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Parathyroid Function in Rats Treated with Growth Hormone

A Morphometric Study*

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Summary. Male rats weighing 220–250 g were injected i.m. daily with 0.1 mg commercial human growth hormone for 3 days or 12 days. The serum concentration of total calcium, phosphorus and alkaline phosphatase were significantly elevated for some days and returned to normal values at the end of the test period. The parathyroid glands, as studied by light and electron microscopical morphometry showed signs of reduced activity at the 4th day and also at the 13th day: a lowered nucleo-cytoplasmic ratio and a decrease of rough endoplasmic reticulum, of Golgi apparatus and of plasmalemmal tortuosity. The findings suggest a hypercalcemic effect of growth hormone involving peripheral organs of calcium metabolism, especially kidney and bone, and a secondary suppression of parathyroid glands by hypercalcemia.

The regulation of parathyroid gland (PTG) function by the serum calcium concentration is generally accepted (Raisz et al., 1965; Sherwood et al., 1968; Potts et al., 1968). A somewhat similar effect was noticed for magnesium (Buckle et al., 1968; Targovnik et al., 1971; Altenähr and Leonhardt, 1972). To what extent the nerve supply of the PTG has a control function is unknown (Altenähr, 1971; Norberg et al., 1975).

Whether higher endocrine control mechanisms also play a part in the hormone synthesis and secretion of the PTG is disputed. Krstic (1965, 1967) suspects an inhibitory effect of the pineal gland on the PTG. Pituitary effects on the PTG were suggested by Hertz and Cranes (1934), Anselmino et al. (1968), Zileli et al. (1968) and Salzer (1971). They mostly describe a decrease in size or inactivation of the PTG after hypophysectomy and an enlargement or stimulation of PTG after giving pituitary extracts. This would mean a parathyrotropic effect of the pituitary gland. Other authors have either doubted or rejected the evidence for an effect of the pituitary gland on the PTG (Eger and Grothe, 1954; Copp, 1969).

Under the influence of growth hormone (GH) similar peripheral effects to those produced by parathyroid hormone (PTH) were observed in calcium homeostasis, including renal and intestinal effects. Hereby a mobilization of calcium and an increase in urine hydroxyproline output took place (Hennemann et al., 1960; Corvilain and Abramov, 1964; Gershberg et al., 1967; Siebenmann et al., 1971). Montz et al. (1973) found similar changes to those in primary hyperparathyroidism in patients with acromegaly (apart from slight hypercalcemia) when radiocalcium kinetic studies were carried out. Salzer (1971) also points out that in

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pluriglandular syndromes with involvement of the parathyroid and the hypophysis mainly eosinophilic pituitary adenomas are associated with acromegaly. The question remains open whether the effect of GH on calcium homeostasis is a parathyrotropic one or if there is a direct GH effect on calcium metabolism which is independent of the PTG (Siebenmann et al., 1971). This paper is concerned with this problem.

Material and Methods

18 male Wistar rats weighing 220–250 gm were divided into three groups:

a) 6 rats were injected daily with GH i.m. for three days,

b) 6 rats were injected daily with GH i.m. for twelve days,

c) 6 rats were injected daily with saline i.m. for twelve days (controls).

The dose per injection for group a) and b) was 0.1 mg commercial GH which was isolated from human pituitary glands and stabilized with glycocol. The pH was set at 6.7–7.3 (Deutsche Kabi GmbH). This dose corresponds to 2 mg GH per m^2 body surface. The animals were fed with a normal standard diet (Altromin) and received tap water ad libitum.

Calcium, phosphorus and alkaline phosphatase in serum were measured on the first day before the test and then on the 2nd, 3rd, 4th, 8th, 11th and 13th days. Calcium was measured by flame photometry, phosphorus with the Zilversmit and Davis method (1950) and alkaline phosphatase according to Bessey et al. (1946).

The PTG were dissected in group a) on the 4th day and in group b) and c) on the 13th day of the experiment. They were fixed with 3% glutaraldehyde in 0.1 M cacodylate buffer and afterwards postfixed with 1.3% osmic acid and embedded in Epon. For light microscopical morphometry semithin (1 μ) sections were prepared and stained with toluidine blue. For ultrastructural morphometry ultrathin sections (400 Å) were cut on a Reichert OM U2 ultramicrotome and stained with uranyl acetate and lead citrate.

