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Storage and degradation of secretory proteins in adenomatous and secondary hyperplastic parathyroid cells

An immunoelectron microscope study

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Summary. Parathyroid hormone (PTH) and chromogranin A/secretory protein-I (SP-I) have been localized on immunoelectron microscopy in double-fixed tissues from adenomatous and secondary hyperplastic parathyroid glands. Storage organelles, identified on the basis of their consistent labelling, included two morphologically distinct varieties of granules/vesicles; the mature granules and the progranules. The former consisted of dense, mostly rounded, medium to large-sized bodies which were strongly labelled and predominant in the proximity of the cell membrane. The other variety of body included a spectrum of small pale vesicles/granules which were mainly located in the Golgi area. Because their morphology and their labelling pattern varied other bodies were assumed to be engaged in degradation or cleavage of the secretory proteins. These bodies comprised crinophagic structures, that is to say multivesicular bodies and large Golgi-related vesicles, as well as a number of atypical solid bodies. Whereas most of the granulated cells stored a mature or a maturing population of vesicles/granules, the process of maturation appeared to be either absent or incomplete in a number of cells from some glands. The major defects were frequently associated with an unusual labelling pattern of the Golgi area and selectively affected groups of cells from all the transitional oxyphil cell adenomas. The minor defects concerned individual cells of different types present in both categories of glands. The present data suggest that in hyperfunctioning glands, the type of hormone processing depends on the capacity of each cell in progranule maturation and that the maturation capacity may decrease dramatically in adenomatous or chronically hyperstimulated cells of the transitional oxyphil type.

Key words: Parathyroid – Storage granule – Parathormone – Chromogranine A – Immunoelectron microscopy

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Introduction

Parathyroid cells from adenomatous or hyperplastic glands show morphological alterations when compared with the cells of normal parathyroid glands (Cinti et al. 1986; Rudberg et al. 1986). In the normal glands chief cells predominate, whereas pathological glands often contain an increased proportion of mitochondria-rich cells. The latter include transitional oxyphil cells (considered hormonally active) and fully developed (possibly inactive) oxyphil cells. In addition, chief cells from the abnormal glands are often present in the active phase of their secretory cycle, with an hypertrophic Golgi apparatus, abundant rough endoplasmic reticulum, scarce cytoplasmic lipids and frequent interdigitations of the plasma membrane. In contrast, hardly anything is known of the fine morphology of compartments concerned with storage and/or degradation of the secretory proteins. This is rather disappointing because hormone processing in these cells is probably regulated by different mechanisms, including a controlled degradation of the stores and the existence of two functionally distinct secretory pools (for review see Cohn and Elting 1983 and Habener et al. 1984). It has been shown that secretory protein-I (SP-I), a glycoprotein produced and secreted along with PTH, is very similar to chromogranin A, a protein with a widespread occurrence in endocrine peptide-secreting cells (Morrissey et al. 1980; Cohn et al. 1983, 1984;

Koneki et al. 1987). PTH and SP-I/chromogranin A have been localized in human and bovine parathyroid cells by means of post-embedding immunoelectron microscopy (Ravazzola et al. 1978; Futrell et al. 1979; Varndell et al. 1985; Arps et al. 1987) and appear to be costored within large granules. Because of glutaraldehyde-free and/or osmium-free fixation, fine subcellular structures could not be investigated in these studies. Although these proteins especially human PTH are unstable to osmification and unosmification the recent use of sensitive methods combined with adequate etching procedures has allowed the immunodetection of PTH (Setoguti et al. 1985; Inoue and Setoguti 1986; Shoumura et al. 1988) and chromogranin A (Hearn 1987) in more conventionally fixed tissues from animals (rat and rabbit) and man respectively.

These observations prompted us to investigate a number of adenomatous and secondary hyperplastic parathyroid glands by immunoelectron microscopy, using a polyclonal antiserum to PTH and a monoclonal antibody to chromogranin A/SP-I. We developed technical procedures suitable for the detection of each protein in well-preserved cells and now describe the morphology and distribution of the immunoreactive organelles engaged in the storage and/or degradation of the secretory proteins.

Material and methods

Rabbit anti-bovine PTH serum (cat. n° 512525) was obtained from Calbiochem. (La Jolla, USA). By radioimmunoassay, it showed the following cross-reactivities (data provided by the manufacturer): C-terminal PTH 100%; N-terminal PTH less than 0.1%.

Monoclonal mouse anti-human chromogranin A antibodies LK2H10 were available as purified IgG from Hybritech (San Diego, USA). 5 nm or 10 nm colloidal gold probes were obtained from Janssen (Beerse, Belgium).

Tissues investigated were obtained at operation from patients with hyperparathyroidism. They included 12 secondary hyperplastic glands (from patients with chronic renal failure) and 12 cases of solitary parathyroid enlargement (adenoma) predominantly composed of either chief cells (7 cases) or transitional oxyphil cells (5 cases).

For electron microscopy tissues (5 samples per gland) were fixed at 4° C either in 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.3, or in buffered picric acid – 4% formaldehyde solution (PAF) (Zamboni and De Martino 1967) and usually post-fixed in 1% osmium tetroxide. After routine dehydration, they were embedded in Epon 812. In addition, some non-osmicated samples were embedded in LR White resin, according to the rapid procedure.

In ultrastructural immunocytochemistry preliminary experiments were carried out in order to determine the effects on the tissue morphology and on the labelling intensity of the following variables; the type of fixative, the type of resin and etching of the sections with 5% or 10% H202 for some seconds or minutes. PTH immunolabelling was done with the immunogold method. The sections were successively floated on drops of the following reagents; 1) normal goat serum 2) anti-PTH serum diluted 1:2000 with Tris-buffered saline (TBS) pH 8.2, containing 1% BSA (incubation time 18 h) 3) goat anti-rabbit IgG coating 5 nm or 10 nm gold particles.

Chromogranin A immunostaining was performed according to the protein A – gold method (adapted from Hearn (1987)). The sections were floated on drops of TBS, pH 8.2, containing 1% BSA (fraction V, globulin-free, Sigma, St-Louis, USA), then incubated for 18 h at 4° C on drops of LK2H10 antibody diluted 1:2000 with 1% BSA – TBS. After washing, they were incubated for 1 h with rabbit anti-mouse IgG (Jackson Immunoresearch Lab., West Grove, USA), washed again and further incubated for 1 h with protein A – 5 nm or 10 nm gold. Finally, all immunostained sections were contrasted with 2% aqueous uranyl acetate (10 mn and lead citrate (5 mn).

