

Morphological identification of the lipid-storing cells in golden hamster parathyroid glands after vitamin A treatment

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Summary. We investigated hamster parathyroid glands of different ages using electron microscopy and found a new cell type in young, adult and senile hamsters. These special cells were located in interstitial tissues and invariably contained several lipid droplets within the cytoplasm. The cells showed an elongated spindle with some cell processes. The cells contained small Golgi complexes and moderate cisternae of the granular endoplasmic reticulum. The morphological characteristics of these cells were mostly the same as those of lipid-storing cells in other organs (Yamada and Hirose, 1976). After vitamin A administration, the lipid droplets in these cells markedly increased in number and also in volume density. The other morphological features of these cells resembled those of the control animals. We called these cells parathyroid lipid-storing cells. They may incorporate and store vitamin A within the lipid droplets. They can be classified as one of the cellular components in hamster parathyroid gland.

Key words: Parathyroid gland, Lipid-storing cell, Ultrastructure, Golden hamster, Vitamin A

Introduction

Vitamin A plays a crucial role in the physiology of vision and has important roles in regulating genes involved in cell morphogenesis, differentiation, and proliferation (Evans and Kaye, 1999; Dawson, 2000). It also functions as antioxidants that can prevent carcinogenesis by decreasing the levels of the free-radicals that cause DNA damage (Evans and Kaye, 1999; Dawson, 2000). However, excess vitamin A has a toxic action for animals. In mammals, vitamin A is primarily stored in special cells in certain organs or

tissues. Nakane (1963) first confirmed with fluorescence microscope that the Ito cell in the liver contained vitamin A within the lipid droplets. Following this discovery, similar cells were identified in liver, lung, intestine, kidney, adrenal gland, pancreas and other organs in normal as well as vitamin A-treated animals (Yamada and Hirose, 1976; Kusumoto and Fujita, 1977; Hirose and Yamada, 1978; Yamamoto et al., 1978; Watari et al., 1989; Nagy et al., 1997). The common morphological features of lipid-storing cells in different organs showed that they invariably contained several lipid droplets in the cytoplasm and these lipid droplets had a capacity to store vitamin A (Yamada and Hirose, 1976; Hirose and Yamada, 1978). It was postulated that lipid-storing cells bear some role in the metabolism and storage of vitamin A, since these cells are found in organs where metabolic activity of vitamin A is high (Hirose and Yamada, 1978). However, information on the morphology of the parathyroid lipid-storing cells has been lacking not only in human, but also in other animal species. The present study was therefore undertaken to determine whether such lipid-storing cells exist in hamster parathyroid glands from neonatal to senile periods in normal condition and after vitamin A administration.

Materials and methods

In the present study, we used 1-, and 10-day, 1-, 3-, and 18-month-old golden hamsters of both sexes of 10 each for control groups without any treatment. One- and 3-month-old hamsters of 10 each were used for experimental groups. Animals of the experimental groups were daily injected intramuscularly with vitamin A palmitate at 5000 IU for 5 days. The parathyroid glands were removed under sodium pentobarbital anesthesia 24 hours after the last injection. They were fixed in a mixture of 2.5% glutaraldehyde and 2% OsO₄ in Millonig's buffer at pH 7.4 for 1 hour, dehydrated through ascending concentrations of acetone and embedded in Epon 812. Ultrathin sections were prepared on a Porter-Blum MT-1 ultramicrotome. After being

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stained with uranyl acetate and lead salts, the sections were examined under a Hitachi H-800 electron microscope. Ten micrographs at final magnification of 11,000 were taken from different regions of the parathyroid glands of each animal from all groups. The number of lipid-storing cells in 100 micrographs from each group was counted. The areas of the cytoplasm and lipid droplets in lipid-storing cells were measured. The diameter and the number of lipid droplets per $100 \mu\text{m}^2$ of the cytoplasm in lipid-storing cells were also calculated with the aid of an image measuring system (Finetec). The areas of the cytoplasm, Golgi complexes, cisternae of the granular endoplasmic reticulum and lipid droplets in parathyroid chief cells were also estimated. Statistical analysis by one-way analysis (ANOVA) followed by Fisher's PLSD test was done using Stat View J-4.5 (Abacus Concepts). A p value <0.05 was considered statistically significant.

