

# Electron microscopic study of the parathyroid gland of the calcium-treated hamster subjected to hypergravity environment

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**Summary.** The ultrastructure of the parathyroid glands of calcium-treated golden hamsters subjected to 5 gravity environment was studied. In the calcium-treated animals exposed to hypergravity environment, the Golgi complexes and cisternae of the granular endoplasmic reticulum were significantly decreased compared with those of the animals exposed to hypergravity environment only and appeared to increase compared with those of the calcium-treated animals, but were almost similar to those of the control animals. In addition, many chief cells contained some prosecretory granules in the Golgi areas, some secretory granules situated close to the plasma membrane and many lysosomes. The morphology of the parathyroid glands in the calcium-treated animals exposed to hypergravity environment resembled that of the control animals. These results suggest that the parathyroid glands suppressed by treatment of calcium and stimulated in response to hypergravity environment may indicate the secretory activity of the parathyroid glands of the control animals.

**Key words:** Parathyroid, Calcium, Hypergravity, Hamster, Ultrastructure, Stereology

## Introduction

It is well known that the serum calcium concentration of animals is elevated after administration of calcium. Numerous morphological studies have suggested alteration in the parathyroid gland under high concentrations of serum calcium. Most of these studies have been concerned with treatment of calcium (Roth and Raisz, 1964, 1966; Nakagami, 1967; Murakami, 1970; Capen, 1971; Stoeckel and Porte, 1973; Nunez et al., 1974; Roth and Capen, 1974; Boquist and Fahraeus,

1975; Chertow et al., 1975; Wild and Becker, 1980; Setoguti et al., 1981, 1985, 1988; Wild et al., 1982).

Many reports have shown the effects of a hypergravity environment on bone (Rosenfeld et al., 1973; Fosse et al., 1974; Amtmann and Oyama, 1976; Amtmann et al., 1976; Jaekel et al., 1977; Smith, 1977; Gazit, 1980; Pace et al., 1985; Simon et al., 1985; Wunder et al., 1987) and an increase in the bone density of animals subjected to hypergravity environment has been documented (Amtmann and Oyama, 1976; Amtmann et al., 1976; Jaekel et al., 1977; Pace et al., 1985; Simon et al., 1985). In addition, some morphological works have dealt with the problem concerning the effects of hypergravity environment on the parathyroid gland (Sannes and Hayes, 1975; Shoumura et al., 1989, 1990).

However, there is no study on the effects of hypergravity environment on the ultrastructure of the parathyroid gland of calcium-treated animals. We investigated ultrastructural changes of the parathyroid glands of calcium-treated golden hamsters exposed to hypergravity environment.

## Materials and methods

Five-month-old male golden hamsters with an average body weight of 180 g were divided into 4 groups of 5 animals each: the control group was given 0.45 ml distilled water intraperitoneally once daily for 3 days; the calcium (Ca) group was given 4% calcium chloride solution intraperitoneally once daily at a dose of 100 mg/kg body weight for 3 days; the 5G group was given 0.45 ml distilled water once daily for 3 days and then placed in a chamber mounted on a 1.5 meter radius centrifuge, rotating at a constant speed of 53.4 r.p.m. (5x gravity) 19 hours after the last injection; the Ca-5G group was given 4% calcium chloride solution once daily at a dose of 100 mg/kg body weight for 3 days and then placed in the same chamber, rotating at a constant speed of 53.4 r.p.m. 19 hours after the last injection. The

parathyroid glands of the control and Ca groups were removed 24 hours after the last injection, and the glands of the 5G and Ca-5G groups 5 hours after centrifugation under sodium pentobarbital anesthesia. The glands were immersed in a mixture of 2.5% glutaraldehyde and 2% OsO<sub>4</sub> in Millonig's buffer at pH 7.4 for 1 hour, dehydrated through ascending concentrations of acetone and embedded in Epon 812. Thin sections were cut on a Porter-Blum MT-1 ultramicrotome, stained with uranyl acetate and lead salts, and examined with a Hitachi H-700 H electron microscope.

