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Suppression of Parathyroid Gland Activity by Magnesium

Morphometric Ultrastructural Investigation*

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Summary. Hypocalcaemia (and hypophosphataemia) with significant activation of parathyroid glands (PTG) in rats is produced by a calcium and phosphate deficient diet. During administration of a 0.1 M solution of calcium gluconate as drinking water, plasma calcium levels remain within normal limits; administration of 0.1 M magnesium aspartate results in hypermagnesaemia in addition to the hypocalcaemia. The activation of PTGs is inhibited by substitution of calcium as well as by administration of magnesium; glandular cells show normal activity ultrastructurally. Morphometric analysis of cellular organelle contents demonstrate a quantitatively equal inhibition of PTG activity by both electrolytes. It appears that calcium and magnesium are freely interchangeable in their suppressive action on PTGs.

Zusammenfassung. Bei Ratten wurde mit einem calcium- und phosphorarmen Futter eine Hypocalcämie (und Hypophosphatämie) mit deutlicher Aktivierung der Nebenschilddrüsen (NSD) erzeugt. Bei gleichzeitiger Substitution mit einer 0,1 M Lösung Calcium-Gluconat als Trinkwasser blieben die Plasmacalciumwerte normal; bei gleichzeitiger Gabe einer 0,1 M Lösung Magnesium-Aspartat entwickelte sich neben der Hypocalcämie eine Hypermagnesiämie. Sowohl durch die Substitution mit Calcium als auch durch die Gabe von Magnesium wurde die Aktivierung der NSD verhindert; die Drüsenzellen sind ultrastrukturell normaktiv. Die morphometrische Analyse des Organellengehaltes ergab quantitativ eine gleich starke Hemmung der NSD-Aktivität durch beide Lösungen. In ihrer suppressiven Wirkung auf die NSD sind Calcium und Magnesium offenbar frei austauschbar.

Introduction

Regulation of parathyroid hormone (PTH) secretion by plasma calcium concentration has been proven in vivo as well as in vitro (Sherwood *et al.*, 1968, 1969). Furthermore, numerous investigations of recent years have demonstrated that magnesium ions also exert a regulatory influence on synthesis and secretion of PTH. An indirect indication of a stimulation of parathyroid glands (PTGs) is the hypercalcemia which is produced by experimental magnesium deprivation in vivo and which is dependent on intact PTGs (Heaton, 1965; Gitelman *et al.*, 1968a). On the other hand PTG dependent hypocalcaemia caused by experimental hypermagnesaemia has been demonstrated indicating a reduced PTH secretion (Gitelman *et al.*, 1968b; Massry *et al.*, 1970). A raised magnesium concentration in plasma or culture media reduces the incorporation of amino acids into PTG tissue indicating a diminished protein and proteohormone synthesis (Au *et al.*, 1966; Raisz, 1967). Care *et al.*, (1966) as well as Buckle *et al.* (1968) perfused in vivo isolated PTGs with blood of different magnesium concentrations and measured the plasma PTH concentration of the venous blood using the radioimmunoassay.

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In these experiments both groups observed a reversed correlation of magnesium concentration in the perfused blood and plasma PTH levels in the venous blood. In vitro it was possible to compare quantitatively the regulatory influence of calcium and magnesium concentrations on PTG secretion using the radioimmunoassay technique. In these experiments calcium and magnesium exerted an equivalent inhibitory influence on hormone secretion. When calcium and magnesium were mixed, merely the sum of the concentrations determined the inhibitory influence (Targovnik *et al.*, 1971).

So far Roth and Raisz (1964) only studied the influence of magnesium on PTGs electronmicroscopically. These authors observed in their in vitro investigations typical electronmicroscopical signs of cell activation in low calcium culture medium. Addition of strontium or magnesium, however, did not produce any morphological signs of a reduction of increased gland activity. Our dietetic experiments were planned to investigate if an in vivo inhibition of PTG activity by magnesium can be demonstrated electron microscopically. The ultrastructural findings have been evaluated visually as well as by morphometry.

