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Ultrastructural Pathology of Parathyroid Glands*

Eberhard Altenähr

With 18 Figures

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A. Introduction

The normal and pathological anatomy of the parathyroid glands (PTG) as seen by light microscopy is well known (BARGMANN, 1939; CASTLEMAN, 1952; ROTH, 1962; ALTENÄHR *et al.*, 1969; SEIFERT and ALTENÄHR, 1969). However, the cellular mechanisms of hormone production and secretion have not yet been clarified to any great extent. Therefore the aim of ultrastructural studies of PTG was primarily to analyse in more detail the function of PTG under different physiological and pathological conditions. In addition, a more refined cytological diagnosis of PTG would offer new prospects regarding clinical, diagnostic, and therapeutical questions, by determining whether a primary change in the PTG causes the disturbance in calcium metabolism or whether the altered PTG ultrastructure is secondary to calcium metabolism disturbances of other aetiology.

B. Functional Cytology and Ultrastructural Pathology of Parathyroid Glands in Animal Experiments

The electron microscopic studies of animal PTG in different species published so far are summarized in Table 1. There are observations under normal conditions, special physiological conditions (growth, pregnancy, lactation, laying hens, hibernation, metamorphosis of amphibians), as well as under pathological and experimental conditions.

Knowledge of the morphological equivalents of cellular hormone synthesis, storage, and secretion is an essential prerequisite for diagnostic conclusions. In addition, there is the problem of morphologic definition and staining of parathyroid hormone. To solve these questions, comparative experimental studies of stimulated and suppressed PTG were performed.

The dominating cell in PTG of all species is the *chief cell*. Detailed descriptions of their nuclei have only been published by MONTSKO *et al.* (1963) and ZAWISTOWSKI (1966). Ultrastructural changes in the nuclei, dependent on functional activity, have not been satisfactorily investigated. As in other glands synthesizing protein hormones, it can be assumed that ribosomes, rough endoplasmic reticulum and Golgi complex are involved in hormone synthesis and storage. The hormone or its precursors are synthesized by ribosomes, transported to the Golgi complex via the endoplasmic reticulum and packed into hormone-containing vesicles and granules in the Golgi complex (Fig. 1a). The morphological indications for this process are the proliferation of ribosomes and the increased size of the rough endoplasmic reticulum and Golgi complex when PTG are overactive or experimentally stimulated (Figs. 2 and 3). A corresponding reduction and involution of these cellular components is observed in experimental suppression or inactivity (Fig. 4) (LEVER, 1959; ROTH and RAISZ, 1964, 1966; CAPEN *et al.*, 1965a; STOECKEL and PORTE, 1966b; NAKAGAMI, 1967; MAZZOCCHI *et al.*, 1967b; ALTENÄHR, 1970; and others). MELSON

(1966), LEVER (1958), and NAKAGAMI (1967) have described proliferation of mitochondria following stimulation of the PTG and MAZZOCCHI *et al.* (1967b) observed an increase in mitochondrial size. Following stimulation, the number of lipid bodies is reduced (ROTH and RAISZ, 1964; MAZZOCCHI *et al.*, 1967b) while it is increased following suppression (MURAKAMI, 1970) (Fig. 2).

The cytoplasm of active chief cells appears dark due to an increase in cell organelles. As a result, stimulated PTG mainly consist of dark chief cells, rich in cell organelles (Fig. 3). The cytoplasm of inactive chief cells, especially when suppressed, is light and shows fewer organelles (Fig. 4) (LEVER, 1958; CAPEN *et al.*, 1965a; NAKAGAMI, 1967; MAZZOCCHI *et al.*, 1967b; HARA and NAGATSU, 1968; ALTENÄHR, 1970; ALTENÄHR and LIETZ, 1970). STOECKEL and PORTE (1966a) consider the different electron density of the ground plasma to be a fixation artefact. We, however, think the electron density of the ground plasma may depend on differences in the fixation lability caused by different functional cell activity.

The described findings in animal PTG are somewhat more complex in the individual species because of differences in cell type differentiation. Apart from chief cells, monkey PTG also contain oxyphil cells (TRIER, 1958; NAKAGAMI, 1965), characterized by a special prevalence of mitochondria. Cell type differentiation of equine PTG appears to be most similar to human PTG. FUJIMOTO *et al.* (1967) observed light and dark oxyphil cells, dark, light and vacuolized chief cells, as well as water-clear cells in horse PTG. Light chief cells, light vacuolized chief cells, and water-clear cells are characterized by an increased amount of glycogen in their cytoplasm. CAPEN and ROWLAND (1968b) described a glycogen increase after stimulation in cat PTG.