The specificity of labelling for C-terminal PTH was assessed by the following controls: replacement of the primary antiserum with normal rabbit serum and incubation of the sections with the primary antiserum previously treated for 24 h at 4° C with excess PTH sequences, followed by the gold reagent. Human PTH 1-84 (99% pure Sigma, St-Louis, USA) and human PTH 1-34 (Cambridge Research Biochemical, England) were used at the respective concentrations of 0.2 nmole and 3 nmoles per ml of diluted antiserum.

Controls for monoclonal antibody LK2H10 consisted of substituting the mouse anti-keratin antibody KL1 (Immunotech., Luminy, France), which is the same IgG1 isotype as LK2H10.

Results

In both Epon and LR White systems, fixation of the tissues with 2.5% glutaraldehyde caused a marked decrease in labelling for PTH, even when post-fixation was omitted. Thus, this fixative was avoided in favour of PAF for PTH detection. In contrast, both glutaraldehyde and PAF were suitable for chromogranin A localization. PAF fixation (without post-fixation), followed by Epon or LR White embedding, resulted in a strong labelling for both proteins but was unsatisfactory in terms of morphological preservation of the tissues. Chromogranin A was optimally detected in glutaraldehyde-fixed, osmium post-fixed, Epon embedded tissues, after a brief etching of the sections (H202 5% for 60 s). When insufficiently diluted, the LK2H10 antibody, but not the KL1 control antibody, labelled intracellular aggregates of glycogen. This staining was considered non-specific since it was strongly attenuated at 1:2000 working dilution. PTH was detected in less good but acceptable conditions, provided that the tissues were fixed in PAF followed by osmium and recently (less than 6 months) embedded in Epon and that the sections were etched using H202 10% for 4–6 min.

No labelling was obtained when the anti-PTH serum had been inactivated with hPTH 1-84, whereas the addition of an excess of hPTH 1-34 did not modify the reaction.

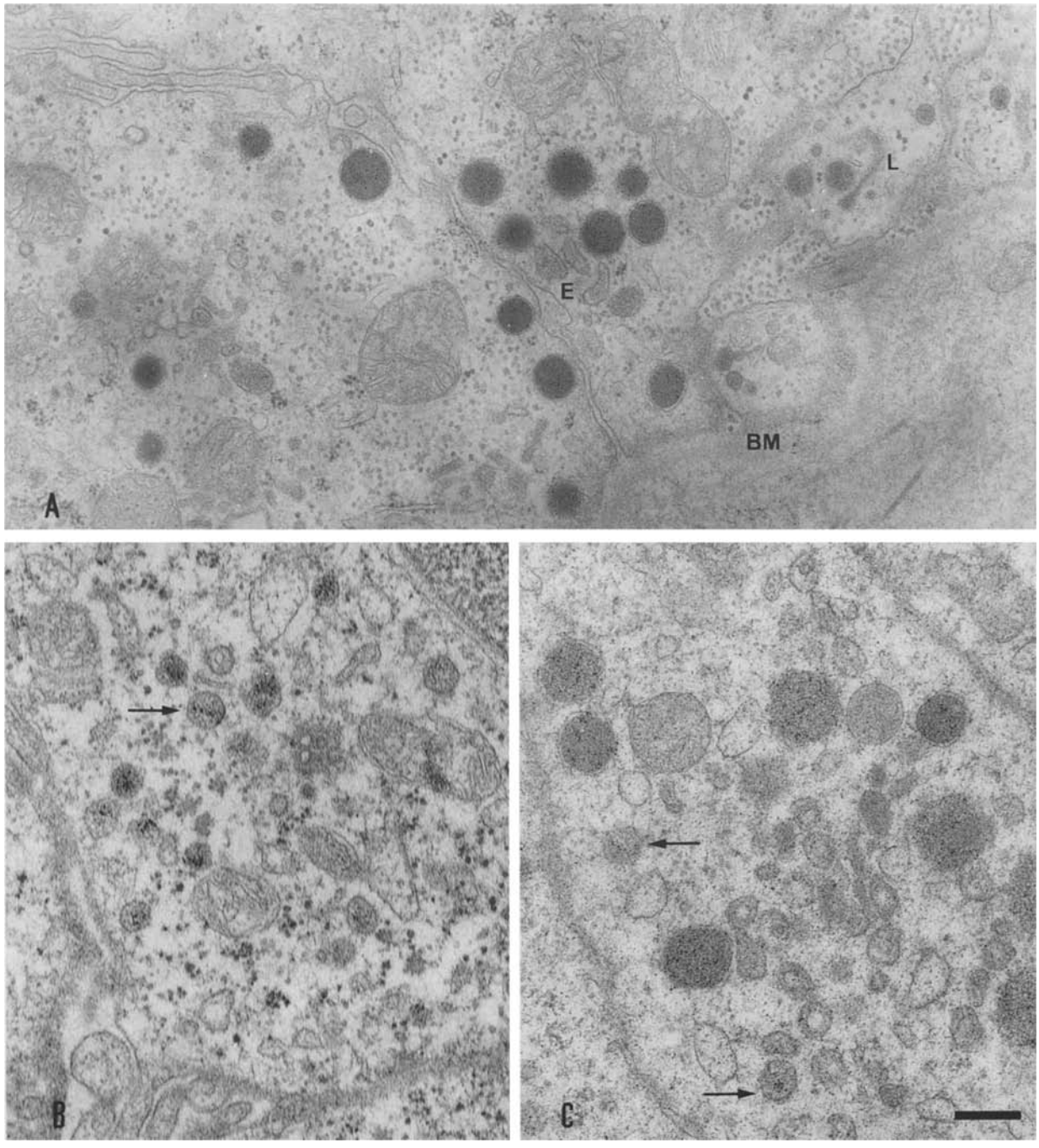

Fig. 1. Secretory granules/vesicles labelled for chromogranin A (**A, B**) or for PTH (**C**). In **A** large dense mature granules are concentrated at the vascular pole of the parathyroid cells (BM: basement membrane) and along the cell membranes. Lysosomal bodies (L) and endocytic vesicles (E) are not labelled. (**B**) illustrates small pale immature vesicles (*arrow*). N: nucleus. In (**C**) mature granules and immature vesicles (*arrows*) are labelled for PTH, but, because of technical conditions, morphology is imperfect. × 35000, scale bar: 285 nm; 5 nm gold (**A**) or 10 nm gold (**B and C**)

Two varieties of well-defined granules/vesicles were consistently labelled in presence of each antibody. Gold labelling was abundantly scattered over large granules of the solid round type, encompassed by a very thin halo, just inside the membrane (Figs. 1A and 1C). These were present in variable, often low, amounts in many cells and were principally concentrated in the peripheral part of the cytoplasm or in cell processes, especially at the vascular pole. Their section diameter varied