Material and Methods

40 male Wistar rats weighing 100 to 120 g have been used. 4 groups of 10 animales were formed. For five weeks these animals were fed either normal food or a calcium and phosphate deficient diet contaning 0.07% calcium (= 7% of the requirement) and 0.10% phosphorus (= 15% of the requirement) in the dry substance; this diet was prepared from 71.5% maize flour, 22.5% casein, 4.0% yeast, and 2.0% NaCl in destilled water. As drinking fluid the animals received either tap water, destilled water, 0.1 M calcium gluconate, or 0.1 M magnesium aspartate ad libitum (Table 1).

Table 1. Experimental conditions

Group	Food	Drinking fluid
I	normal food	tap water
II	Ca and P deficient diet	destilled water
III	Ca and P deficient diet	0.1 M calcium gluconate
IV	Ca and P deficient diet	0.1 M magnesium aspartate

Body weight, plasma levels of calcium, phosphorus and magnesium were determined in all animals prior to the experiment and afterward in weekly intervals. Calcium was determined using flame photometry; phosphorus and magnesium were determined colorimetrically using the "Biochemica Test Combination Phosphorus and Phosphatides" of Boehringer Mannheim G.m.b.H. and the test combination "Merckotest Magnesium" of Merck A.G. Darmstadt, respectively.

Parathyroidectomy was performed under ether anesthesia after five weeks. PTGs were fixed for 2 hours in 3% glutaraldehyde in 0.1 M cacodylate buffer pH 7.3, washed in cacodylate buffer and postfixed in 1.3% osmic acid in cacodylate buffer. After dehydration in increasing alcohol concentrations and propylenoxide the PTG were embedded in Epon 812. Blocks were trimmed using a LKB -Pyramitome[1]; ultrathin sections were cut with a Reichert-ultramicrotome OM 2 and contrasted with uranyl acetate and lead citrate. Electron microscopy was carried out using a Philips EM 300.

1 The LKB Pyramitome is a kindly gift of the Otto-Foundation.

The quantitative ultrastructural analysis was performed according to the method described by Reinhardt *et al.* (1969) with minor modifications. Using random sample conditions, 5 micrographs each were taken from different regions of the PTGs of 5 animals from each group at a primary magnification of 3200, photographically enlarged, resulting in a final magnification of 10000. A reticle with 400 crossing points was projected onto the photography and the cell structures hit were counted. The following cell structures were considered: 1. nucleus, 2. Golgi complex including prosecretory granules and secretory granules (the latter are rarely visible in rat PTGs), 3. rough endoplasmic reticulum and areas with aggregated ribosomes, 4. mitochondria, 5. cell membranes and regions of interdigitations of cell membranes, 6. ground cytoplasm and not identifyable elements. Interstitial spaces, capillaries, and intramitochondrial vacuoles (group II) were not counted. Nuclei and cell membrane areas were related to total cell volume (%), Golgi complex, rough endoplasmic reticulum, mitochondria, and ground plasma to the total cytoplasm (= total cell volume minus nucleus minus cell membrane areas).

Results

A. General

Animals of groups II to IV (calcium and phosphate deficient diet) showed a significant reduction in their general condition. Weight gain was considerably less as compared to the normal controls. The fur was bristly. The most pronounced changes of the general condition were found in the animals of group II.

B. Plasma Electrolytes (Fig. 1)

Plasma calcium levels were within normal limits during the 5-week-period in animals from group I (normal controls) and group III (calcium gluconate substituted animals). Animals receiving the calcium and phosphate deficient diet without substitution (group II) and animals treated with magnesium aspartate (group IV) showed a significant hypocalcaemia.

Plasma phosphorus levels of normal controls were between 7 and 8 mg/100 ml. All animals on calcium and phosphate deficient diet (group II-IV) showed significantly reduced levels. The lowest plasma phosphorus levels were observed in calcium gluconate substituted animals (group III).

Plasma magnesium level was low in animals of group II and III but not significantly reduced compared to normal controls. Animals on magnesium aspartate (group IV) exhibited a significantly raised plasma magnesium level.

C. Electron Microscopical Findings

PTGs of animals on calcium and phosphate deficient diet without substitution (group II) showed the typical signs of a cellular activation with increased protein synthesis (Fig. 3) in comparison to animals of control group (Fig. 2). An extension of the rough endoplasmic reticulum and the Golgi complex with increased number of prosecretory granules were observed. Mitochondria were numerous, enlarged, their structure less dense, often associated with hydropic vacuoles and swelling. Cell membranes showed an increased tortuosity with multiple interdigitations.