Vesicles with a moderately electron-dense homogeneous or loose granular content located inside or close to the Golgi complex are characteristic for protein hormone production (Fig. 1a). Their diameter is between 30 and 200 mμ, and they are generally called immature secretory or *prosecretory granules*, although they most probably contain completed parathyroid hormone ready for secretion. The cells of stimulated glands contain a significantly increased number of these prosecretory granules.

The membrane-surrounded bodies containing more densely packed fine granular material have a larger diameter (100–700 mμ) than the prosecretory granules of the corresponding species and are called *secretory granules* by most authors (Fig. 1b). They are derived from prosecretory granules by condensation of their content (NAKAGAMI, 1967; NEVALAINEN, 1969) through the fusion of several prosecretory granules, possibly via an intermediate multivesicular body (DAVIS and ENDERS, 1961). It is commonly believed that these "mature" secretory granules are hormone storage granules.

The number of secretory granules in cells of normally active PTG is species-dependent. The PTG cells of cows and mice, for example, contain relatively numerous secretory granules (CAPEN *et al.*, 1965a; STOECKEL and PORTE, 1966a) (Fig. 1b). Cat PTG show a moderate number of secretory granules (CAPEN and ROWLAND, 1968), while PTG of most other species, especially rat

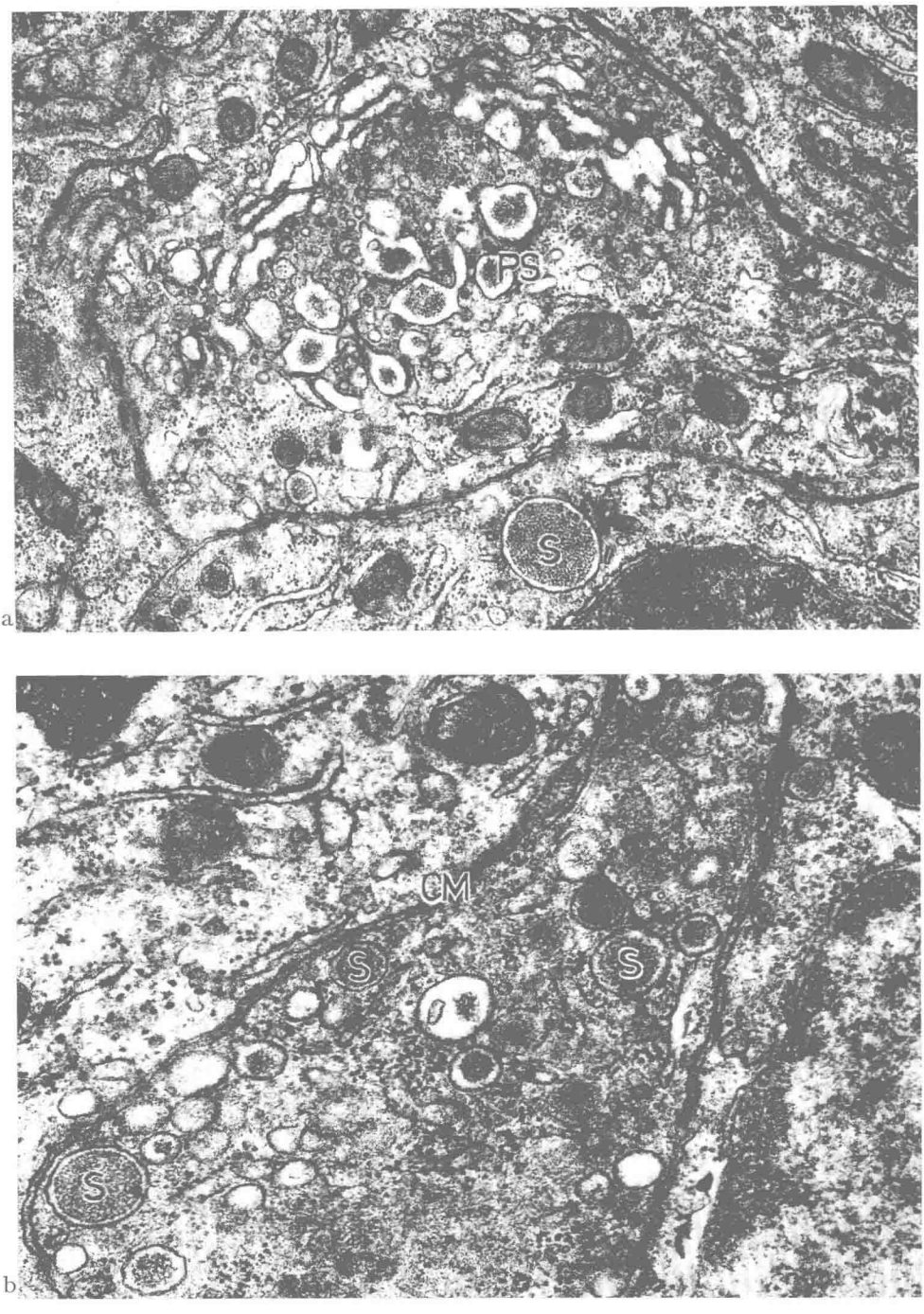

Fig. 1. Normal mouse PTG: a) Formation of prosecretory granules (*PS*) in Golgi vesicles by packing of electron-dense, fine granular material. *S* = secretory granule. 32000 ×. b) Secretory granules (*S*) with varying diameters, often close to the cell membrane (*CM*). 41000 ×

PTG, contain very few, their number differing even from cell to cell. The quantitative distribution of secretory granules during different functional stages is not uniform. For example, MAZZOCCHI *et al.* (1967b) described an increased number of storage granules in the stimulated PTG, while others (ROTH and RAISZ, 1964; MELSON, 1966; CAPEN and ROWLAND, 1968b) observed a degranulation. In suppressed PTG, ROTH and RAISZ (1964) and MURAKAMI (1970) found an increased amount of storage granules, while NAKAGAMI (1967) observed a reduced number. Other authors have not found significant differences in the number of storage granules (ALTENÄHR, 1970) or have not commented on this problem at all. There seems to be a lysosomal digestion of storage granules (HARA and NAGATSU, 1968; ROHR and KRÄSSIG, 1968; ALTENÄHR, 1970). Our own observations (ALTENÄHR, 1970) indicate that hormone storage and secretion of stored hormone do cccur, but are not an important functional principle of PTG. In this respect the PTG are quite unlike the C-cells of the thyroid gland, their most obvious functional changes being degranulation, storage in granules and phagolysis of granules (LIETZ, 1970; ALTENÄHR and LIETZ, 1970).