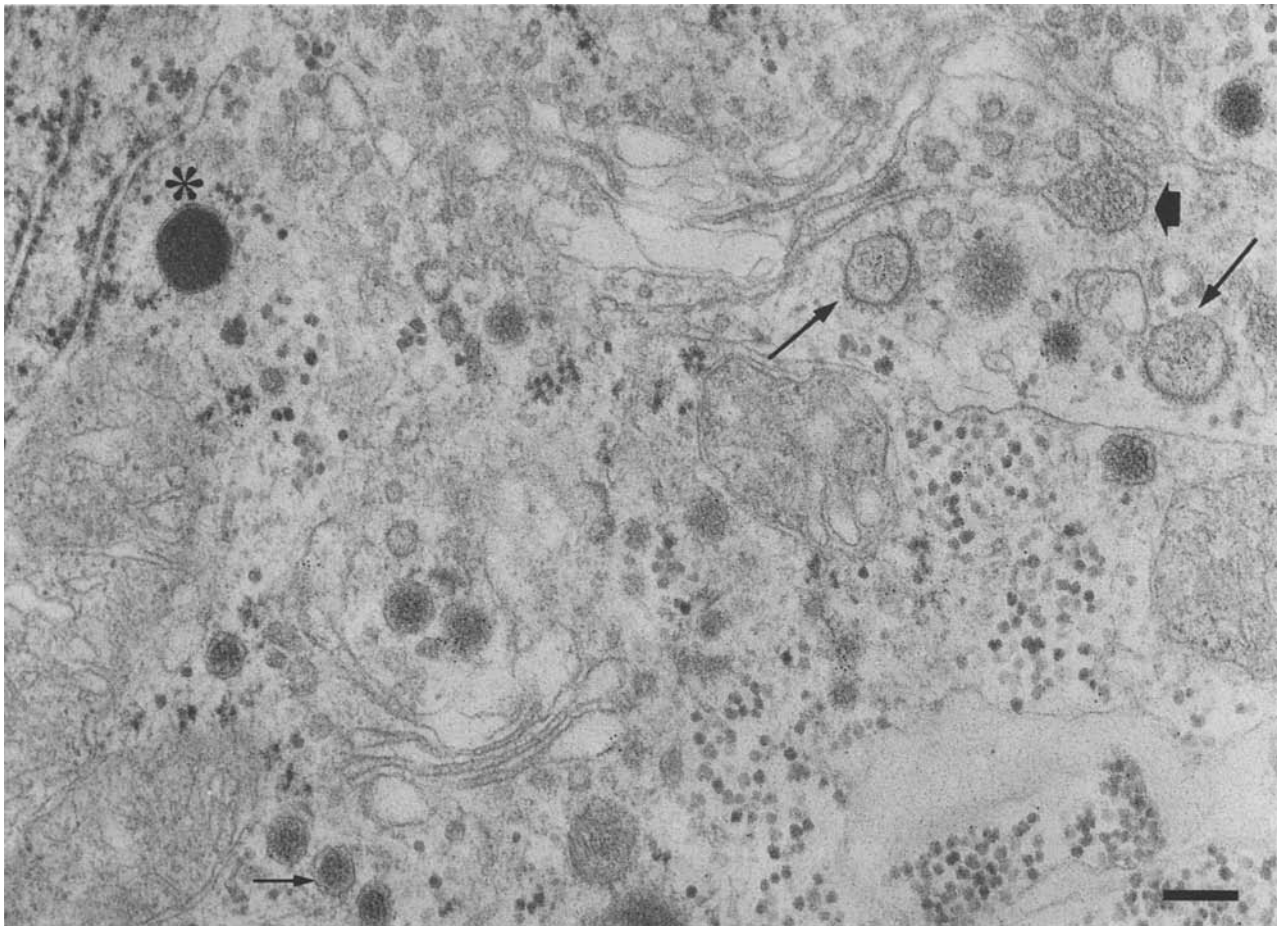

Fig. 2. Immunolabelling for chromogranin A of the Golgi area in an active chief cell. Two gold particles are present over the pale content of an early secretory vesicle budding off the end of a stack (*thick arrow*). Two focally coated vesicles (*long arrows*) and a number of pale or haloed progranules (*arrow*) are also labelled. One mature granule is present at a distance (*asterisk*). Note that the background labelling is restricted to glycogen aggregates. ×49000, scale bar: 202 nm, 5 nm gold

between 200 nm and 250 nm. Some larger specimens (approximately 300 nm in diameter) were inconstantly present but strikingly abundant in the strongly granulated cells that were found in a number of hyperplastic glands. In contrast, medium-sized granules (150–200 nm in diameter) predominated in many transitional oxyphil cells and in uncompletely developed oxyphil cells. The mitochondria-rich cells also stored strongly immunoreactive granules of elongated or ovoid shape. The solid granules were never seen in actual fusion with the cell membrane. Other organelles frequently found in close proximity to the granule clusters were unlabelled. They consisted principally of coated and uncoated vesicles of varying size (including endocytic-like sacs) of lysosomal bodies (characterized by an irregular contour and a moderate electron opacity) and of residual bodies (larger in size and more heterogeneous in structure).

A distinct labelling, best shown by the use of 5 nm gold, was also demonstrated over a variety of small (150 nm in diameter) pale vesicles/granules (Figs. 1 B and 1 C). Included were Golgi-associated vesicles, some of which were budding off cisternae at the concave face. Inside the vesicles, the gold particles decorated a floculent material or a pale homogeneous content. A bristle-coated patch was sometimes observed on the vesicle membrane (Fig. 2). The Golgi stacks were unreactive, as were coated or uncoated microvesicles. Transitional forms were frequently seen between secretory vesicles and small haloed granules. The latter showed a distinct labelling over their moderately dense core. They were mostly found in the Golgi area, but in some cells were scattered throughout the cytoplasm and/or accumulated at the peripheral part of the cell (Figs. 2 and 7). Other bodies differed from the typical granules/vesicles in that they varied greatly in both morphology and label-