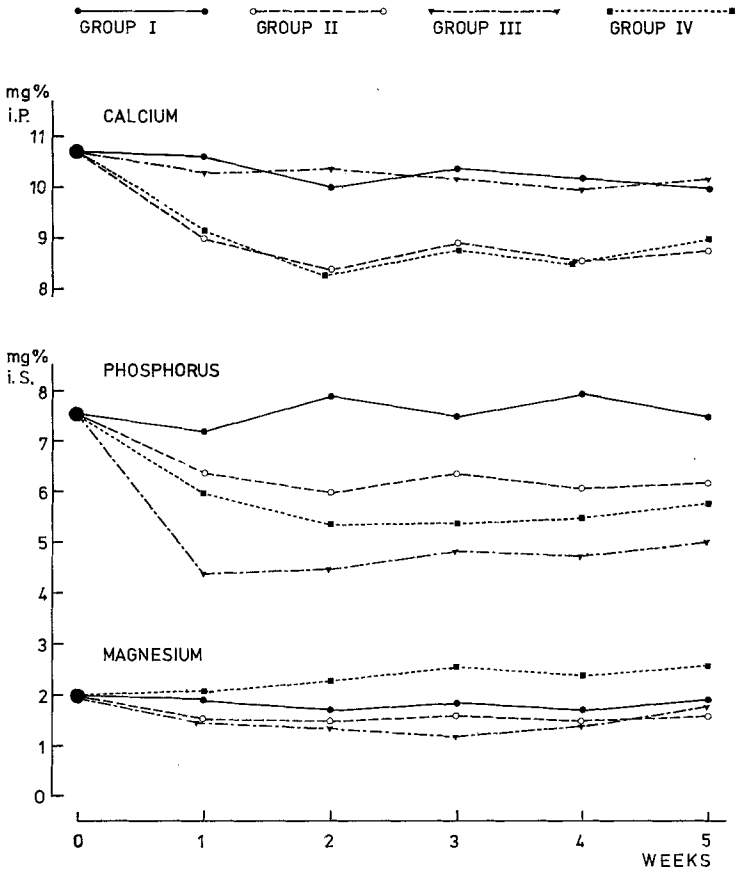

Fig. 1. Plasma levels of calcium, phosphorus, and magnesium during the 5-week-experiment in groups I to IV

Organelle free cytoplasm was relatively diminished. In relation to the general increase in cytoplasm the nuclear volume appeared reduced.

PTGs isolated from animals on calcium and phosphate deficient diet substituted with 0.1 M calcium gluconate (group III, Fig. 4) as well as from animals on calcium and phosphate deficient diet treated with magnesium aspartate (group IV, Fig. 5) did not differ from normal controls as regards to the organelle contents of the PTG cells. These PTGs demonstrated a normal cellular activity.

Morphometrically determined values of the nucleus and cell membrane areas were related to the total cell volume (Fig. 6); the values for rough endoplasmic reticulum, Golgi complex, mitochondria, and ground plasma are given as percentage of the total cytoplasm (= total cell volume minus nucleus minus cell membrane areas) (Fig. 7). The visual impressum of organelle distribution was verified by this quantitative analysis. Differences in nucleus/plasma relation are not yet significant considering the limited number of evaluations performed and the fact that they can not be determined exactly on ultrathin