The assumption that the described prosecretory and storage granules in PTG cells contain hormone is based on their similarity to secretory granules of other glands producing protein hormones, and also on the changes observed under experimental conditions. L'HEUREUX and MELLIUS (1956) found parathyroid hormone activity in a corpuscular fraction of similar size obtained after differential centrifugation of bovine PTG tissue homogenate. However, they have not examined these corpuscules by electron microscopy. Immunocytochemical proof of parathyroid hormones has not so far been obtained. Cellular secretion of synthesized hormone packed by the Golgi complex to prosecretory and secretory granules has not been completely clarified morphologically in spite of numerous attempts. Often, secretory granules are located near the plasma membrane (Fig. 1b) and prosecretory granules, too, show a transfer from the Golgi complex to the periphery of the cell, especially following stimulation. In some PTG a preferential location in peripheral areas of the cytoplasm and at cell membranes adjacent to narrow intercellular spaces is observed (e. g. mouse PTG; STOECKEL and PORTE, 1966a); in others, they are located near cell surfaces next to widened intercellular spaces or towards the interstitial space (e. g. human PTG: ALTENÄHR and SEIFERT, 1971). Most authors assume a fusion between granule membrane and plasma membrane (STOECKEL and PORTE, 1966a; MELSON, 1966; NAGAGAMI, 1967; HARA and NAGATSU, 1968; TANAKA, 1969; YOUSHAK and CAPEN, 1970). An increased incorporation of granule membranes into the cell membrane could be the reason for the enlargement of the cell surface and the increased tortuosity of plasma membranes in stimulated PTG. It is very difficult, however, to obtain an electron micrograph of such a fusion and exocytosis (Fig. 6b), which definitely excludes a tangential section. Probably, the hormone is liquified when secreted from the cell. Rarely, isolated granules have been described in the interstitial spaces (human PTG: MUNGER and ROTH, 1963; cow PTG: CAPEN *et al.*, 1965a; pig PTG: FETTER

and CAPEN, 1968). The situation in pig PTG, however, is different, because cytoplasmic processes containing secretory granules reach through the basement membrane into the perivascular space. They seem to be subsequently detached from the cell.

The frequently observed widened intercellular spaces contain a moderately electron-dense colloidal homogeneous or fine granular material, and communicate with the perivascular space via a system of channels (ALTENÄHR, 1970; SETOGUTI *et al.*, 1970; COLEMAN, 1969). Colloidal storage of hormone in such intercellular spaces would seem possible, and would be consistent with the findings of PERKIN *et al.* (1968) obtained by immune fluorescence microscopy.