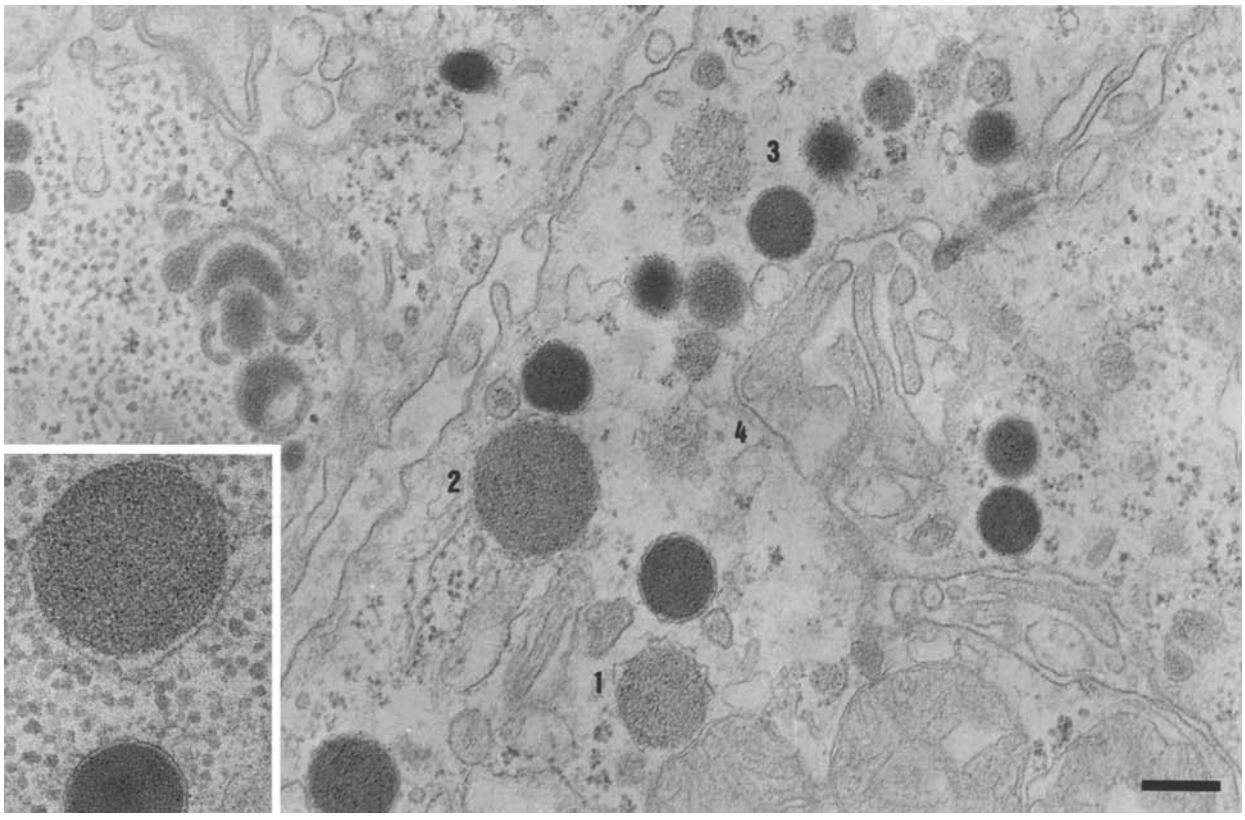

Fig. 3. Atypical (granulolytic) bodies labelled for chromogranin A. Bodies 1 and 2, supposed to be early stages, are strongly immunoreactive. Bodies 3 and 4, supposed to be advanced stages, are poorly labelled and lack a limiting membrane. ×42000, scale bar: 243 nm; 5 nm gold: *Inset*: Detail of a body of the former variety. Note the eccentric membrane and the rather pale content. ×70000, 5 nm gold

ling pattern. In spite of the etching artefacts, they could also be identified in sections labelled for PTH and stained positively for the hormone. Atypical solid bodies were scattered in small numbers in most of the cells that stored a moderate to high amount of compact granules. Compared with the latter, they were larger (350–400 nm in diameter) and their finely particulate content was less electron-dense and reacted more variably for chromogranin A and PTH. The submembranous space was wider and often eccentric and the limiting membrane was poorly defined (Fig. 3 inset). Transitional forms were seen between these bodies and final structures made up of a granulo-filamentous weakly labelled material without any distinct membrane.

Focal labelling for chromogranin A and/or PTH also occurred over large vesicles of varying and often complex morphology, present in most of the granulated cells (whatever the type of the predominating granules). The most characteristic ones were multivesicular bodies (Figs. 4A and 4B). Each of them consisted of a vesicle measuring 300–600 nm in diameter, delimited by a well-defined membrane, which sometimes harbored a coated segment, and containing a number of very small vesicles. Approximately 50 per cent of these bodies showed a single inclusion within the internal matrix. When it consisted of a dense material, the inclusion resembled a fragment or a remnant of secretory granule core and was strongly labelled. The labelling was weaker over floculent or poorly defined inclusions. These bodies were sometimes surrounded by and connected to tubular extensions or microvesicles, or appeared simplified in that they lacked the internal vesicles and/or the dense matrix (Figs. 4C and 4D).

Taking the ratio of large dense to small pale granules/vesicles as an index of the actual state of granule maturation allowed us to categorize the cells as follows:

(1) Virtually agranular cells. No valid estimation of the index was possible for these cells. Included were (a) the fully developed oxyphil cells, (b) a number of glycogen-rich chief cells (which exhibited varying amounts of synthesizing organelles),