In each hamster from the 4 groups, 20 micrographs at a final magnification of 22,000 were taken from different regions of the parathyroid glands. The area of cytoplasm, the Golgi complexes, cisternae of the granular endoplasmic reticulum, secretory granules, lipid droplets, large secretory granules, large vacuolar bodies, lysosomes and enlarged intercellular spaces containing floccular or finely particulate material was estimated with the aid of an image analyser (Mutoh, Digigrammer G-6).

All data are presented as the mean  $\pm$  SEM. One-way analysis of variance (ANOVA) was used to detect significant differences between the 4 groups and then Duncan's multiple range test was used to determine differences between pair of means. Significance was accepted at  $p < 0.05$ .

## Results

### *Ultrastructure of the parathyroid gland*

**Control group.** The morphology of the parathyroid gland of the control group resembled that of normal hamsters, as reported earlier (Emura et al., 1984; Shoumura et al., 1988a,b,c, 1989, 1990). Most chief cells were oval or polygonal in shape. The plasma membranes of adjacent cells pursued a slightly or moderately tortuous course with occasional interdigitations (Fig. 1). The intercellular spaces were generally narrow, and slightly enlarged intercellular spaces were sometimes present and contained floccular or finely particulate material. The chief cells had an oval or polygonal nucleus with occasional indentations. The cytoplasm was scattered diffusely with free ribosomes and randomly with abundant mitochondria. Cisternae of the granular endoplasmic reticulum were distributed at random or in parallel arrays. Most Golgi complexes were relatively well developed and associated with some prosecretory granules (Fig. 1). Round or oval secretory granules of 150-300 nm in diameter which were filled with a finely particulate material were scattered in the Golgi areas as well as in the peripheral cytoplasm (Fig. 1). Some secretory granules were located near the plasma membrane. Large secretory granules of 350-600 nm in diameter (Fig. 1), large vacuolar bodies of 350-750 nm in diameter (Fig. 1), lysosomes and lipid droplets were sometimes observed in the cytoplasm. Large

secretory granules filled with a finely particulate material showed a lower electron density than the secretory granules. Large vacuolar bodies contained floccular material and/or vesicles. Transitional forms between the large secretory granules and large vacuolar bodies were occasionally present (Fig. 1). Several vesicles surrounded some of the large secretory granules, large vacuolar bodies and transitional forms.

**Experimental groups.** Many chief cells contained rich free ribosomes and abundant mitochondria, and secretory granules and large secretory granules were sometimes found in the cytoplasm. In the 5G group, well-developed Golgi complexes associated with numerous prosecretory granules were frequently present (Fig. 2) and cisternae of the granular endoplasmic reticulum were often arranged in parallel arrays (Fig. 2). Many secretory granules were located in the peripheral cytoplasm and several granules were situated close to the plasma membrane (Fig. 2). Lipid droplets and large vacuolar bodies were sometimes seen in the cytoplasm. Enlarged intercellular spaces containing floccular or finely particulate material were frequently present. In the Ca group, cisternae of the granular endoplasmic reticulum were occasionally arranged in parallel arrays and poorly-developed Golgi complexes associated with a few prosecretory granules were frequently observed in the cytoplasm (Fig. 3). Some secretory granules were located in the peripheral cytoplasm. Large vacuolar bodies were occasionally seen, and lipid droplets and lysosomes were frequently observed in the cytoplasm (Fig. 3). In the Ca-5G group, cisternae of the granular endoplasmic reticulum were randomly distributed or sometimes arranged in parallel arrays and many Golgi complexes having some prosecretory granules were relatively well developed (Fig. 4). Some secretory granules were situated close to the plasma membrane. Lipid droplets and lysosomes were frequently found and large vacuolar bodies were sometimes observed in the cytoplasm (Fig. 4). Enlarged intercellular spaces containing floccular or finely particulate material were often observed (Fig. 4, inset).