A direct secretion of hormone from the gland cell into the capillary does not seem possible because the capillary wall and the endocrine cells have no direct contact. Therefore, the hormone must reach the blood via intercellular and perivascular spaces. The fenestrated capillary endothelium with numerous pores is especially well suited for an intense exchange of substances. The great pinocytotic activity of the endothelial cells could also be of importance for hormone transport across the capillary wall. Whether the electron-dense granules in the endothelial cells actually represent secretory granules has not yet been proved (MUNGER and ROTH, 1963; CAPEN *et al.*, 1965a; MELSON, 1966; FETTER and CAPEN, 1968).

Because of the function-dependent change in PTG cytology, a *functional cycle* of the endocrine cells is assumed with a primarily inactive resting chief cell, which is stimulated and activated and thereafter returns to the resting phase (ROTH and RAISZ, 1966; MAZZOCCHI *et al.*, 1967b). This cycle is supposed to be essentially the same in all species (Fig. 2). In the modified and summarized scheme the tortuous cell membranes with numerous indentations following stimulation have been considered, as well as the fact that active cells secrete mainly "immature" prosecretory granules. The described reduction of glycogen after stimulation (ROTH and RAISZ, 1966) does not occur in all species (cat PTG: CAPEN and ROWLAND, 1968; horse PTG: FUJIMOTO *et al.*, 1967; human PTG: ALTENÄHR and SEIFERT, 1971). In addition, the scheme shows cells of human PTG during chronic secondary hyperparathyroidism (activated light chief cell, small water-clear cell, transitional oxyphil cell) and the extreme cell types of human PTG (large water-clear cell, oxyphil cell) (Fig. 2).

The experimental ultrastructural investigations have confirmed that the calcium concentration of serum or culture medium is the essential factor regulating the endocrine activity of the PTG.

A *reduction in serum calcium* results in *stimulation* of PTG cells (MONTSKO *et al.*, 1963; ROTH and RAISZ, 1964, 1966; CAPEN and ROWLAND, 1968; ROTH *et al.*, 1968; ALTENÄHR, 1970; ALTENÄHR und LIETZ, 1971):

A low-calcium diet produces hypocalcaemia and ultrastuructural signs of activation in the PTG (ROTH *et al.*, 1968; CAPEN and ROWLAND, 1968b; ALTENÄHR, 1970) (Fig. 3).

Administration of phosphates also results in stimulation of PTG (LEVER, 1958; LANGE and von BREHM, 1963; MELSON, 1966; STOECKEL and PORTE,