Morphometry was done by volumetric analysis according to Rohr (1970). In each parathyroid 30 fields of vision were studied with the light microscope at 1,000 times magnification and with a multipurpose screen (42 test points) the following structures were analysed: Cell nuclei of the PTG parenchymal cells; cytoplasm of the PTG parenchymal cells; extracellular space and interstitial tissue. Cell nuclei plus cytoplasm of PTG parenchymal cells were set as 100% and the relative volume of nuclei and cytoplasm to total cell volume were calculated. The extracellular space and the interstitial tissue were not considered.—Electron microscopically, on each animal 10 fields of vision of a PTG were photographed at 3,600 times magnification and then enlarged a further 6 times. The photographs were evaluated with a quadratic screen of 514 test points. The following compartments were differentiated: rough endoplasmic reticulum; Golgi apparatus + prosecretory granules; mitochondria; lysosomes + vacuolar bodies; ground plasma free of organelles; plasmalemmal areas including plasmalemmal interdigitations. These compartments were set as 100% cytoplasmic volume of PTG parenchymal cells and the relative volume of each compartment to the total cytoplasmic volume was calculated. The cell nuclei were not considered in ultrastructural volumetry, nor were the extracellular space and interstitial tissue. Previous pilot measurements had shown that examinations each with 10 fields of vision per parathyroid had only an error probability of 1.1% per cell compartment compared with examinations of 40 fields of vision.—The relative volume percents were expressed as mean values and standard deviations, calculated by the student—*t*- test.

Results

The serum calcium concentration was significantly increased from the 3rd to the 8th day of GH application and then returned to the initial value. The serum phosphorus concentration was increased from the 2nd to the 4th day of treatment with GH, on the 8th day it was significantly lowered and returned to normal values on the 11th and 13th day. The alkaline phosphatase concentration in serum