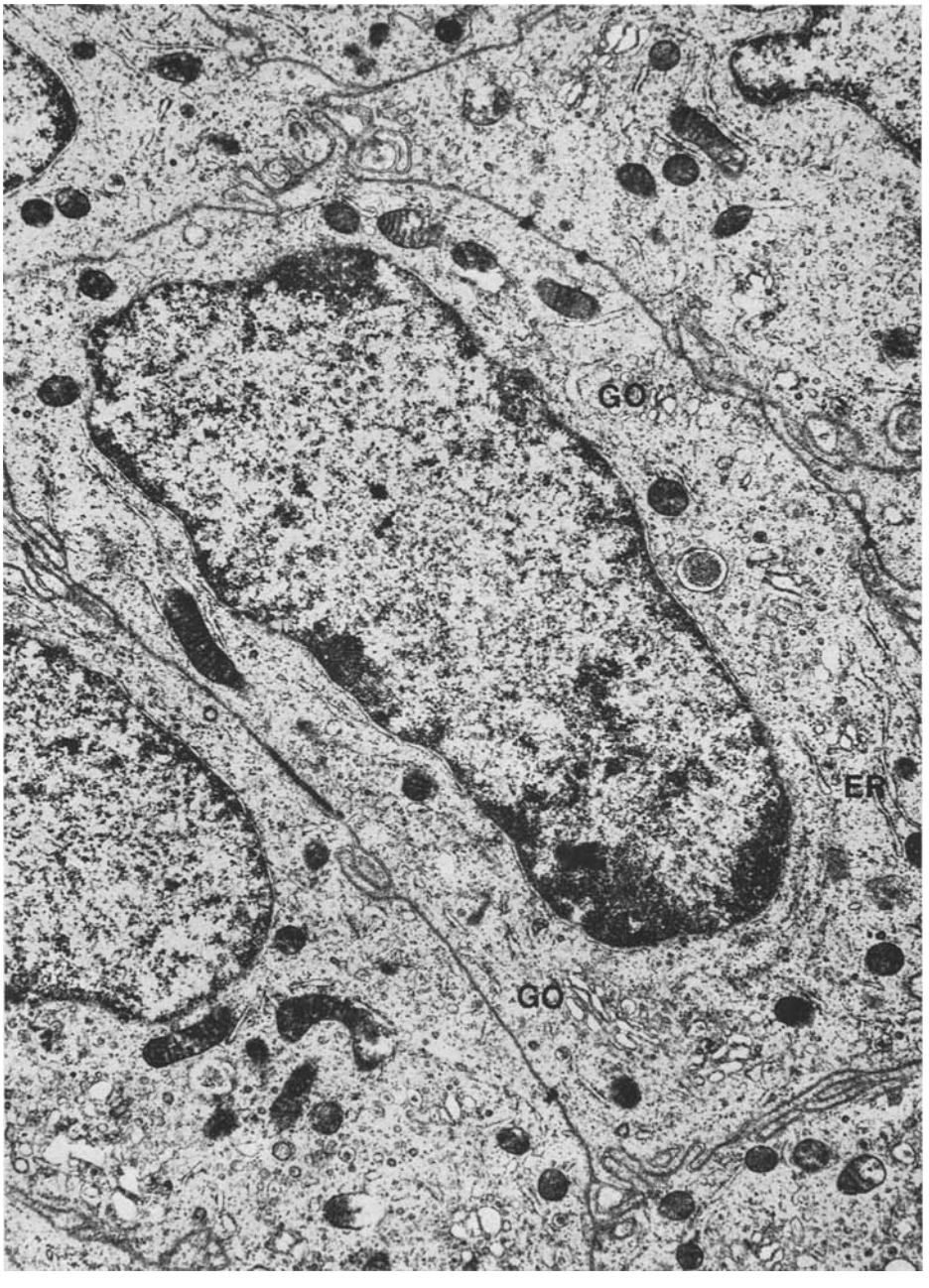

Fig. 2. Normal controls (group I): Normally active PTG cells. Little extension of endoplasmic reticulum (*ER*) and Golgi complex (*GO*). Mainly staight, only occasionally tortuous cell membranes. ×13500