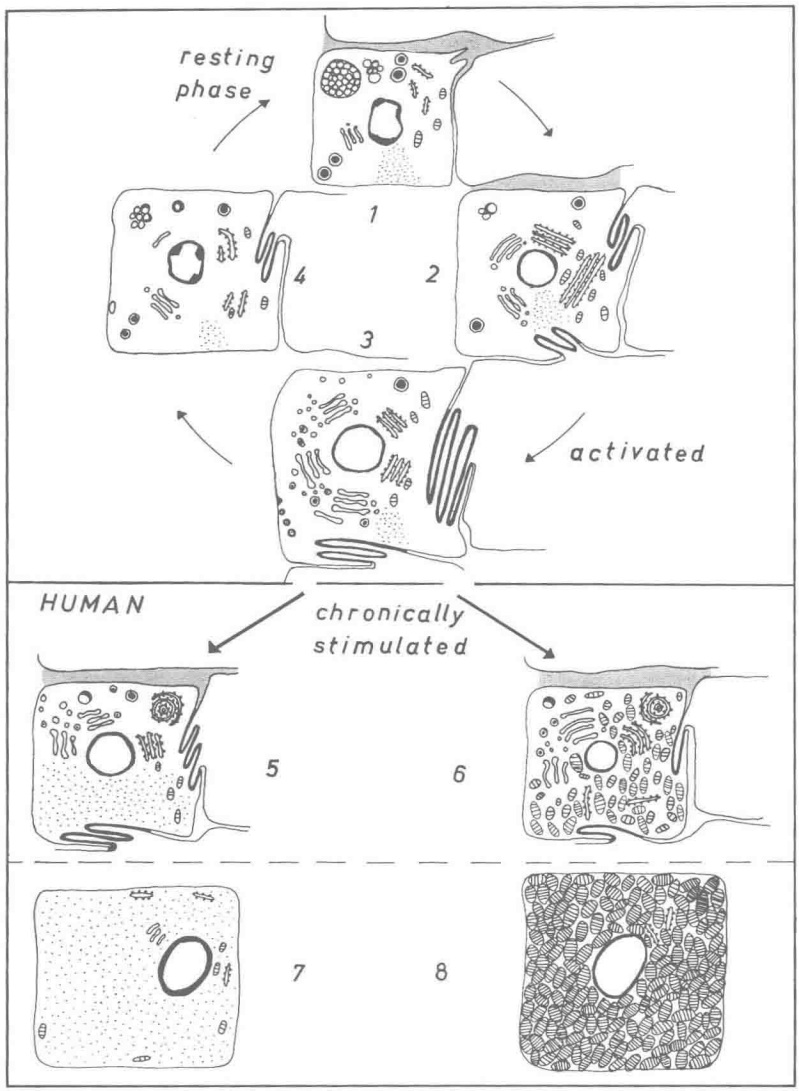

Fig. 2. Diagram demonstrating the secretory cycle and functional changes of PTG cells: During transformation of PTG cells from the resting phase (1) to the active phase (*2*, *3*) the endoplasmic reticulum first increases in size, followed by an increased Golgi complex, the number of prosecretory granules subsequently becoming more numerous. The tortuosity of cell membranes and interdigitations increases. At the same time the number of lipid vacuoles in the cytoplasm diminishes.—During regression (*4*) from the active to the resting phase (*1*) the size of the protein-synthesizing apparatus decreases and lipid vacuoles become more numerous again. Tortuosity of cell membranes diminishes.—Under conditions of chronic stimulation (secondary hyperparathyroidism), human PTG show glycogen-rich light chief cells and small water-clear (*5*), containing in addition an extended protein-synthesizing apparatus. Oxyphil chief cells or transitional oxyphil cells (6) containing numerous mitochondria may also exhibit a prominent protein-synthesizing apparatus in secondary hyperparathyroidism.—Extreme cell forms of human PTG are the large water-clear cell (*7*) and the typical oxyphil cell (*8*) with an inconspicuous or absent protein-synthesizing apparatus

1966b; ALTENÄHR, 1970), obviously by producing hypocalcemia (AURBACH and POTTS, 1969; ALTENÄHR, 1970).

Following repeated applications of thyrocalcitonin, PTG also show ultrastructural signs of activation (ALTENÄHR, 1970). Since no specific changes in the ultrastructure were observed, as compared to other hypocalcemic conditions, it can be concluded that calcitonin also indirectly activates the PTG by a transient hypocalcemia, and that it does not exert a direct stimulating influence on the PTG (ALTENÄHR, 1970).

Ultrastructural studies of PTG in animals with experimental *renoprived hyperparathyroidism* demonstrated the same changes of increased cellular activity (LEVER, 1958; DAVIS and ENDERS, 1961; MAZZOCCHI *et al.*, 1967b). The stimulus for this activation has not been definitely clarified so far.

An *elevation of serum calcium* produced by calcium infusions, oral calcium, parathyroid hormone (Fig. 4), or dihydrotachysterol causes the ultrastructural changes of inactivation of the PTG cells (MONTSKO *et al.*, 1963; ROTH and RAISZ, 1964, 1966; STOECKEL and PORTE, 1966b; HARA and NAGATSU, 1968; ALTENÄHR, 1970; ALTENÄHR and LIETZ, 1970).

Controversial results have been obtained following *vitamin D administration* and in *experimental rickets*. CAPEN *et al.* (1965b, 1968) describe inactivation and atrophy of the PTG, following administration of vitamin D to normal, pregnant and lactating cows. KLOTZ *et al.* (1966), however, did not observe any changes in dog PTG. Following administration of a rachitogenic diet to rats, MAZZOCCHI *et al.* (1967b) observed activation of the PTG, while ROTH *et al.* (1968) could not find any influence of vitamin D or rickets on the ultrastructure of PTG.

Drinking water containing high doses of *fluoride* causes hyperplasia in sheep PTG and ultrastructural changes typical of stimulation (FACCINI and CARE, 1965). FACCINI (1969) interprets this observation by assuming a diminished resorption of calcium from the fluoroapatite-containing bone, and thus an increased demand for parathyroid hormone. However, RAISZ and TAVES (1967), using biochemical methods, did not find increased PTG activity after fluoride administration.

LUPULESCU *et al.* (1968) studied dog PTG in *experimental isoimmune hypoparathyroidism* following repeated injections of emulsions of dog PTG together with Freund's adjuvant over a period of four months. Using light microscopy, they observed atrophy and disorganization of the cellular pattern, lymphoplasmocytic infiltration and progressive sclerosis. Electron microscopically, these glands showed atrophy of the endoplasmic reticulum, irregular swollen and vacuolized mitochondria with ruptured cristae and an irregular ragged nuclear membrane. The number and size of secretory and prosecretory granules were reduced. These changes explain the reduction in the hormone secretion rate and the resulting disturbance of phosphate and calcium metabolism in these animals. – No morphological studies of experimental immune parathyroiditis caused by highly purified parathyroid hormone have yet been published. Experimental investigation of immune parathyroiditis will become more important, because human idiopathic hypoparathyroidism is now considered to