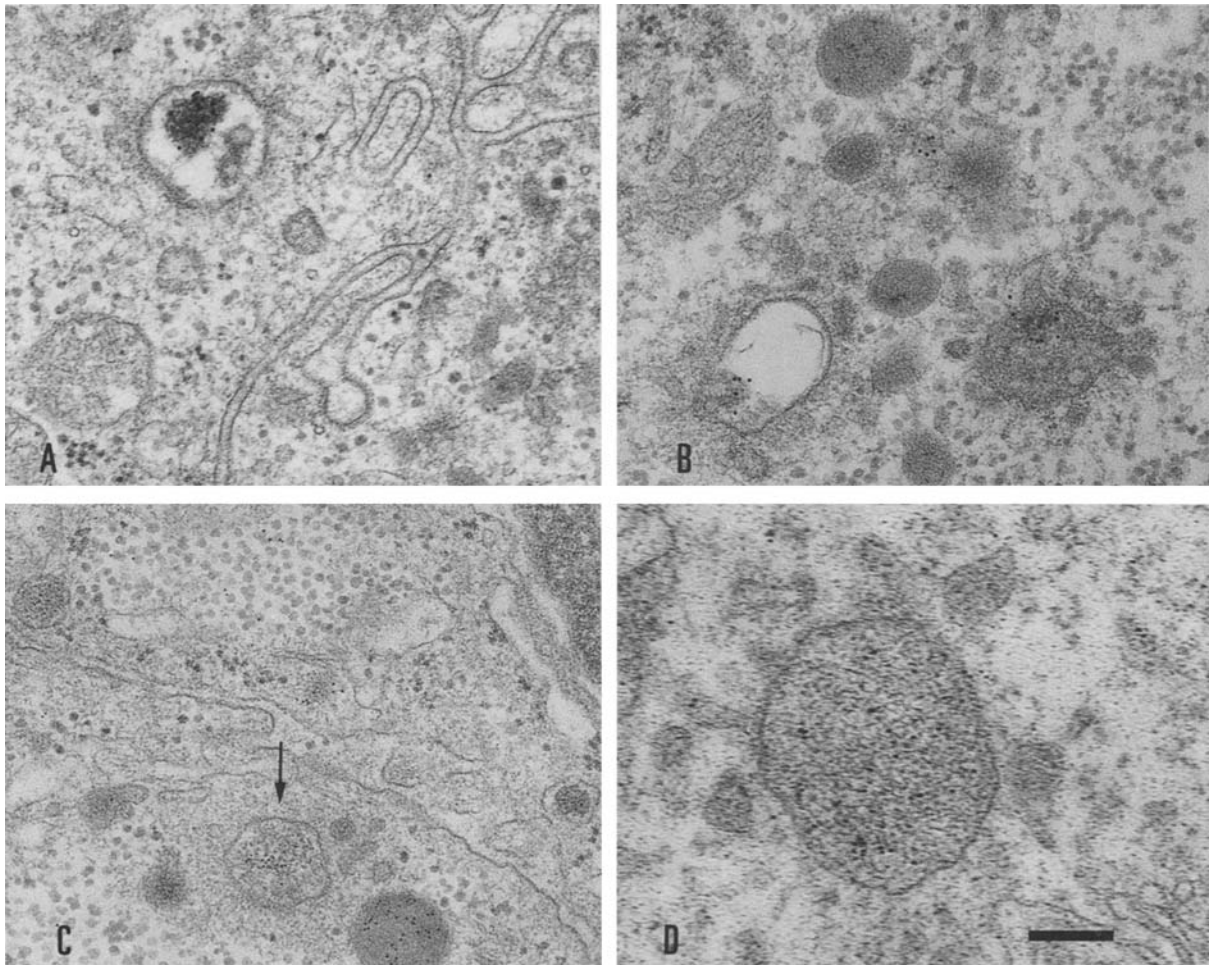

Fig. 4. Crinophagic organelles immunolabelled for chromogranin A (**A, B, C**) or for PTH (**D**). (**A**) shows a typical multivesicular body containing a dense inclusion, to which the labelling is restricted. In (**B**) two bodies are shown; one is surrounded by radiating tubules and contains a dense focally labelled matrix, masking the internal microvesicles; the other is partially emptied. (**C**) illustrates the labelling over a simplified body (*arrow*), which lacks internal vesicles. (**D**) reveals PTH immunoreactivity within a large vesicular body surrounded by radiating tubules. × 52000, scale bar: 169 nm; 10 nm gold (**A, B, D**) or 5 nm cold (**C**)

and (c) some moderately active chief cells characterized by a peripheral clustering of lysosomal bodies, especially at the vascular pole. The virtually agranular cells could be found in both groups of glands; but, in contrast to subtype a, subtypes b and/or c sometimes made up the whole population of cells available for study (this was found in three patients with an adenoma).

(2) Cells storing both varieties of granules or vesicles, with the small pale ones restricted to the Golgi area and the others peripherally distributed. This category comprised a large number of chief or transitional oxyphil cells from both groups of glands. These cells displayed various signs of endocrine stimulation, including the Golgi labelling pattern described above.

(3) Granulated cells containing only the small pale variety of vesicles/granules. The latter were abundantly scattered from the Golgi area to the peripheral part of the cytoplasm and gathered along the cell membrane (Fig. 5). No granule of the large compact type was seen. The Golgi was prominent. The ends of some stacks were strikingly and extensively dilated and contained a floculent material reactive to chromogranin A antibodies (Fig. 6A). A dense strongly immunoreactive inclusion was sometimes present within large, smooth-surfaced or partly coated vesicles (Figs. 6B and 6C), which in some sections appeared continuous with the cisternae. This pattern of labelling characterized a number of cells classified as transitional oxyphil cells because they were rich in both mitochondria and synthesizing organelles. Except for a few present in hyperplastic glands, these cells were principally found in tissues from the 5 transitional oxyphil cell adenomas. They were sometimes asso-

