7. Intercellular area in hyperplastic parathyroid gland showing electron dense precipitation between interdigitating cell membranes. ×28000

Discussion

The findings show that calcium-containing precipitation occurs in human parathyroid parenchymal cells. In preceding experimental studies (Boquist and

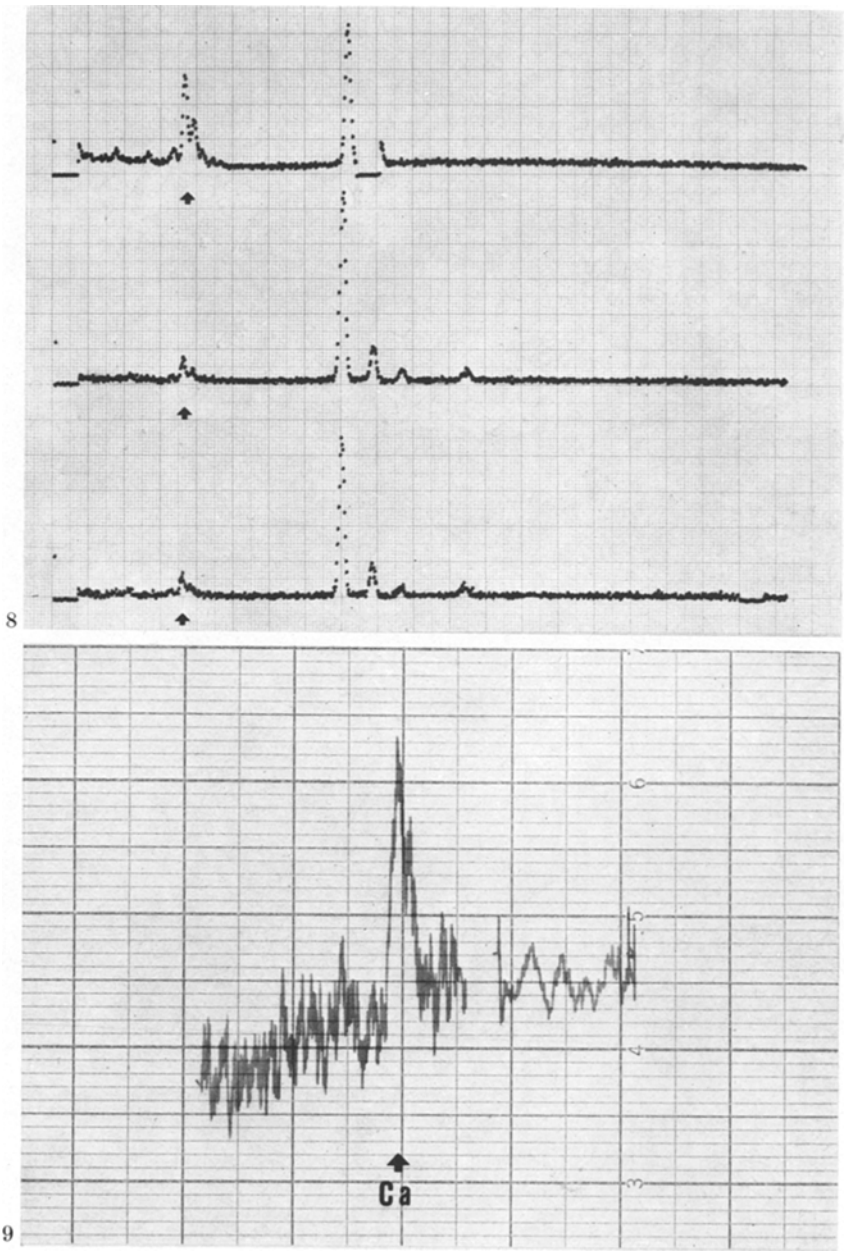

Fig. 8. Data plots of energy dispersive spectra of pyroantimonate precipitations in three different parathyroid chief cells showing peaks for calcium/antimony (arrows). To the right of this are shown the peaks for copper ($Cu_{K\alpha}$ and $Cu_{K\beta}$) from the grids used

Fig. 9. Wave-length dispersive spectrum of pyroantimonate precipitation in stimulated parathyroid chief cell showing peak for calcium

Fåhraeus, 1975; Boquist and Lundgren, 1975), it was tentatively suggested that the calcium-containing precipitation could be of functional significance since the amount and distribution of this precipitation was distinctly different in active, stimulated and suppressed chief cells. The present study does not allow any definite appraisal of the possible association between functional activity and calcium-containing precipitation in human parathyroid cells. However, since the distribution of the precipitation in the human parathyroid cells was similar to that recorded in gerbilline cells, the possibility remains that the calcium-containing precipitation in the human parathyroid cells is of functional significance. In another experimental study (Boquist, 1975), occurrence of oxyphil cells was recorded in parathyroid glands maintained at a high calcium concentration; it was suggested that oxyphil cells at least under certain conditions might develop in parathyroid glands subjected to a high calcium concentration and that the mitochondria of these cells had accumulated calcium. Since a rich mitochondrial calcium-containing precipitation was found in the oxyphil cells also in the present study, the suggested mode of oxyphil cell formation is worth of consideration also as to human parathyroid glands. Calcium is well known to play an important role in the function of endocrine cells. Thus, extracellular calcium is an absolute requirement for the induction of insulin release from the pancreatic B-cells. It is also known that the ambient calcium concentration regulates the secretion of parathyroid hormone. Further studies are needed in order to clarify the role of the calcium-containing precipitation in the human parathyroid cell types.

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