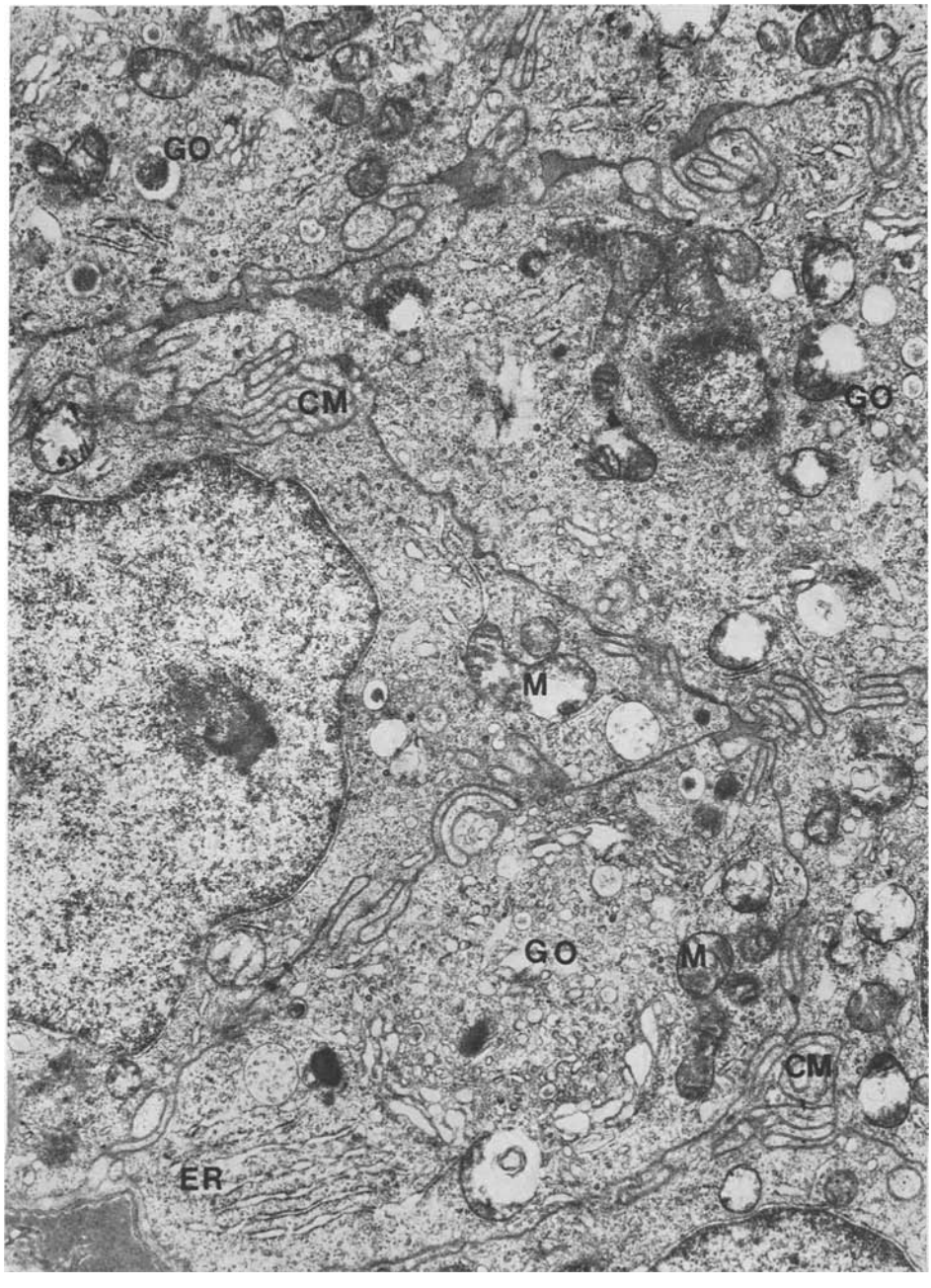

Fig. 3. Calcium and phosphate deficient diet (group II): Activated PTG cells with organelle rich cytoplasm. Rough endoplasmic reticulum (*ER*) and Golgi complex (*GO*) extended. Mitochondria (*M*) enlarged and partly hydropically swollen. Cell membranes (*CM*) displaying increased tortuosity. ×13500