Results

The parathyroid chief cells in 1-day-old hamsters contained large lakes of glycogen particles and poorly-developed Golgi complexes. Secretory granules were hardly found in the cytoplasm. In 10-day-old hamsters, chief cells contained moderate Golgi complexes, small lakes of glycogen particles and a few secretory granules (Fig. 1). In the parathyroid chief cells of 1-month-old hamsters, the prominent feature was the abundance of lipid droplets in the cytoplasm (Fig. 2). The volume density occupied by lipid droplets in 1-month-old hamsters was the highest among hamster parathyroid glands of all ages (Table 1). Golgi complexes and the cisternae of the granular endoplasmic reticulum were well developed. In the parathyroid chief cells of 3-month-old hamsters, well-developed Golgi complexes and the cisternae of the granular endoplasmic reticulum

Table 1. Volume density of lipid droplets in parathyroid chief cell (LD/CC) and lipid-storing cell (LD/LSC), the number (N.LD) and diameter (D.LD) of lipid droplets in lipid-storing cell and the number of lipid-storing cells (N.LSC).

AGE OF ANIMALS	LD/CC	LD/LSC	N.LD	D.LD (μm)	N.LSC
1-day	0.23 \pm 0.05				0
10-days	0.28 \pm 0.05	0.61 \pm 0.17	10.9 \pm 3.2	0.69 \pm 0.25	7
1-month	0.56 \pm 0.07*	0.76 \pm 0.15	13.8 \pm 2.5	0.86 \pm 0.18	15
3-months	0.14 \pm 0.03	0.69 \pm 0.09	15.1 \pm 2.8	0.83 \pm 0.19	28
18-months	0.25 \pm 0.04	0.62 \pm 0.18	15.8 \pm 4.3	0.77 \pm 0.28	31

Values are mean \pm SEM. Volume density is presented as percentage of the cytoplasm. The number of lipid droplets is presented as per $100 \mu\text{m}^2$ of the cytoplasm. *: $p < 0.05$ vs 1-day, 10-day, 3-month-, and 18-month-groups.

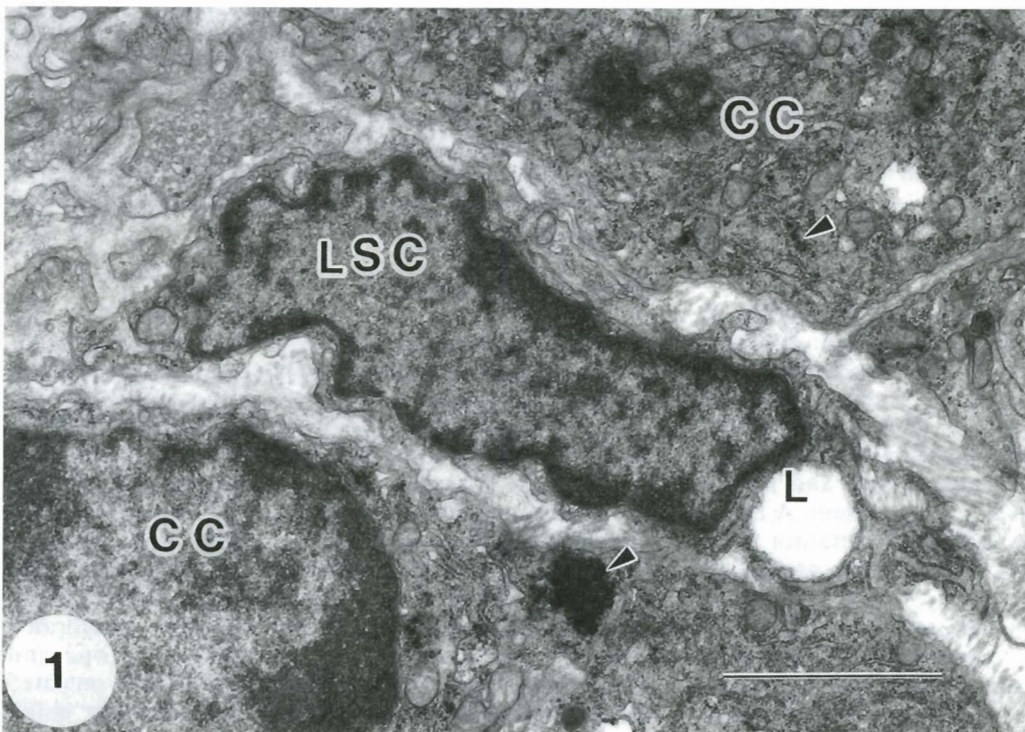


Fig. 1. Parathyroid gland of 10-day-old control hamster. A lipid-storing cell (LSC) containing a lipid droplet (L) is observed in the interstitial tissue. Parathyroid chief cells (CC) contain some glycogen particles (arrowheads). Bar: $2 \mu\text{m}$.