### *Stereological analysis of the parathyroid gland*

The results of the stereological investigations are given in Table 1. In the Ca-5G group the volume density occupied by the Golgi complexes and cisternae of the granular endoplasmic reticulum was significantly decreased ( $p < 0.05$ ) as compared to that of the 5G group and appeared to increase as compared to that of the Ca group, but was almost similar to that of the control group. In addition, there were significant differences ( $p < 0.05$ ) among the control, 5G and Ca groups with regard to the Golgi complexes and cisternae of the granular endoplasmic reticulum. In the Ca-5G group the volume density occupied by large vacuolar bodies was significantly increased, ( $p < 0.05$ ) as compared to that of the Ca group, but was similar to that of the control and

**Table 1.** Volume densities of the Golgi complex (G), granular endoplasmic reticulum (ER), secretory granule (SG), lipid droplet (LD), large secretory granule (LG), large vacuolar body (VB), lysosome (Ly) and enlarged intercellular space containing floccular or finely particulate material (EIS).

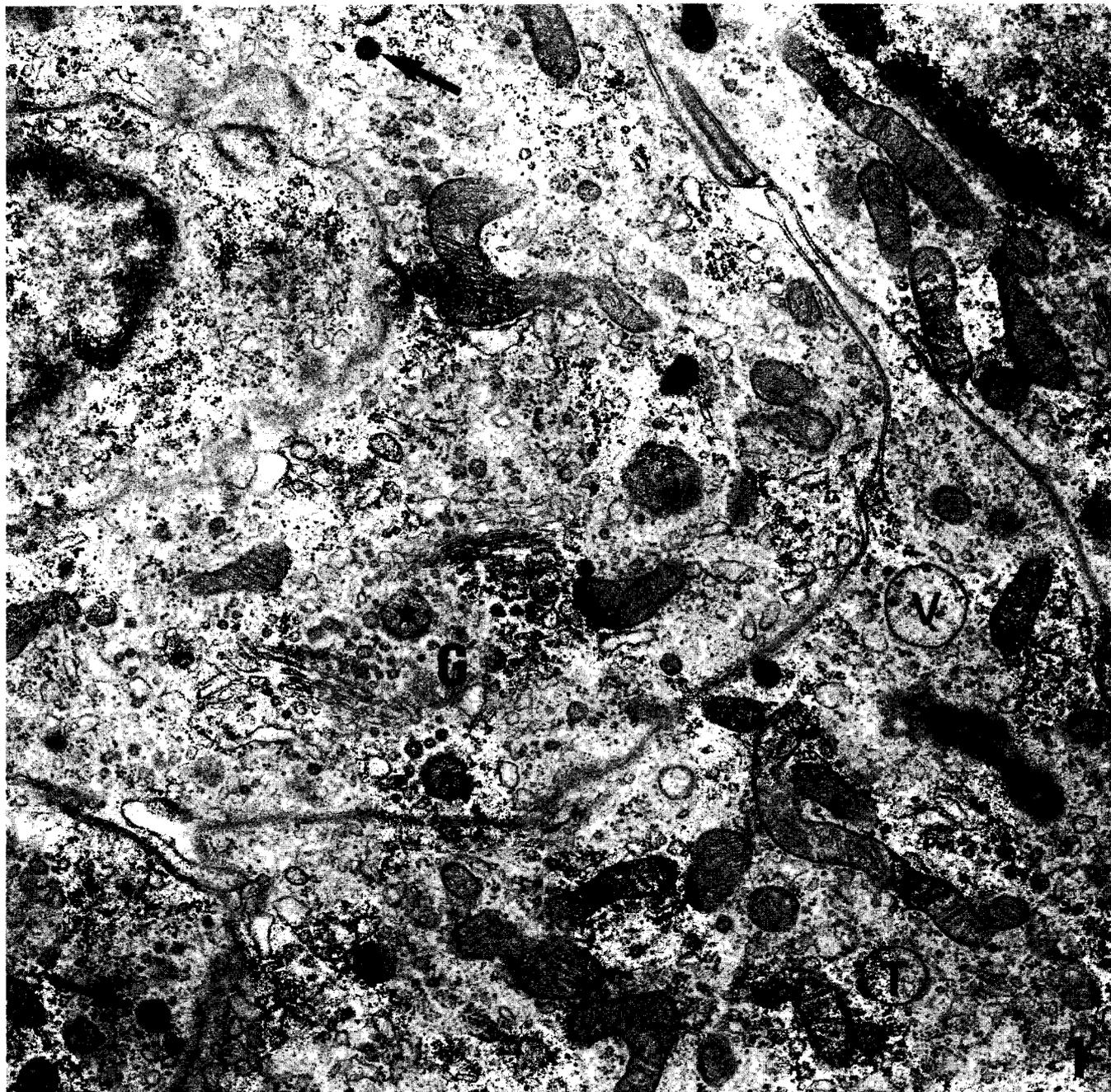
Group	Golgi	ER	SG	LD	LG	VB	Ly	EIS
Control	5.69 ± 0.34	3.81 ± 0.43	0.61 ± 0.10	0.27 ± 0.03	0.10 ± 0.07	0.23 ± 0.03	0.68 ± 0.13	0.013 ± 0.002
5G	6.95 ± 0.23 <sup>a</sup>	4.96 ± 0.23 <sup>a</sup>	0.56 ± 0.06	0.50 ± 0.05	0.11 ± 0.04	0.27 ± 0.03	0.72 ± 0.05	0.027 ± 0.005 <sup>a</sup>
Ca	4.73 ± 0.07 <sup>ab</sup>	2.76 ± 0.32 <sup>ab</sup>	0.52 ± 0.04	1.03 ± 0.54	0.08 ± 0.03	0.13 ± 0.01 <sup>ab</sup>	1.05 ± 0.08 <sup>ab</sup>	0.013 ± 0.001 <sup>b</sup>
Ca-5G	4.98 ± 0.27 <sup>b</sup>	3.48 ± 0.41 <sup>b</sup>	0.53 ± 0.02	0.76 ± 0.28	0.14 ± 0.02	0.23 ± 0.01 <sup>c</sup>	1.08 ± 0.07 <sup>ab</sup>	0.024 ± 0.005 <sup>c</sup>