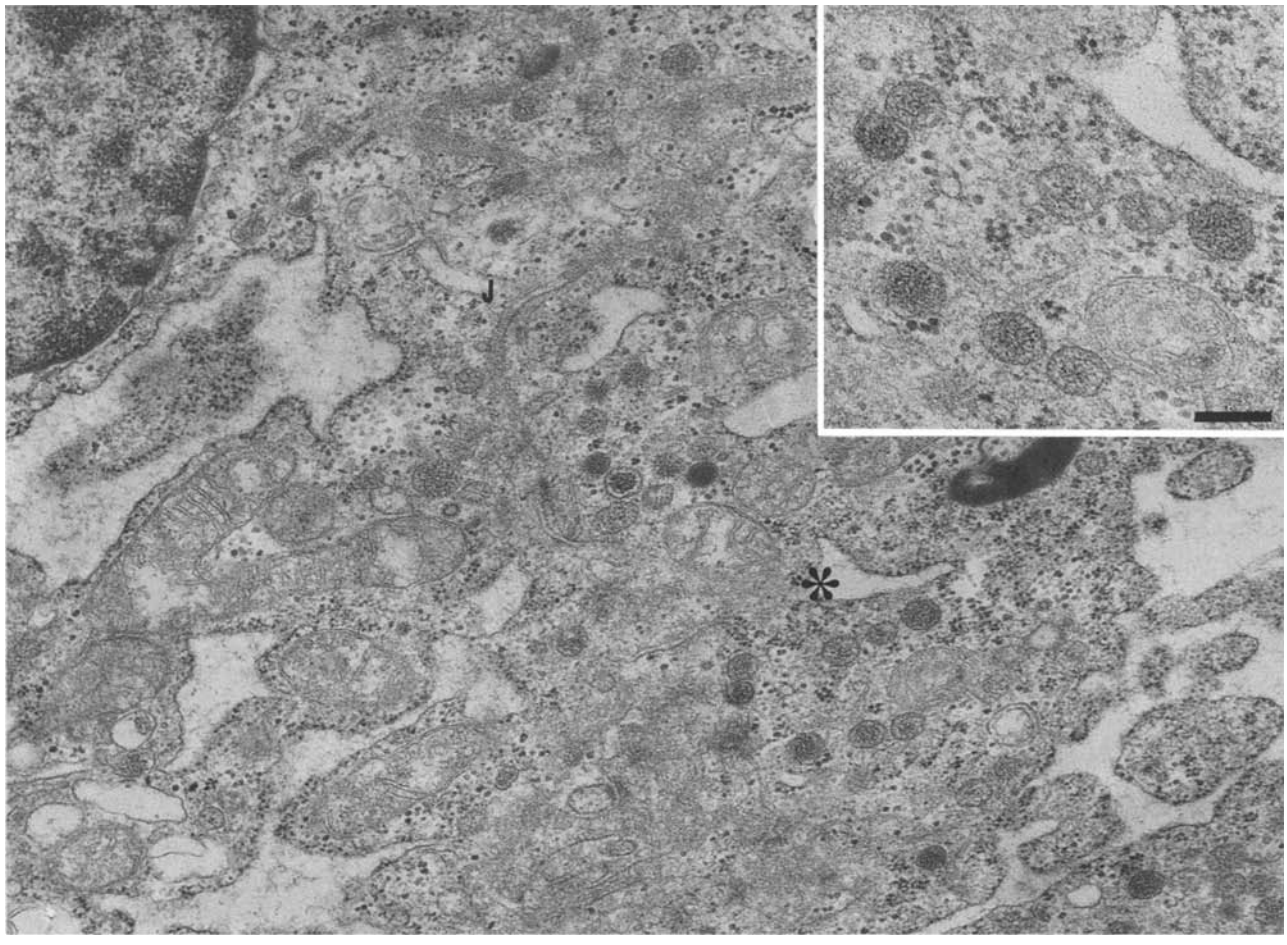

Fig. 5. The non-maturing population of (pro)granules in a group of cells from a transitional oxyphil cell adenoma immunolabelled for chromogranin A. The progranules are accumulated along the cell membranes (J: junctional complex). Mature granules are not present. ×28000, 5 nm gold. *Inset*: Detail of the small pale or haloed progranules present in proximity to the *asterisk*. Note that they are distinctly labelled. ×58000, scale bar: 172 nm; 5 nm gold

ciated with cells storing both varieties of granules, but more usually were grouped together and constituted the majority of the cell population present in some sections.

(4) Cells storing predominantly small pale granules/vesicles. The granules, most of which were of the haloed subtype, were located in the Golgi area and along the cell surface and strikingly accumulated in cell processes. A number of compact granules of medium size was also present in these clusters and often displayed a stronger labelling. "Ghost" vesicles similar in size to the small granules, but unlabelled and surrounded by a wrinkled membrane, were frequently associated with the immunoreactive granules in proximity to the cell surface (Fig. 7). This pattern of labelling was found in a number of chief cells or transitional oxyphil cells from both groups of glands. The cells were either grouped together or, more usually, scattered in the tissue sections, adjacent to other types of granulated or agranular cells. They showed ultrastructural signs of endocrine stimulation but the Golgi pattern described above was not seen.

Discussion

Both antibodies used in the present study proved valid for the specific localization of secretory proteins, either PTH or SP-I/chromogranin A, in osmicated sections of human parathyroid glands. As shown by radioimmunoassay data and inactivation tests, the polyclonal antiserum n° 512525 is directed against the C-terminal biologically inert region of bovine or human PTH. Since hyperfunctioning glands store and secrete mainly intact PTH and C-terminal fragments (Schettler et al. 1984; Hanley and Ayer 1986; MacGregor et al. 1986a, b), it would appear that this antiserum recognizes most of the intracellular hormone stores. Whether it cross-reacts significantly with proPTH, which

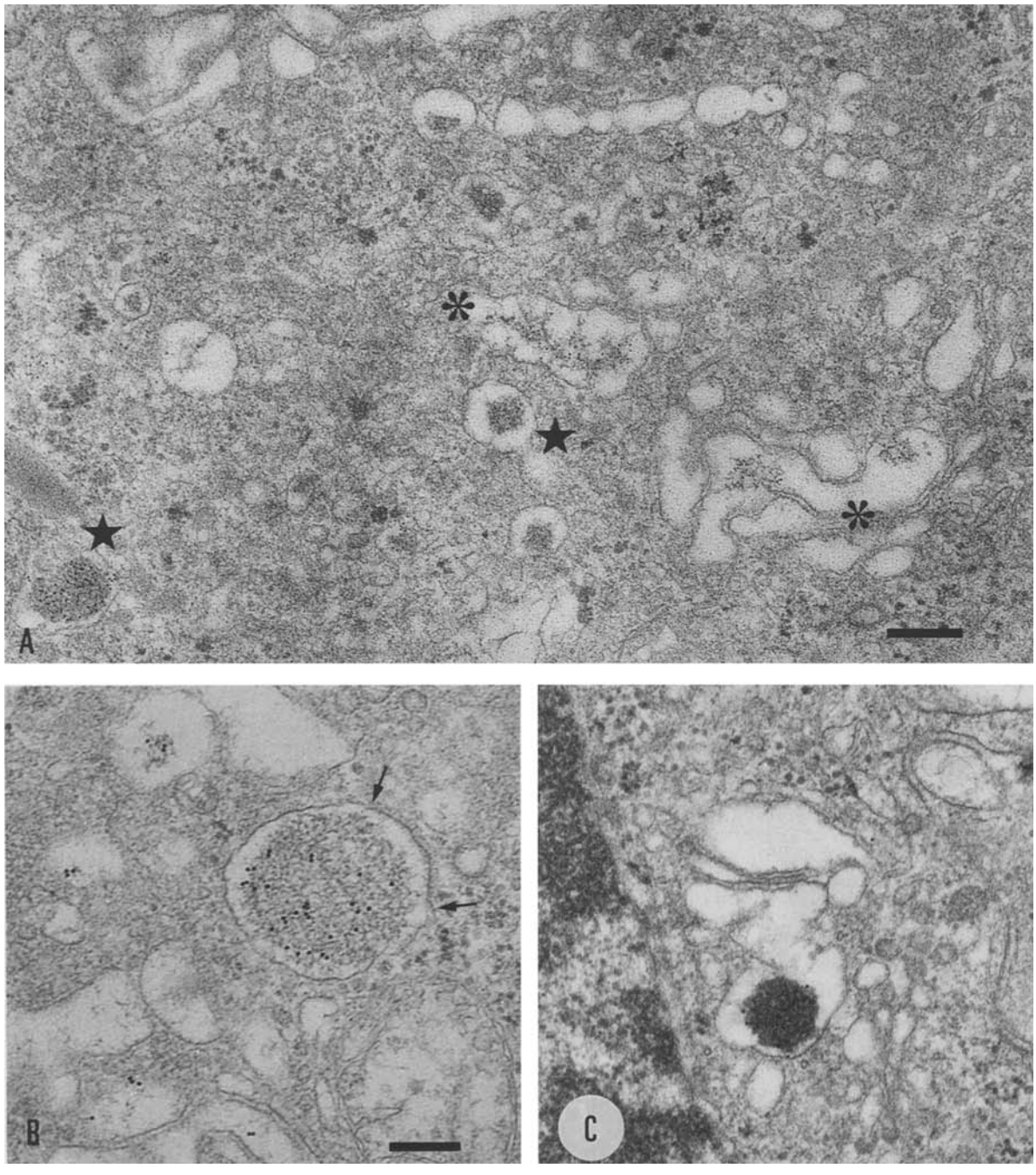

Fig. 6. The Golgi area in a maturation-defective cell immunolabelled for chromogranin A. In (**A**) note the extensive swelling of the cisternae, some of which contain an immunoreactive material (*asterisks*). Large vesicles with a more concentrated material are present at a distance (*stars*). ×49000, scale bar: 202 nm; 5 nm gold. (**B** and **C**) illustrate the material stored in large partly coated (*small arrows*) vesicles (**B**) or in saccules (**C**). ×52000, scale bar: 169 nm; 10 nm gold (**A**) or 5 nm gold (**B**)