Hamster parathyroid lipid-storing cell

were well developed. In the parathyroid chief cells of 3-month-old hamsters, well developed Golgi complexes and the cisternae of the granular endoplasmic reticulum were widely dispersed in the cytoplasm. Some secretory

granules were located close to the plasma membranes (Fig. 3). The number of lysosomes and secretory granules in 18-month-old animals was the highest among hamster parathyroid glands of all ages.

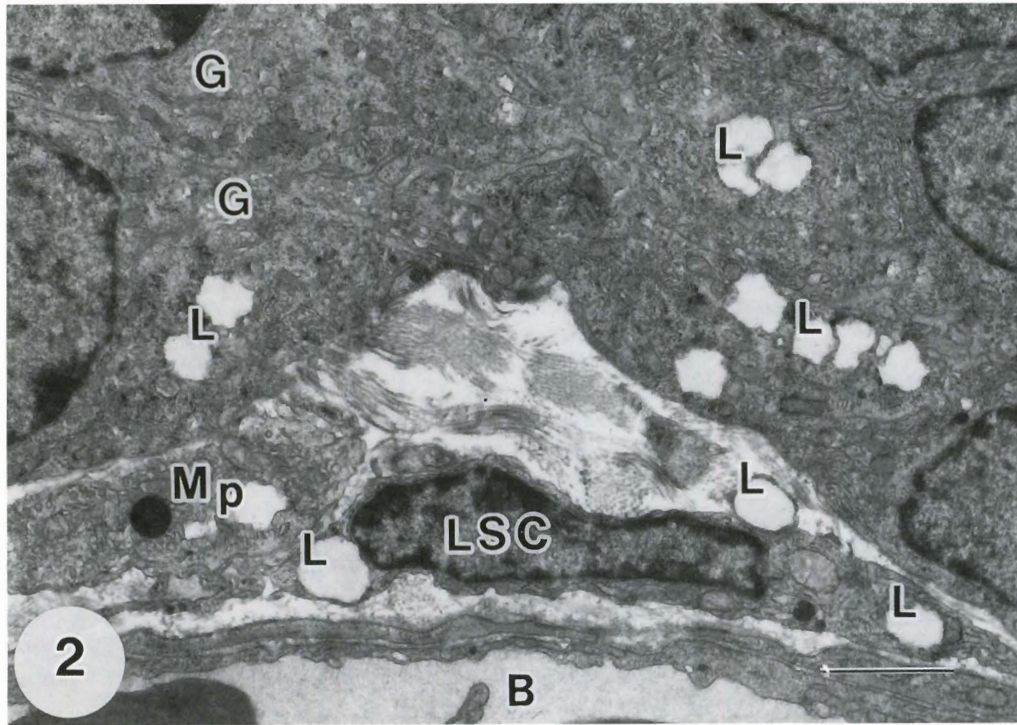


Fig. 2. Parathyroid gland of 1-month-old control hamster. A lipid-storing cell (LSC) containing 3 lipid droplets (L) is observed in the interstitial tissue near the blood vessel (B). The cytoplasm of parathyroid chief cells contains relatively well-developed Golgi complexes (G) and numerous lipid droplets (L). Mp: macrophage. Bar: 2 μ m.