The volume densities are presented as percentage of cytoplasmic volume. Values are means ± SEM.

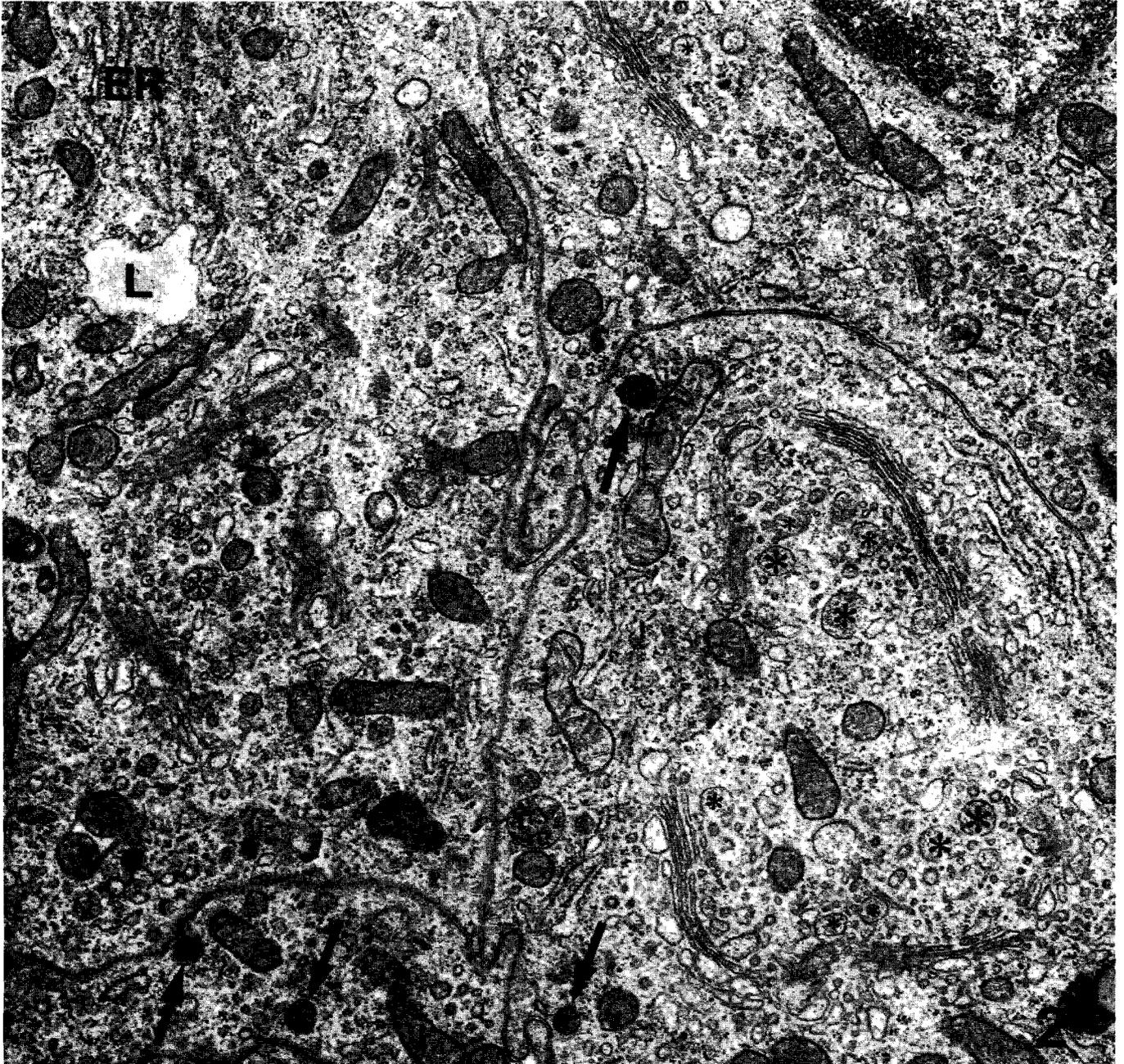
<sup>a</sup>Significant difference from control group (p < 0.05).

<sup>b</sup>Significant difference from 5G group (p < 0.05).

<sup>c</sup>Significant difference from Ca group (p < 0.05).



**Fig. 1.** Parathyroid chief cells of the control hamster. Golgi complex (G) is relatively developed. Round secretory granules are filled with a finely particulate material (arrow). Large secretory granules (\*), large vacuolar granule (V) and transitional form (T) between them are seen. × 22,500