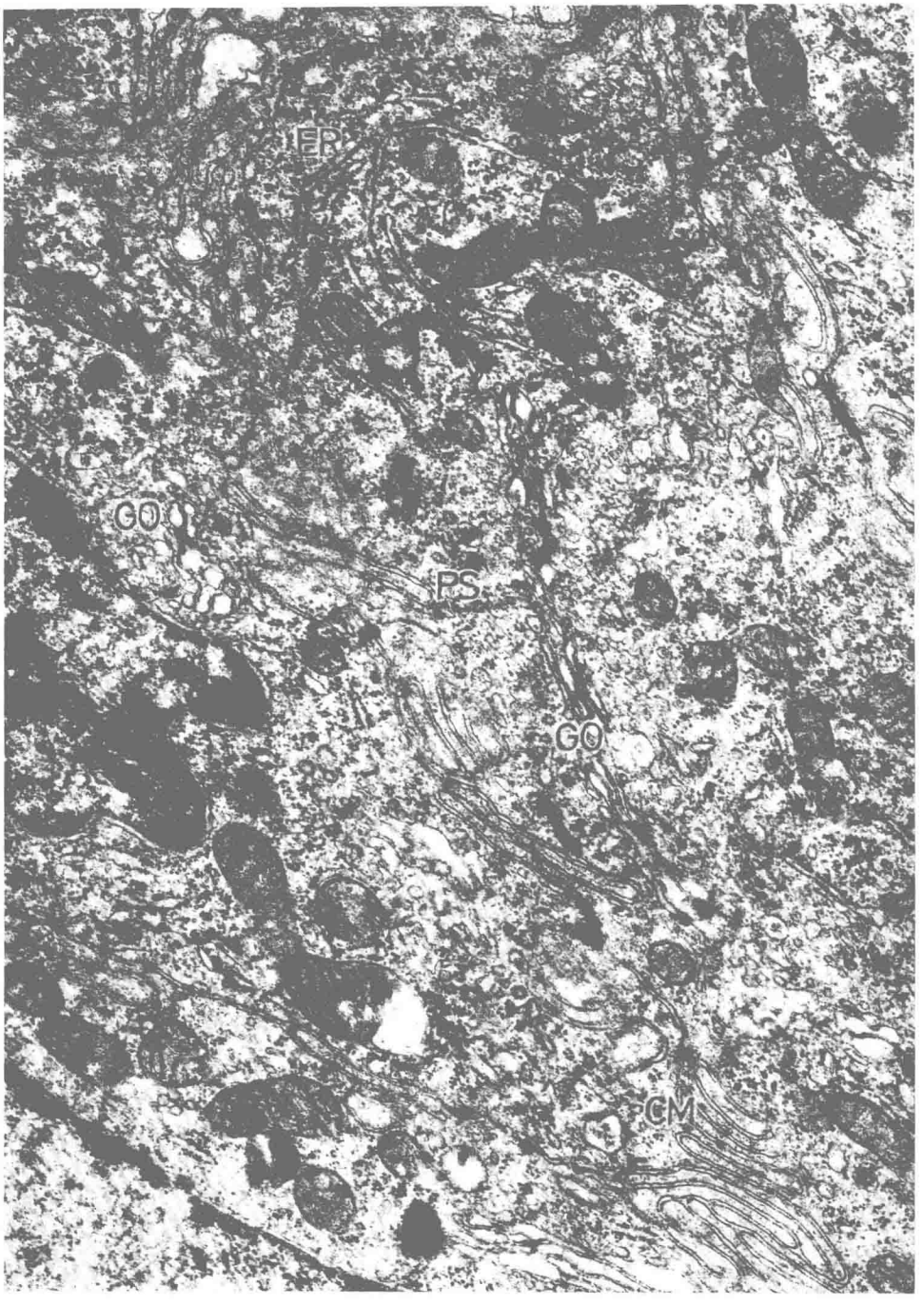

Fig. 3. Rat PTG stimulated by administration of a low-calcium, low-phosphate diet for 4 weeks: Dark cytoplasm, rich in cell organelles, with numerous ribosomes, extended rough endoplasmic reticulum (*ER*) and Golgi complex (*GO*). *PS* = prosecretory granules, *CM* = tortuous cell membrane. 32000 ×