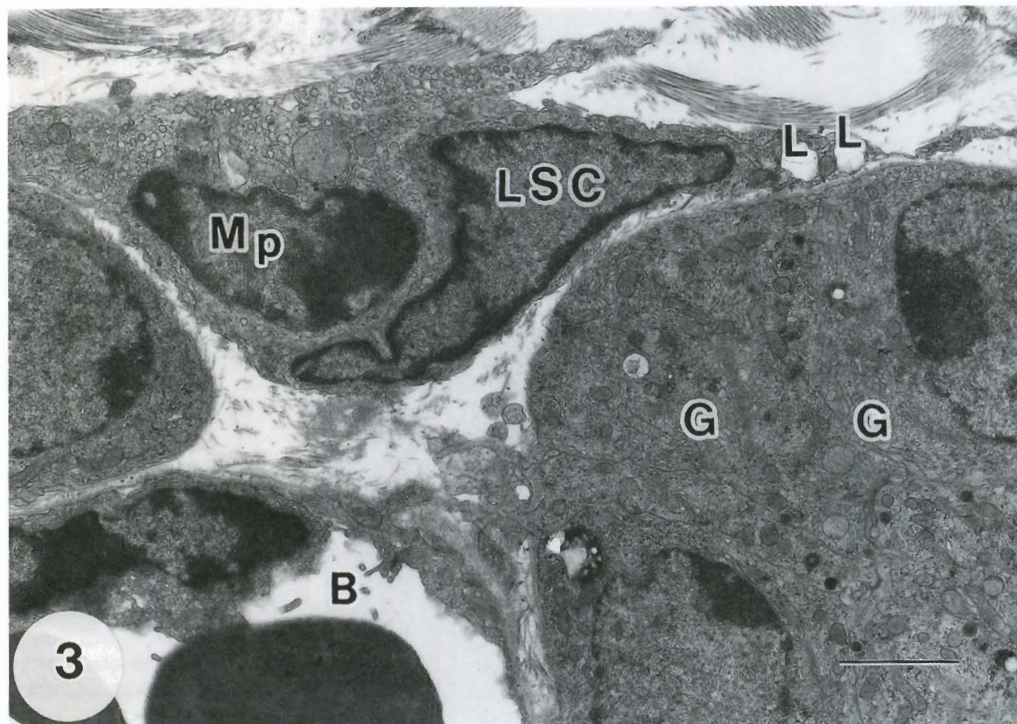


Fig. 3. Parathyroid gland of 3-month-old control hamster. A lipid-storing cell (LSC) having close contact with macrophage (Mp) is observed in the interstitial tissue. Well-developed Golgi complexes (G) are seen in the cytoplasm of parathyroid chief cells. L: lipid droplets; B: blood vessel. Bar: 2 μ m.

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In 1-day-old hamsters, we did not find any cells containing lipid droplets in the interstitial tissues of parathyroid glands. In the parathyroid gland of 10-day-old hamsters, there were a few special cells in the interstitial tissues near the blood vessel, which contained several lipid droplets within the cytoplasm (Fig. 1). This cell type was quite similar to lipid-storing cells in other organs (Yamada and Hirose, 1976; Kusumoto and Fujita, 1977; Hirose and Yamada, 1978; Yamamoto et al., 1978; Watari et al., 1989; Nagy et al., 1997). In the parathyroid glands of 1-, 3-, and 18-month-old hamsters, these cells were present more frequently than those in 10-day-old hamsters (Figs. 2-4). The number of the cells was increased with age. The number of these cells in 100 micrographs of the parathyroid glands of 1-, 10-day, 1-, 3-, and 18-month-old hamsters was 0, 7, 15, 28 and 31, respectively (Table 2). The shape of these cells varied from spindle to irregular, usually it was simple elongated

spindle with tapering cytoplasmic projections (Figs. 1-4). The nucleus had an elliptical or elongated contour. The cisternae of the granular endoplasmic reticulum were moderate. Some of them were dilated and contained amorphous substances. These cells also contained a number of free ribosomes, mitochondria and small Golgi complexes. There were 1 to 3 small round lipid droplets within the cytoplasm (Figs. 1-4). The mean diameter and the number of lipid droplets per 100 μm^2 of the cytoplasm in the cells are shown in Table 1. There was no significant difference among the different ages with regard to the number, diameter and volume density of lipid droplets in these cells. Some cells were surrounded by various amounts of collagen fibers (Figs. 2-4). Some cells occurred in proximity to macrophage (Fig. 3), lymphocyte, mast cell (Fig. 4), plasma cell and nerve fiber. These cells showed no specialized contacts with parathyroid chief cells.