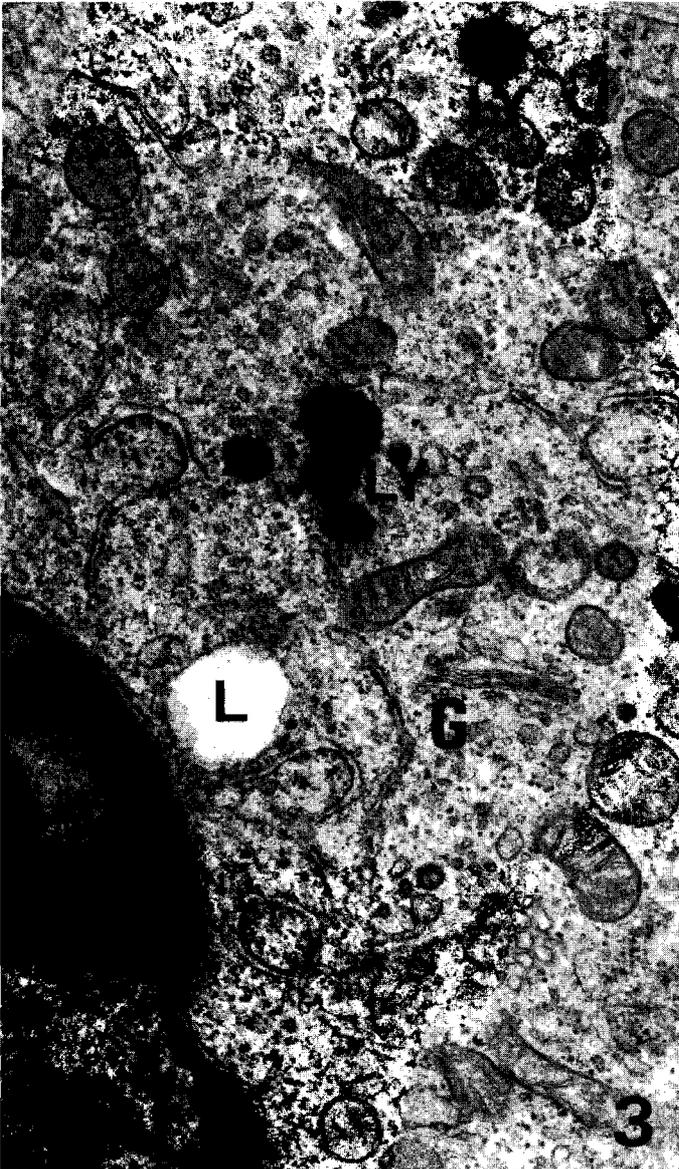
**Fig. 2.** Chief cells of the 5G group. Well-developed Golgi complexes are associated with numerous prosecretory granules (\*) and cisternae of the granular endoplasmic reticulum are arranged in parallel arrays (ER). Many secretory granules are located in the peripheral cytoplasm (arrows). L: lipid droplet.  $\times 22,500$

5 G group. In the Ca-5G group the volume density occupied by lysosomes was significantly increased ( $p < 0.05$  as compared to that of the control and 5G groups, but was similar to that of the Ca group. In the Ca-5G group the volume density occupied by enlarged intercellular spaces containing floccular or finely particulate material was significantly increased ( $p < 0.05$ ) as compared to that of the Ca group and appeared to increase as compared to that of the control group, but was almost similar to that of the 5G group.

There were no significant differences among the 4 groups with regard to the secretory granules, lipid droplets and large secretory granules.

### Discussion

In the present study, the main changes in the parathyroid glands of the Ca group, compared with the control and 5G groups, were the significant decreases in cisternae of the granular endoplasmic reticulum, the



**Fig. 3.** Chief cells of the Ca group. Poorly-developed Golgi complex (G) associated with a few prosecretory granules is seen. L: lipid droplet, LY: lysosomes.  $\times 22,500$

Golgi complexes and large vacuolar bodies and the significant increases in lysosomes. In addition, many chief cells contained a few prosecretory granules in the Golgi areas and numerous lipid droplets. These findings are fairly consistent with the observations of a decrease in functional activity of the parathyroid gland (Roth and Schiller, 1976; Isono et al., 1977, 1980, 1981, 1982, 1983, 1985; Hayashi et al., 1981; Emura et al., 1984; Iwasaki et al., 1987; Shoumura et al., 1988b, 1990; Ishizaki et al. 1989). Similar findings have also been reported in calcium-treated animals (Roth and Raisz, 1964, 1966; Nakagami, 1967; Murakami, 1970; Capen, 1971; Stoeckel and Porte, 1973; Nunez et al., 1974; Roth and Capen, 1974; Boquist and Fahraeus, 1975; Chertow et