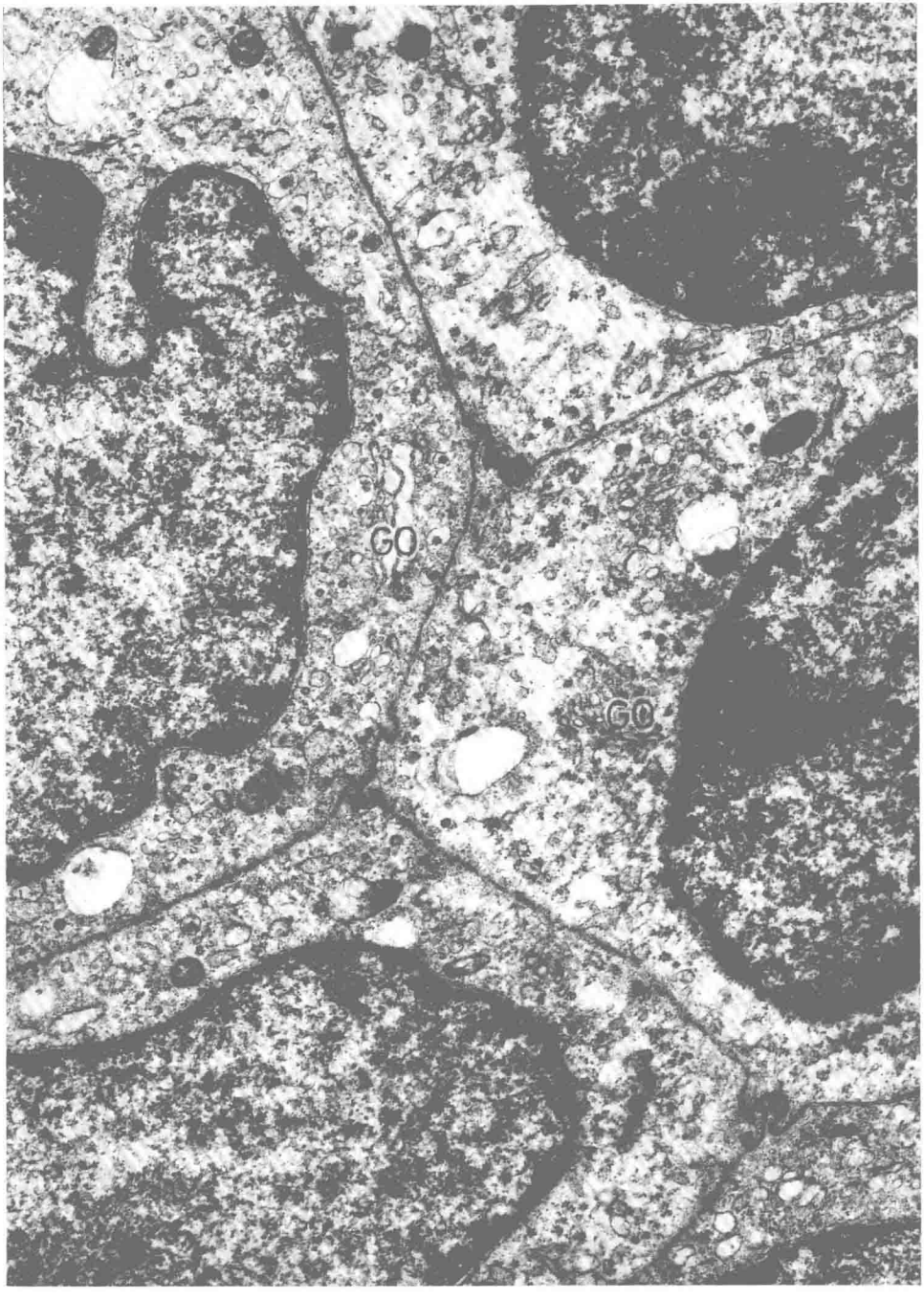

Fig. 4. Suppressed rat PTG after subcutaneous injections of parathyroid hormone for 8 days (twice daily 20 USP Units PTH Lilly): Organelle-depleted light cytoplasm, fewer ribosomes, diminished size of the rough endoplasmic reticulum and of the Golgi complex (*GO*), straight cell membranes. 17000 ×

be an autoimmune disease (BLIZZARD, 1969). Results of ultrastructural studies of human PTG in idiopathic hypoparathyroidism are not yet available.