represents 7% of the total hormone stored in normal parathyroid glands (Habener et al. 1984), is not known. The LK2H10 monoclonal antibody is mainly directed to human chromogranin A/secretory protein-I. That it cross-reacts to some extent with other chromogranin polypeptides (Wilson et al. 1986) is not actually a drawback, since the latter are not to be found in normal or hyperfunctioning parathyroid glands (Hagn et al. 1986; Weiler et al. 1987). The tissue morphology was better preserved in the monoclonal protocol because chromogranin A antigenicity persisted on glutaraldehyde-fixed post-osmicated tissues, whereas the conventional procedure needs to be adapted to re-

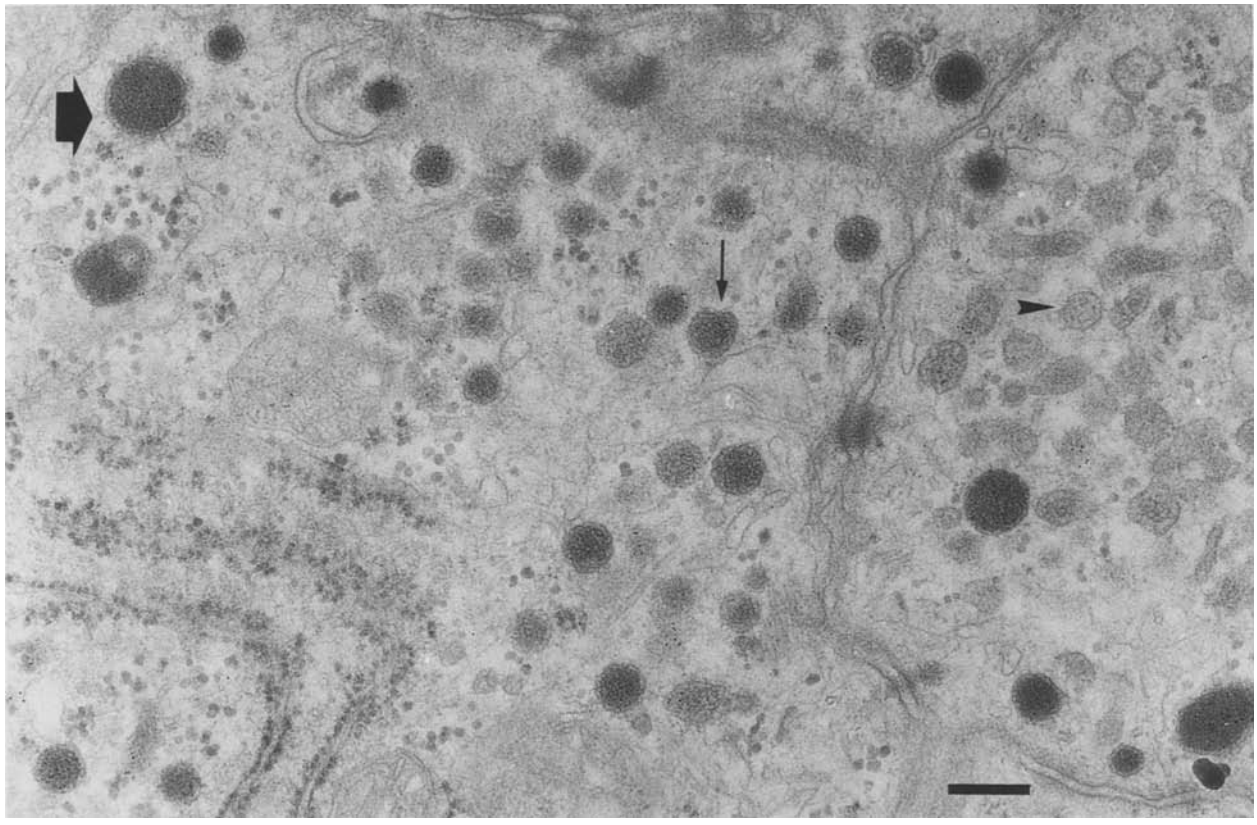

Fig. 7. Uncompleted maturation of peripherally accumulated (pro)granules (*arrow*) in two adjacents chief cells from a hyperplastic gland. Only a few mature granules (*thick arrow*) are present. "Ghost" vesicles (*arrowhead*) are not labelled. Immunolabelling for chromogranin A. ×49000, scale bar: 202 nm; 5 nm gold

store PTH antigenicity. This explains that, although the results were grossly similar using each antibody, some findings were more easily evaluated in the monoclonal procedure.

The strong labelling present over the large round dense granules (usually called secretory, or storage or mature granules) contrasted with the absence of gold particles over pleomorphic bodies of lesser density, similar in aspect to cytochemically defined lysosomes (Nilsson 1977). This observation is of interest because both types of organelles were present in close proximity to each other and to the cell surface. Only the labelling pattern allowed identification of individual bodies of ambiguous morphology, such as elongated secretory granules (as seen in mitochondria-rich cells) and rounded sections of lysosomes. Whereas the population of mature granules was grossly homogeneous in terms of electron opacity and labelling density, the presence of very large granule sections was inconstant. Their increased number in the strongly granulated cells suggests that they are destined for a particular type of storage function. Such storing-cells were principally noticed in secondary hyperplastic tissues and may reflect the therapeutic (suppressive) effect of dialysis on these glands. In contrast, the poorly granulated cells, particularly those of the oxyphil or transitional oxyphil types, stored mainly smaller forms. The other variety of consistently labelled organelles included a range of small vesicles/granules with a pale or floculent content. Their labelling was very helpful in distinguishing them from other smooth-surfaced vesicles frequently spread throughout the cytoplasm, especially in the Golgi region. In earlier ultrastructural studies (Altenähr 1972; Roth and Capen 1974; Thiele 1986), organelles of this type have been referred to as prosecretory or immature or pro-granules, but one paper only (Nilsson 1977) offers a detailed description and a convincing illustration of them.