al., 1975; Wild and Becker, 1980; Wild et al., 1982). In the parathyroid glands of the 5G group, the cisternae of the granular endoplasmic reticulum and the Golgi complexes which had numerous prosecretory granules and enlarged intercellular spaces containing floccular or finely particulate material were significantly increased as compared with those of the control group. Similar results have also been observed in the parathyroid glands of gerbils subjected to 2 gravity environment for 60 days (Sannes and Hayes, 1975) and hamsters exposed to 2, 5, or 10 gravity environment for 5 hours (Shoumura et al., 1988a, 1989, 1990). These results are essentially similar to the observations which indicate an increase in function of the parathyroid gland (Roth and Raisz, 1964; Isono et al., 1971, 1977, 1979a, 1979b, 1986; Isono and Shoumura, 1973, 1980; Nunez et al., 1974; Roth and Capen, 1974; Roth and Schiller, 1976; Capen, 1980; Hayashi et al., 1980; Wild 1980; Emura et al., 1982, 1984; Ishizaki et al., 1983, 1989; Wild and Manser, 1986; Shoumura et al., 1988a,b, 1989).

In the present study, many chief cells in the Ca-5G group contained rich free ribosomes, abundant mitochondria, many lipid droplets and lysosomes, relatively well-developed Golgi complexes associated with some prosecretory granules and cisternae of the granular endoplasmic reticulum, and some secretory granules situated close to the plasma membrane. Enlarged intercellular spaces containing floccular or finely particulate material were often observed. In addition, our study demonstrates that in the Ca-5G group the Golgi complexes and cisternae of the granular endoplasmic reticulum are significantly decreased compared with those of the 5G group and appeared to increase compared with those of the Ca group, but were almost similar to those of the control group. The morphology of the parathyroid glands in the Ca-5G group resembled that of the control group. Therefore, it is considered that the parathyroid glands suppressed by treatment of calcium and stimulated in response to hypergravity environment may indicate the secretory activity of the parathyroid glands of the control group.

It has been reported that in active chief cells secretory granules have a tendency to gather beneath the plasma membrane (Fujii and Isono, 1972; Isono and Shoumura, 1980; Emura et al., 1984; Shoumura et al., 1988a,b, 1989). In the present study, secretory granules were situated close to the plasma membrane and the enlarged intercellular spaces contained floccular or finely particulate material similar to the contents of the secretory granule in the control and experimental groups. These findings suggest the possibility of exocytosis of the secretory granules in the parathyroid gland of the golden hamster, as described in many studies. In the 5G and Ca-5G groups the enlarged intercellular spaces containing floccular or finely particulate material were increased as compared with those of the control and Ca groups. Therefore, it is conceivable that the release of secretory granules may be stimulated in the 5G and Ca-5G groups.