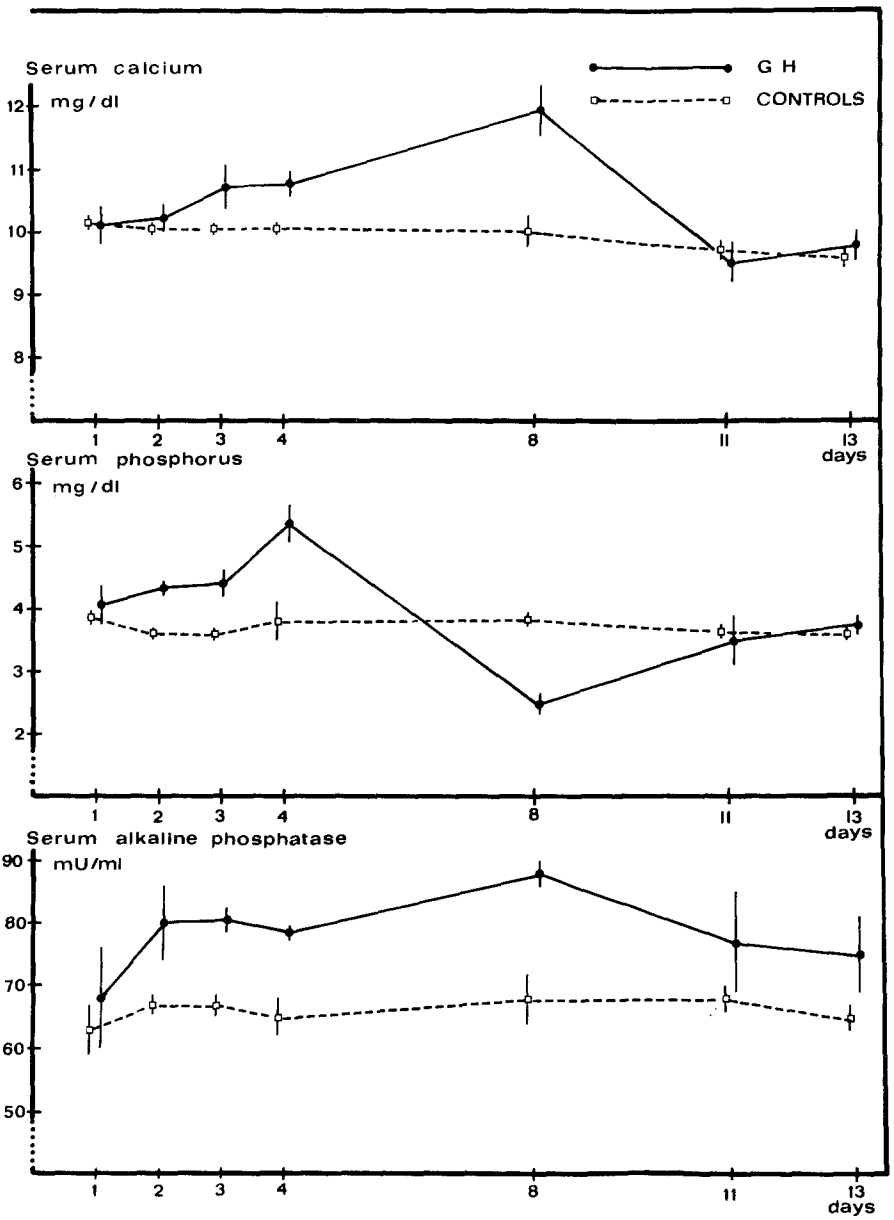

Fig. 1. Serum concentrations of calcium, phosphorus and alkaline phosphatase during the experimental period

was increased significantly from the 2nd to the 8th day of treatment with GH, at the end of the test period it was still elevated but no longer significantly (Fig. 1).

In light microscopic studies of semithin section we at first saw no noticeable differences between PTG of rats treated with GH and control rats. Morphometri-

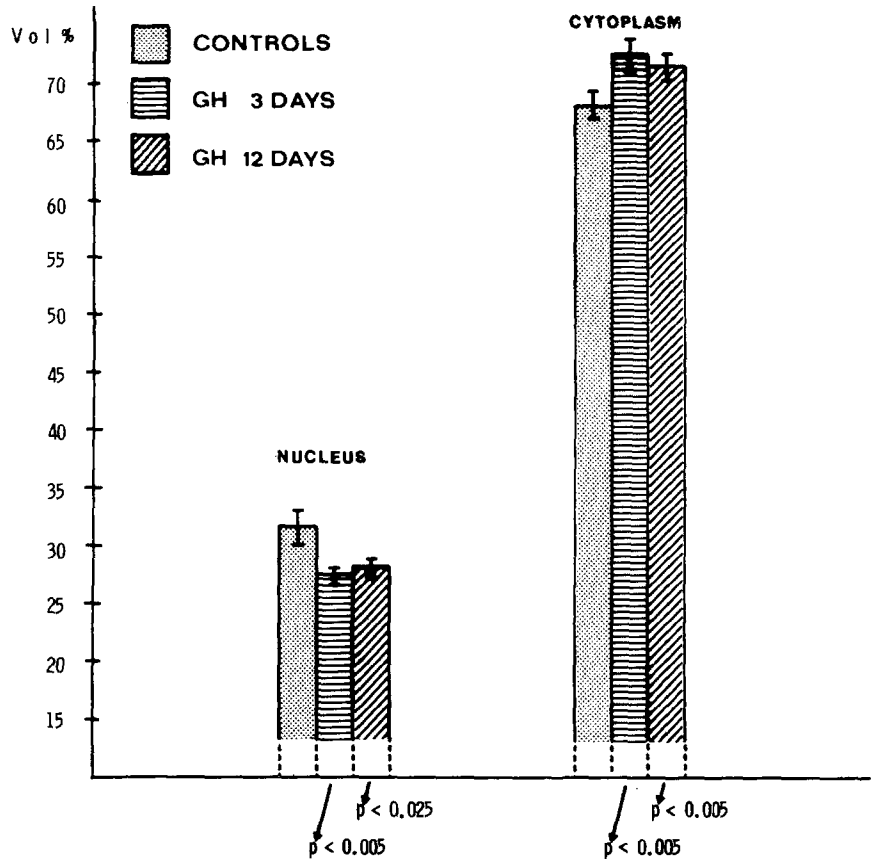

Fig. 2. Volume percents of nucleus and cytoplasm in total PTG cell volume. Significance p is calculated of test groups as against controls

cally after 3 days and also after 12 days of GH application there was a relative decrease in nucleus size and a relative increase in the volume of cytoplasm (Fig. 2).