In the present material, most of the granulated cells contained both immature and mature granules, the former being mainly restricted to the Golgi area and the latter concentrated near the cell surface. In spite of a species-dependent variability in granule morphology, a topographical segregation of two varieties of endocrine granules

also occurs in parathyroid glands in other mammals (Capen 1971). Granules of intermediate electron opacity were scarce; although no morphometric analysis was carried out, this observation suggests that the intracellular maturation of progranules occurs as a brief event. In a number of cells involved in peptide hormone synthesis, the morphological maturation of granules is associated with physico-chemical changes in their peptide content, including a progressive conversion of prohormone to hormone (Varndell et al. 1983; Orci et al. 1987). Since in the present material a typical coat was demonstrated on rare chromogranin A – storing vesicles of the Golgi region, it may be important to recall that in pancreatic B-cells the conversion of proinsulin to insulin is initiated within (clathrin)-coated early vesicles (Orci et al. 1987). However, the conversion of proPTH to PTH is assumed to take place within the Golgi complex itself, as indicated by autoradiographic studies combined with electrophoretic analyses (Habener et al. 1979). This problem needs to be further investigated by direct morphological methods.

Because they showed a diversity of morphological and immunolabelling patterns other types of body were thought to be engaged in the breakdown of hormone stores. In particular a distinct labelling was present over a dense or finely particulate inclusion in some multivesicular bodies (MVB's). This observation corroborates the demonstration of crinophagic MVB's in rat endocrine cells, including the bodies reacting for PTH in parathyroid glands (Inoue and Setoguti 1986), those reacting for insulin in pancreatic B-cells (Orci et al. 1984) and those initially described in anterior putuitary gland (Smith and Farquhar 1966). It is now widely accepted that MVB's are pre-lysosomal organelles of endocytic origin (Mellman et al. 1986). In a number of rat endocrine cells, some MVB's are acid phosphatase-positive (Smith and Farquhar 1966; Orci et al. 1984) but those in human or animal parathyroid cells are not (Shannon and Roth 1974; Nilsson 1977; Setoguti et al. 1985). On the basis of the present labelling, some of them are secondary lysosomes involved in crinophagy. The others may be functionally distinct, for example destined for the controlled degradation of some retrieved secretory granule membranes, as has been demonstrated in chromaffin cells (Patzak and Winkler 1986).

Through a range of morphological and labelling patterns, the atypical solid bodies that we found in most of the mature granule-storing cells were reminiscent of storage granules engaged in a "swelling" process followed by granulolysis. Although some of these bodies were seen in a stage of advanced breakdown, we cannot rule out that others might be secreted. According to histochemical and biochemical data (Dockerty et al. 1984; Orci et al. 1984), enzymes or enzyme precursors, usually found in lysosomes, including peptidases, may become associated with the storage compartment of endocrine cells and some may act in prohormone processing and/or as degradating enzymes. It is noteworthy that in those cells storing progranules exclusively, the atypical bodies were not seen, whereas crinophagic MVB's were frequently observed, as well as large crinophagic-like vesicles derived from the Golgi complex.

Because of the immature granules accumulated under the plasma membrane, a number of cells differed strikingly from most of the others, which stored predominantly mature or maturing granules or were virtually agranular. The non-maturing pattern was mainly restricted to groups of seemingly active mitochondria-rich cells present in all the transitional oxyphil cell adenomas. Thus, we assume that, in these cells, the process of progranule maturation may be persistently or permanently inhibited. That the Golgi area is or becomes implicated in the defect is indicated by the "swelling" cisternae with a secretory content. Morphologically similar features have been described as the effect of proPTH conversion inhibitors (McGregor et al. 1977). Another labelling pattern, the peripheral clustering of mixed but predominantly immature granules, characterized other cells, which were scattered in tissues from both groups of glands and belonged either to the transitional oxyphil or to the chief cell type. This pattern suggests a delay or a minor defect in the process of progranule maturation. Whether it reflects a reversible (cyclic?) or a persistent (permanent?) state of the intracellular hormone processing cannot be inferred from the present study. The immature granules in the defective cells are probably destined for secretion, as suggested by their peripheral location and the frequent association of unlabelled sac-like vesicles of endocytic type. The secretion mechanism needs to be further investigated, as has been done for mature granule exocytosis, using freeze-fracture electron microscopy (Thiele 1980, 1986). Although many questions remain unsettled, our observations lend some morphological support to the actual concept of PTH and SP-I processing by parathyroid cells. Principally based on "in vitro" findings, the scheme indicates that the early proteins, leaving the Golgi area, are destined either for immediate secretion or for storage and/or subsequent secretion (Cohn and Elting 1983; Habener et al. 1984).

The present description of cells with either a maturing or a non (or incompletely) maturing population of granules may account ultrastructurally for the existence of two distinct secretory pathways. The labelling patterns that we noticed correspond to the "regulated" and the "constitutive" pathways proposed by Kelly (1985) for protein secretion by eukaryotes. A by-pass secretion with absence of hormone packaging may also exist, as suggested by "in vitro" studies (Dietel 1982) and by the ultrastructural investigation of adenomas causing acute hyperparathyroidism (Hehrman et al. 1980). Also consistent with this hypothesis are the present findings of functioning adenomas composed of virtually agranular cells.

Recent data also indicate that two types of PTH/SP-I degradation are involved in the regulation of the biologically active hormone (MacGregor et al. 1986a). A presumably lysosome-related system is thought to degrade the hormone extensively or to cleave it into fragments that are not likely to be secreted. In porcine and bovine tissues, the proteases responsible for this degradation are cathepsin B and cathepsin D (MacGregor et al. 1979; Hamilton et al. 1983). In parallel, we localized an intracellular breakdown of secretory proteins within (multi)vesicular or compact bodies engaged in crinophagy and granulolysis. Since the system is likely to operate more actively when secretion is inhibited, we cannot exclude that other organelles may be involved in this process in suppressed (or normal) glands. The other degradative system cleaves PTH into fragments destined for secretion, most of which are C-terminal and biologically inert (MacGregor et al. 1986a). It is thought to act via a number of unidentified proteases that have become associated with the packaging or the storing compartments of the cells. The granules containing these fragments (together with modified or unmodified SP-I) could not be identified by the present methodology; it is uncertain whether they differ morphologically from those storing principally intact PTH and SP-I. Since the secretion of hormone fragments is less sensitive to calcium than is that of PTH and increases proportionally under high calcium concentration (Hanley and Ayer 1986; MacGregor et al. 1986b), the fragments are liable to be stored abundantly in suppressed cells. It could very well be that the large storage granules found in parathyroid cells from dialyzed patients are concerned in this process.

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