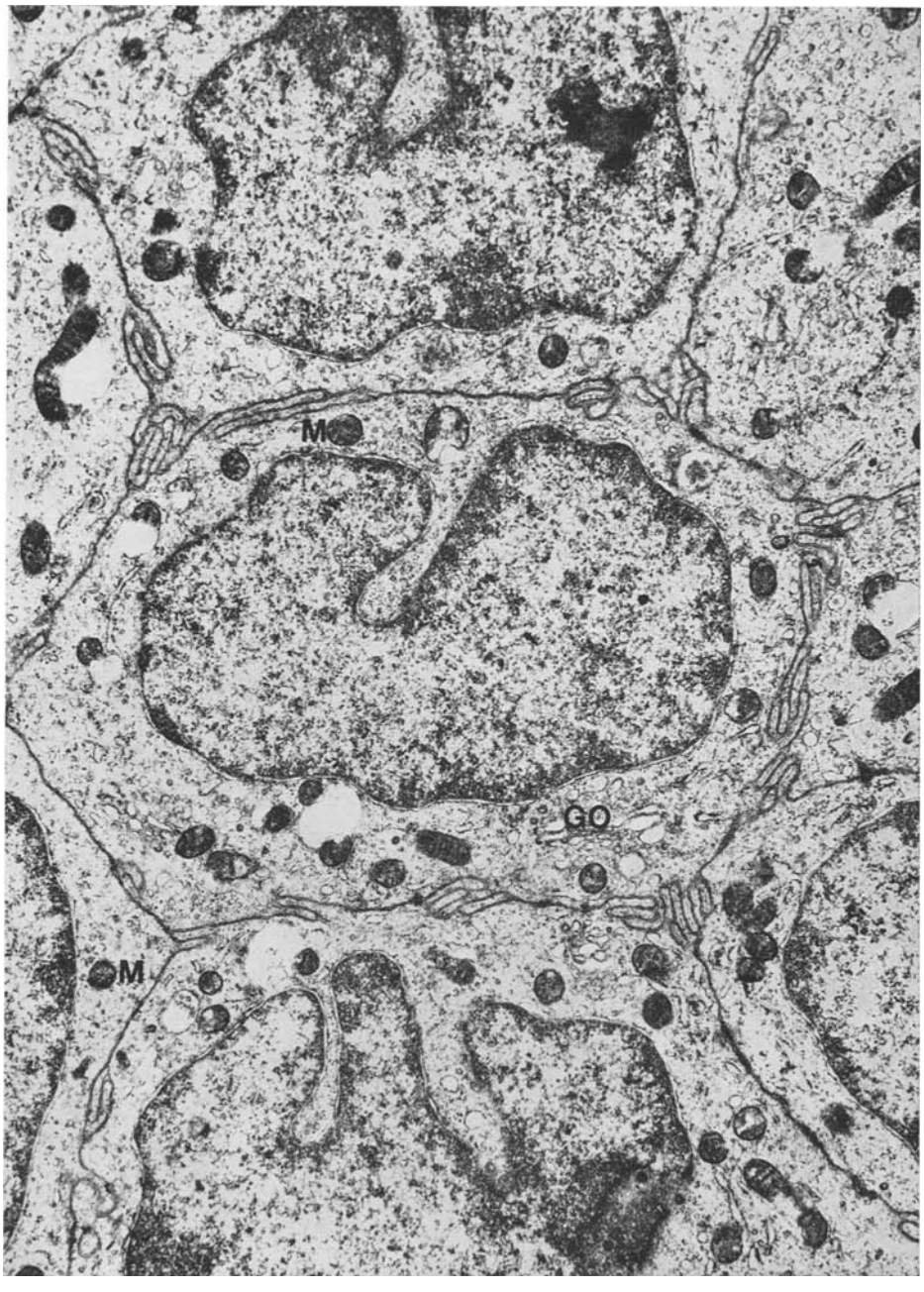

Fig. 4. Calcium and phosphate deficient diet plus calcium gluconate (group III): Normally active PTG cells with small Golgi fields (*GO*), few profiles of endoplasmic reticulum and small mitochondria (*M*). ×13500