After vitamin A treatment, the morphology of the parathyroid chief cells in 1- and 3-month-old hamsters resembled that of the control animals. Chief cells contained well-developed Golgi complexes and moderate cisternae of the granular endoplasmic reticulum (Figs. 5, 6). Numerous lipid droplets were observed in the cytoplasm of the 1-month-old animals (Fig. 5A). There was no significant difference between the control and vitamin A-treated groups with regard to the volume density occupied by Golgi complexes, cisternae of the granular endoplasmic reticulum and lipid droplets in parathyroid chief cells (Table 2). The cells containing lipid droplets were also observed in vitamin

Table 2. Volume density of cell components in parathyroid chief cells.

AGE OF ANIMALS	PROTOCOL	LD	G	ER
1 month	control	0.56±0.07	5.37±0.83	8.08±1.16
	experiment	0.53±0.06	5.42±0.69	8.25±0.97
3 months	control	0.14±0.03	6.21±0.75	8.84±1.05
	experiment	0.14±0.03	6.13±0.80	8.67±1.33

Values are mean±SEM. LD: lipid droplet; G: Golgi complex; ER: cisternae of the granular endoplasmic reticulum. *: $p < 0.05$.

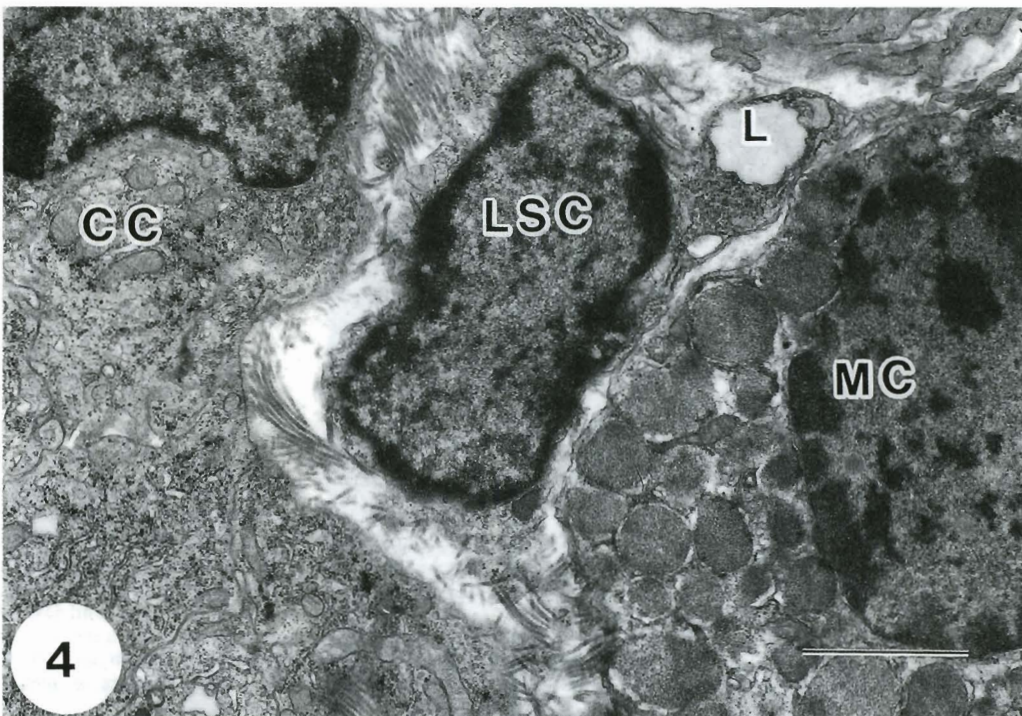


Fig. 4. Parathyroid gland of 18-month-old control hamster. A lipid-storing cell (LSC) and a mast cell (MC) are observed in the interstitial tissue. CC: chief cells. Bar: 2 μm .

A-treated hamsters. The number of these cells in 100 micrographs of the parathyroid glands of 1- and 3-month-old hamsters was 19 and 25 respectively, similar to that of the control animals (Table 3). The cells were irregular in shape and usually had several cytoplasmic

extensions (Figs. 5, 6). The nuclei were oval, some being indented. The morphological features of the cells resembled those of the control animals. Sometimes relatively larger mitochondria were found in the cells (Fig. 6B). Centrioles were also observed in the