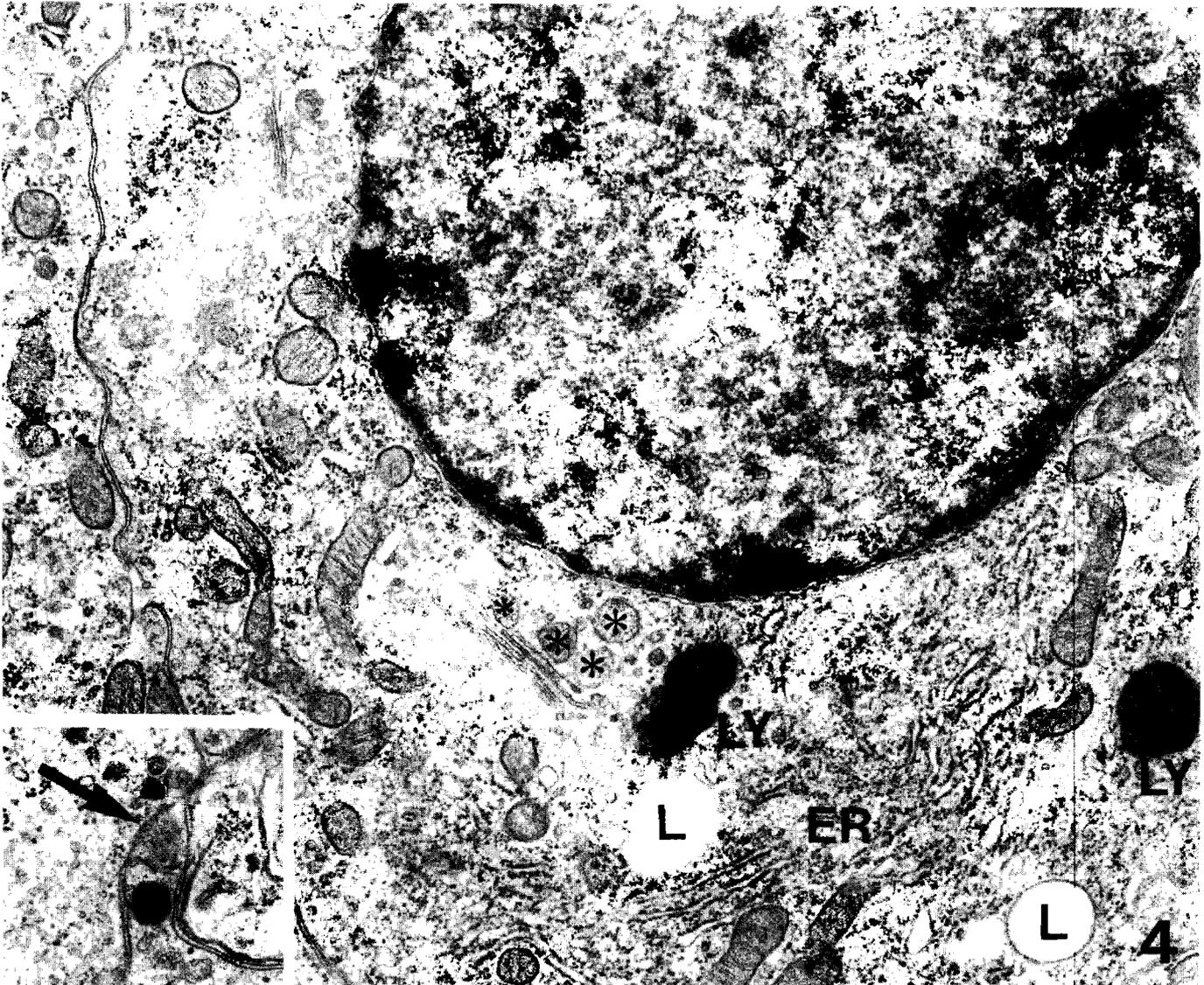


Fig. 4. Chief cells of the Ca-5G group. Cisternae of the granular endoplasmic reticulum are often arranged in parallel arrays (ER). Golgi complexes having some prosecretory granules (\*). Lipid droplets (L) and lysosomes (LY) are frequently found.  $\times 22,500$ . Inset: Enlarged intercellular space contains finely particulate material (arrow).  $\times 28,500$

In the present study, large secretory granules, large vacuolar bodies and transitional forms were present in the control and experimental groups. The contents of the large secretory granules were similar to those of the secretory granules. Protein A-gold particles are detected over the secretory granules and the large secretory granules (Inoue and Setoguti, 1986; Shoumura et al., 1988c,d). We believe that both granule types include parathyroid hormone, as previously reported (Inoue and Setoguti, 1986; Shoumura et al., 1988c,d), and that the large secretory granules are storage granules, also described earlier (Isono and Shoumura, 1980; Isono et al., 1980, 1981, 1982, 1985; Setoguti et al., 1981; Shoumura et al., 1988a,b,c,d, 1989, 1990). Very few protein A-gold particles are noted over the transitional forms but particles are absent over the large vacuolar

bodies (Shoumura et al., 1988c). The present study demonstrates that vesicles surrounded large secretory granules, large vacuolar bodies and transitional forms. We think that some vesicles are incorporated into the large secretory granules to form certain kinds of the large vacuolar bodies and that such transformation involves lysosomal digestion of the storage granules.

*Acknowledgements.* This work was supported by a research grant from the Ministry of Education of Japan.

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Accepted June 6, 1989