Electron microscopically we saw in the control group the same cellular formation and organelle pattern of PTG as had been previously described in the rat (Altenähr, 1970; Fig. 3). The secretory (storage) granules which are relatively scarce in the rat PTG cells were not changed in the test animals, although with some of the test animals one could already perceive a diminution of organelles in the PTG cells in comparison to the control rats (Fig. 4). These changes were not so evident in all animals, thus morphometric evaluation was necessary to prove these results. After 3 days as well as after 12 days of GH application, the endoplasmic reticulum, Golgi apparatus and plasmalemmal areas were significantly reduced. On the other hand the ground plasma free of organelles was markedly increased. The relative volume of lysosomes and mitochondria was not significantly changed (Fig. 5).

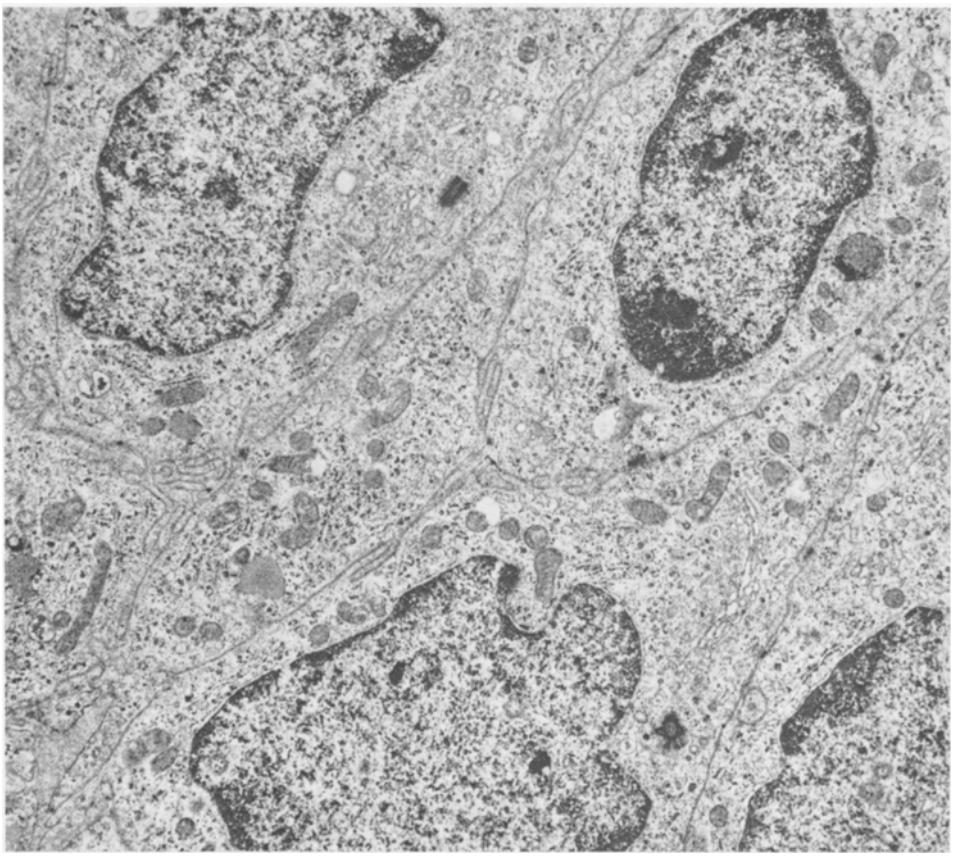

Fig. 3. Ultrastructure of PTG cells in a control rat. Normal organelle pattern of cytoplasm with marked rough endoplasmic reticulum and Golgi apparatus. × 10,000

Discussion

These experiments were performed on rats with human GH which has a heterologous effect on the rat (Daughaday and Parker, 1965). The concentrations of calcium, phosphorus and alkaline phosphatase rose during the first day of the experiments. The rise of the calcium and phosphorus concentrations suggests a simultaneous mobilization of calcium and phosphorus from bone. The rise of alkaline phosphatase points to a metabolic effect on bone cells.