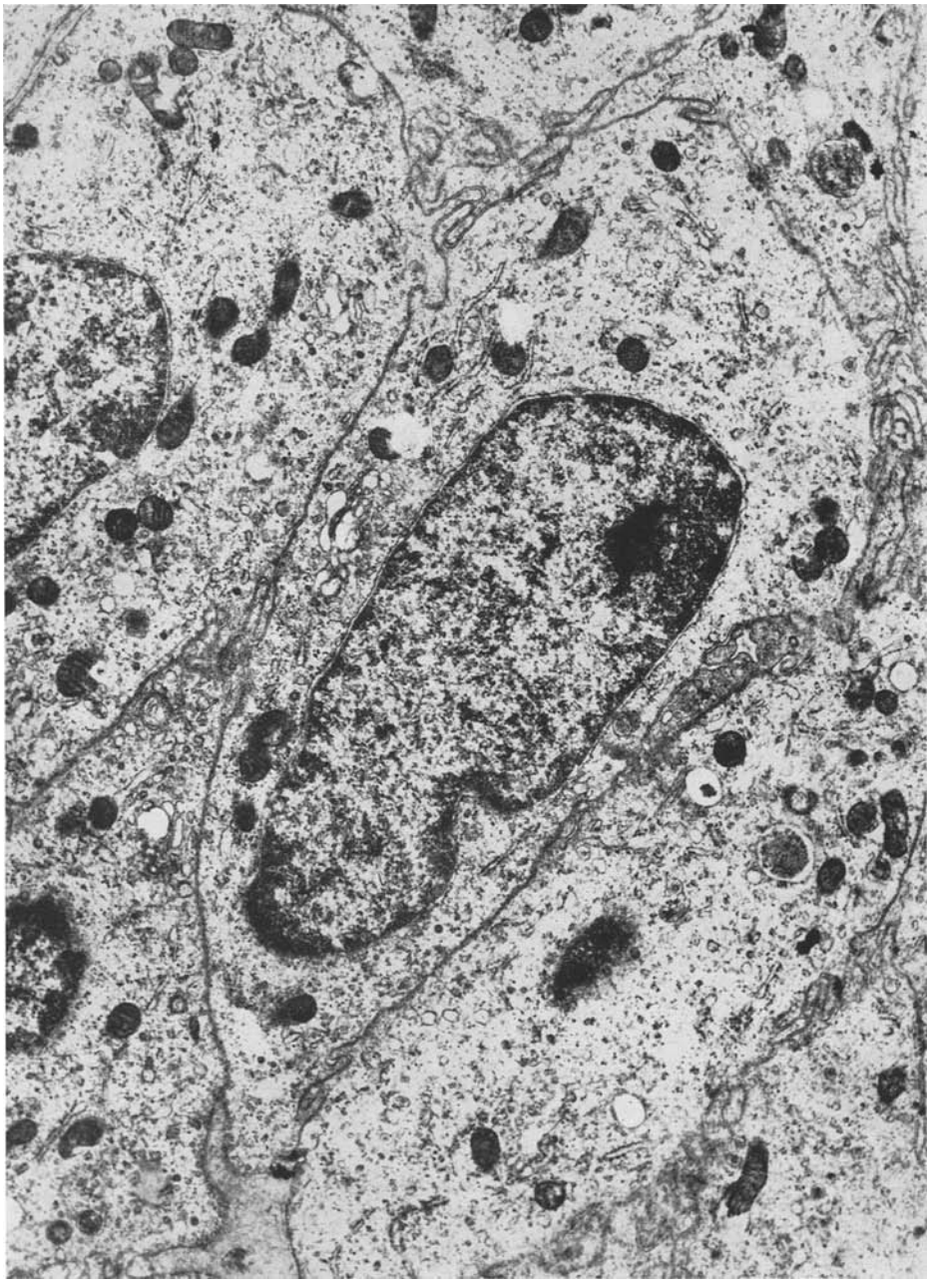

Fig. 5. Calcium and phosphate deficient diet plus magnesium aspartate (group IV): Normally active PTG cells with a relatively organelle deficient cytoplasm. ×13500

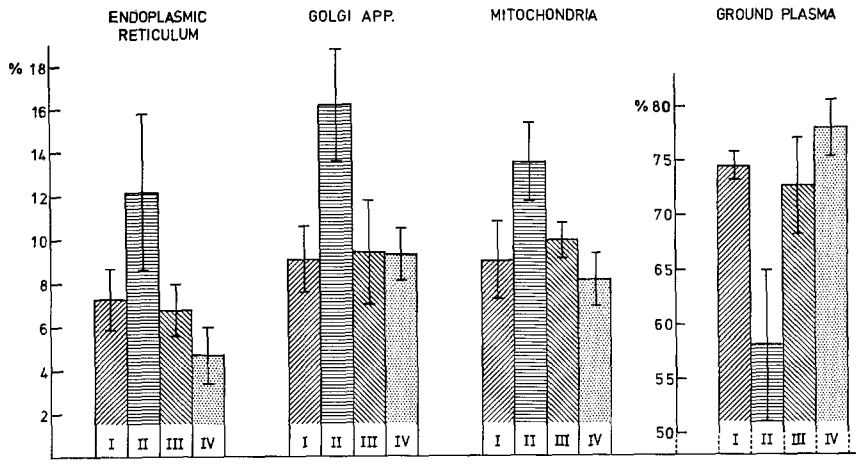

Fig. 6. Percentage of nuclei and cell membrane areas compared to the total cell volume in groups I to IV