Nervous regulation of parathyroid function is unknown. Light microscopically, RAYBUCK (1952) observed unmyelinated nerves in close contact with endocrine cells in rat PTG. Electron microscope studies have repeatedly demonstrated nerves in perivascular spaces of PTG, but no innervation of endocrine epithelial cells has been described by other electron microscopists (ROGERS, 1963; MAZZOCCHI *et al.*, 1967b; NAKAGAMI, 1967). Apart from an innervation of arterioles, we were able to demonstrate neuroepithelial synapses of autonomous neurons with chief cells (ALTENÄHR, 1971). This makes a nervous influence on endocrine functions of PTG cells seem possible, although no corresponding experimental results are as yet available.[1]

C. Ultrastructure of Animal Parathyroid Glands under Special Physiological Conditions

Ultrastructural studies of PTG of *growing* cats (CAPEN and ROWLAND, 1968) and pigs (FETTER and CAPEN, 1970) have shown somewhat differing results. While fast-growing cats show predominantly active chief cells, rich in cell organelles, the authors found mainly inactive chief cells with transparent cytoplasm and fewer organelles in young pigs. These results do not allow any conclusion concerning endocrine function of PTG in growing animals.

At the end of *pregnancy*, immediately before the calculated date of parturition, PTG of cows show a definite activation in their ultrastructure. This continues after parturition and reaches maximum activation at the beginning of lactation, 20 hours after parturition (CAPEN *et al.*, 1965a). These authors consider the rapid calcification of fetal bones at the end of pregnancy and the increased calcium demand of the lactating glands after parturition to be the reason for the PTG activation. Serum calcium level of cows reaches the lower limit of normal at this time and obviously has to be kept at this level by increased PTG function.

Ultrastructural investigations of the PTG of *laying hens* show a special physiological activation (NEVALAINEN, 1969). This is obviously the result of the increased calcium requirement for egg shell formation.

Seasonal changes of PTG ultrastructure in Triturus pyrrhogaster (Boié) have been investigated by SETOGUTI et al. (1970a, b). They observed signs of increased activity in the spring as compared to hibernation.

COLEMAN (1969) studied and discussed PTG changes during the *metamorphosis* of Xenopus laevis (Daudin) in the larva and mature toad. There are striking changes in the ratio of light and dark cells, but the author does not come to definite conclusions in regard to endocrine activity.

[1] *Note added in proof:* Since completion of this article an experimental light and electron microscopical study by G. M. SALZER (Acta endocrin., Copenhagen, **68**, Suppl. 157, 1–64, 1971) appeared, which indicates some influence of pituitary gland on parathyroid glands and C-cells.

D. Ultrastructural Pathology of Animal Parathyroid Glands During Different Spontaneous Diseases

1. Parturient Paresis of Cows with Hypocalcaemia

The parturient paresis of cows with hypocalcaemia represents a spontaneous metabolic disease. It develops in lactating cows during the first few days following parturition and is characterized by hypocalcaemia, tetany, and eventually pareses and coma. The PTG cells of these cows show the morphological changes typical of increased activity and hormone production: increased tortuosity of cell membranes with multiple interdigitating cytoplasmic processes, prominent Golgi complexes with numerous prosecretory granules, lamellar aggregates of granular endoplasmic reticulum, multiplication of ribosomes, reduction in mature secretory granules (CAPEN and YOUNG, 1967a). At the same time, the parafollicular cells of thyroid gland are depleted of secretory granules, and their Golgi complexes and endoplasmic reticula are only poorly developed. The number and size of parafollicular cells are reduced and they are cytologically inactive.

CAPEN and YOUNG (1967a) therefore assume that the abrupt discharge of stored thyrocalcitonin from the parafollicular cells causes this hypocalcaemia and hypophosphataemia. Corresponding to the morphological findings, these animals show a reduction in stored thyrocalcitonin in the thyroid gland as determined by bioassay (CAPEN and YOUNG, 1967b) and an increased serum level of parathyroid hormone as demonstrated by immunoassay (SHERWOOD et al., 1966). These results allow two interpretations of the course and mechanism of this disease:

1. Unknown stimulus (?)—discharge of stored thyrocalcitonin from thyroid C-cells—hypocalcaemia and hypophosphataemia with parturient paresis—activation of PTG. or

2. Subnormal serum calcium level after delivery and during lactation—inactivity of C-cells and activation of PTG—transient hypercalcaemia (?, not demonstrated)—secretion of stored thyrocalcitonin—hypocalcaemia and hypophosphataemia with parturient paresis.

2. Osteopetrosis of Chicken

This disease is characterized by slight hypocalcaemia and irregular subperiosteal and endosteal fibrous bone formation, predominantly at the diaphyses of the long bones. The aetiological agent is considered to be a virus (SIMPSON and SANGER, 1968) which can be demonstrated in and next to osteoblasts and in cells of various endocrine glands. YOUSHAK and CAPEN (1970) electron microscopically observed in the PTG intercellular aggregates of leucosis viruses and occasionally intracellular virus particles. In addition, the PTG show the ultrastructural changes typical of hyperactivity (prominent rough endoplasmic reticulum and Golgi complex, augmented production and secretion of prosecretory granules, reduction of storage granules). The raised