An increase in bone remodelling was also demonstrated by Harris et al. (1972) and Heaney et al. (1972) after a long application of bovine GH in dogs. Delling et al. (1973) also pointed out increased bone remodelling in acromegalic patients. The temporary decrease in serum phosphorus concentration on the 8th day is probably due to an increased renal phosphorus output. We would like to attribute the normalization of the serum parameters at the end of the experiments to a counter-regulation mechanism.

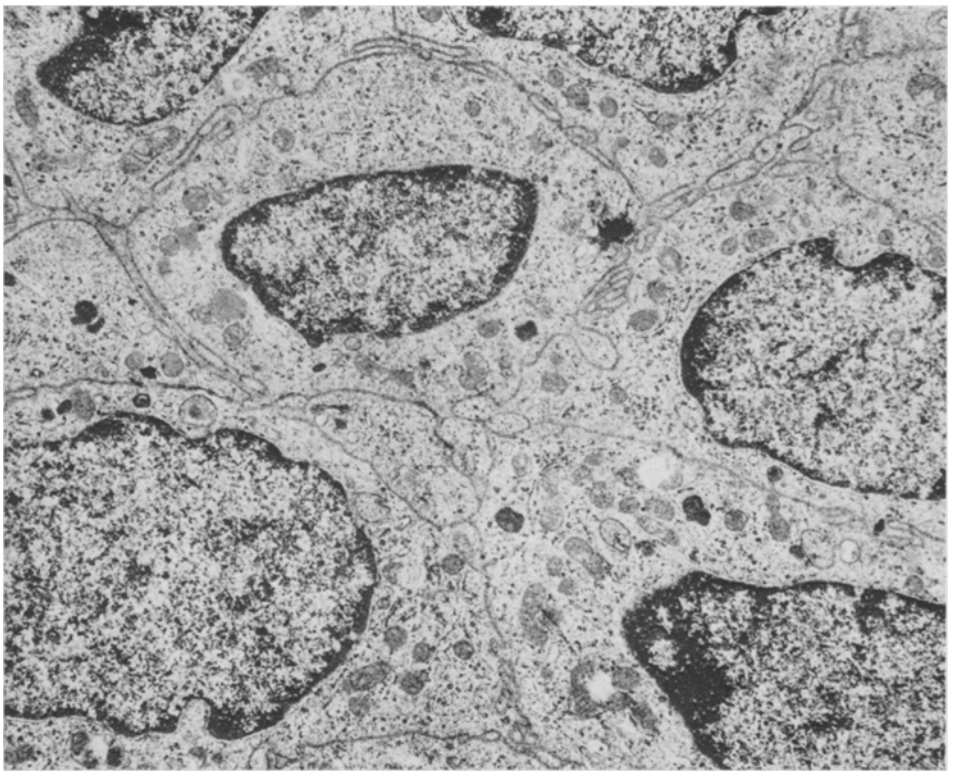

Fig. 4. Ultrastructure of PTG cells in a rat treated 12 days with GH. Relatively light cytoplasm with poor content of rough endoplasmic reticulum, no Golgi apparatus is seen in this field of vision. × 10,000

The volumetric data on the PTG cells include those cell parameters which are important for the estimation of parathyroid function and especially for the proteohormone synthesis. (Sandritter et al., 1955; Roth and Raisz, 1966; Capen, 1971; Altenähr, 1970, 1972). During the application of GH there is a decrease in the nucleocytoplasmic ratio and in the volume of rough endoplasmic reticulum and Golgi apparatus. The plasmalemmal areas are also diminished, which indicates less twisting of plasmalemma and less interdigitation of adjacent cells. The ground plasma is relatively increased. These findings represent a decrease in the endocrine function of the PTG. These morphological findings indicating functional impairment of the PTG do not speak in favour of GH having a positive parathyrotropic effect. The hypercalcemia which occurred during the first days of the test cannot be attributed to a hyperparathyroidism induced by GH. Rather we believe that the inactivation of the PTG is secondary and caused by the hypercalcemia. In contrast to the normalization of the serum parameters, the morphologically demonstrable functional impairment of the PTG remains till the end of the experimental period. One may speculate that inactivation of the PTG plays a role in the proposed counter-regulation and consequently plays a part in the normalization of the serum parameters.