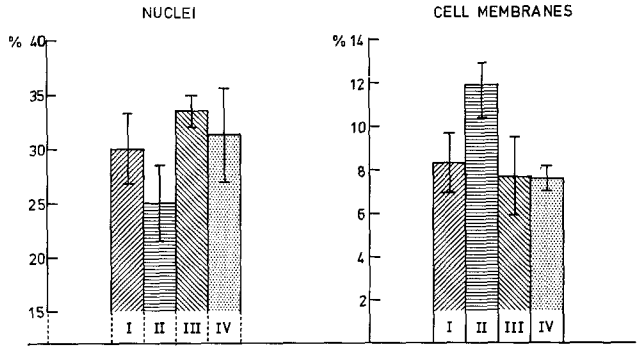

Fig. 7. Percentage of endoplasmic reticulum, Golgi complex, mitochondria and organelle free ground plasma compared to the total cytoplasm (= total cell volume minus nucleus minus cell membrane areas) in group I to IV

sections. It was found that all other cell compartments returned to normal under treatment with magnesium aspartate and calcium gluconate. In some cases magnesium appeared to be slightly more effective than calcium. It can be concluded that 0.1 M calcium gluconate and 0.1 magnesium asparate exerted an overall equal suppressive influence on PTGs.

Discussion

Numerous electronmicroscopical investigations have demonstrated function dependent differences in PTG cells. The organelle contents of the cytoplasm is an indicator of the endocrine activity of PTGs. When activated, mainly the organelles involved in protein and proteohormone synthesis and package of hormone are increased and extend over greater areas of the cytoplasm, i.e. the rough

endoplasmic reticulum with ribosomes, and the Golgi complex with prosecretory granules. The plasma membranes exhibit increased tortuosity and interdigitations (Lever, 1958; Roth and Raisz, 1964, 1966; Stoeckel and Porte, 1966; Mazzocchi *et al.*, 1967; Altenähr, 1970, 1972).

Group II received the same diet as it had been used in earlier investigations (Altenähr, 1970). The animals demonstrated a significant hypocalcaemia and electron microscopical signs of PTG activation. Using the same diet enteral resorption of calcium gluconate in the drinking fluid (group III) appeared to remain satisfactory resulting in a normal plasma calcium level. Enteral resorption of magnesium aspartate (group IV) was also satisfactory producing a hypermagnesaemia in these animals.

In contrast to the in vitro investigations of Roth and Raisz (1964), it was possible, under our experimental conditions in vivo, to suppress the diet induced PTG activation by addition of calcium gluconate as well as magnesium aspartate to the drinking fluid. Our electronmicroscopical results, however, are consistent with the results obtained using indirect and direct criteria of PTG function. Since mainly the organelles involved in hormone synthesis have been evaluated as ultrastructural criteria of glandular function, our results indicate that magnesium exerts an inhibitory influence not only on hormone secretion (Targovnik *et al.*, 1971) but also on hormone synthesis. The quantitative ultrastructural analysis of the cell compartments showed an equal inhibitory influence on PTG activity by 0.1 M magnesium aspartate and by 0.1 M calcium gluconate. Although experiments with varying calcium and magnesium concentrations and determinations of ionized calcium and magnesium have not been carried out, the equally effective inhibition by magnesium and calcium is in good agreement with the free interchangeability of these cations in the suppression of PTGs as demonstrated by Targovnik *et al.* (1971). As an explanation for this finding, a cation binding complex on the PTG cell surface or in it's cytoplasm is assumed, which binds calcium as well as magnesium ions and is saturated at higher cation concentrations (Sherwood *et al.*, 1971).

The inhibitory influence of magnesium on PTGs can now also be considered to be proven morphologically. Contradictory opinions have been published regarding the therapeutic usefulness of hypermagnesaemia in the suppression of secondary hyperparathyroidism in man (Pletka *et al.*, 1971; Stewart and Fleming, 1971).

It seems that C-cells of the thyroid gland respond to acute hypermagnesaemia in a similar way as to hypercalcaemia and that they are responsible for the initial hypocalcaemia in a magnesium overload, while the longer persisting hypocalcaemia in a magnesium overload is the result of PTG suppression (Radde *et al.*, 1969; Care *et al.*, 1971). More recent reports support the assumption that regulation of the PTG activity can also be achieved by hypophyseal (Salzer, 1971) and nervous (Altenähr, 1971) influences in addition to plasma calcium and magnesium concentrations.

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