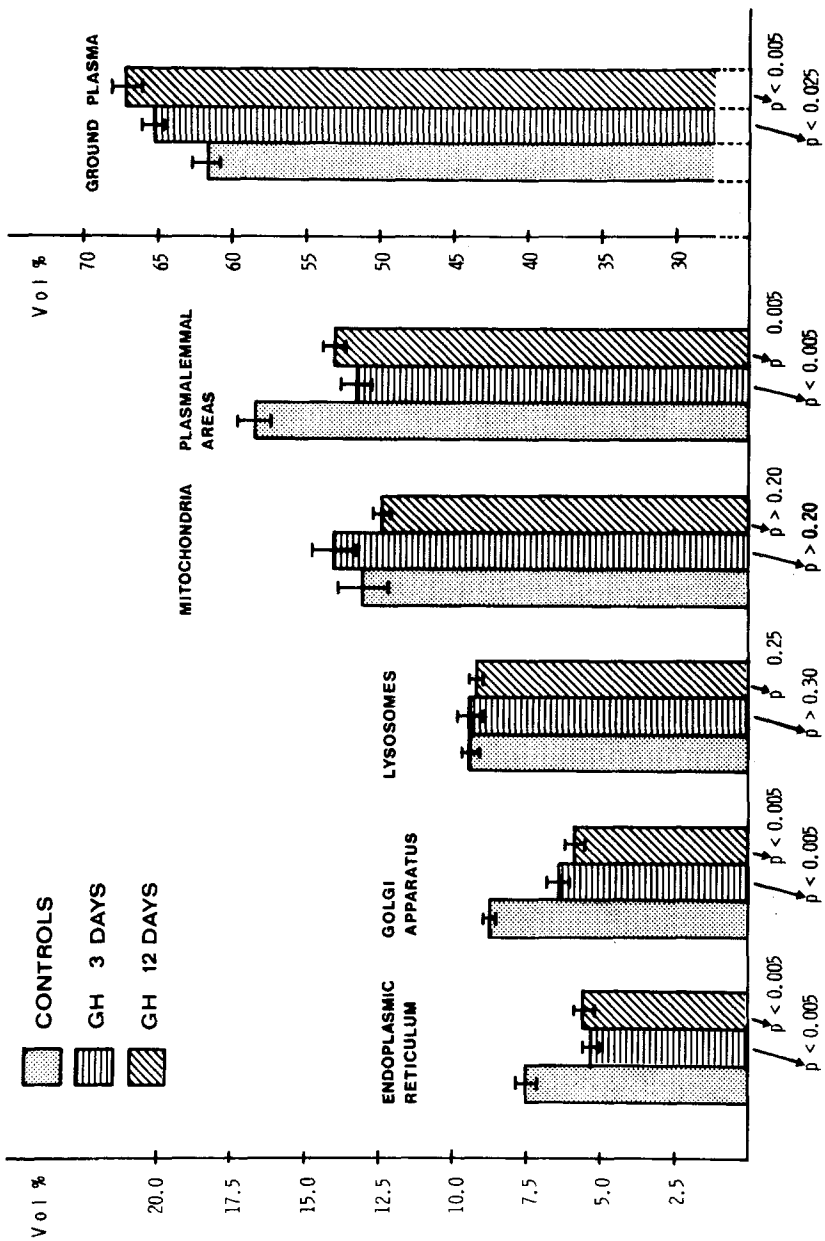

Fig. 5. Volume precents of cytoplasmic compartments in total PTG cytoplasm. Significance p is calculated of test groups as against controls

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