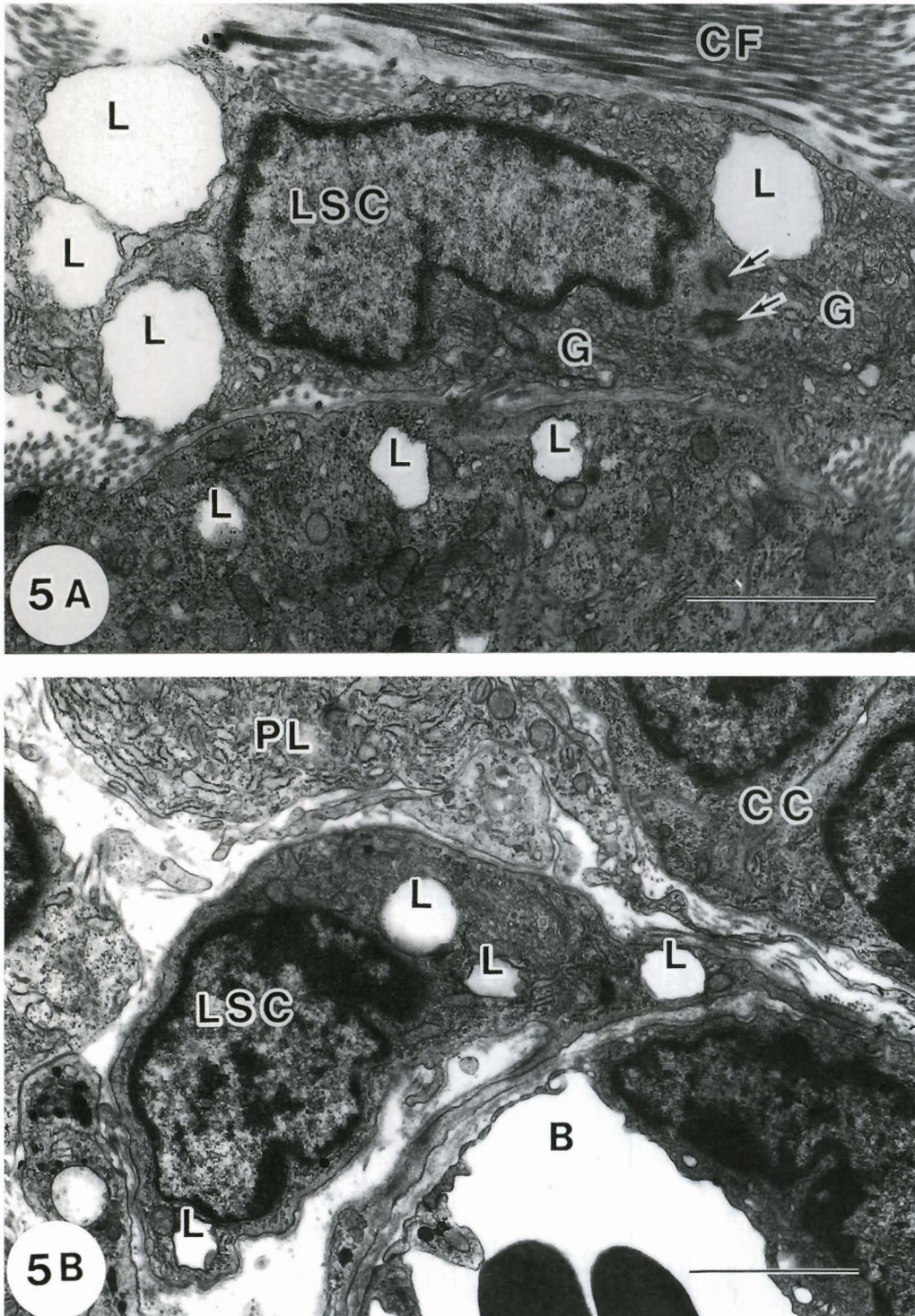


Fig. 5. Parathyroid gland of 1-month-old vitamin A-treated hamster. **A.** A lipid-storing cell (LSC) contains 4 large lipid droplets (L) and relatively well-developed Golgi complexes (G). Parathyroid chief cells contain several lipid droplets (L). CF: collagen fibers; Arrows: centrioles. **B.** A lipid-storing cell (LSC) containing 4 lipid droplets (L) is observed near the blood vessel (B). CC: chief cells; PL: plasma cell. Bar: 2 μ m.

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cytoplasm (Fig. 5A). There were 3 to 5 large lipid droplets in the cell. The lipid droplets were one of the most prominent cellular elements in the cells. As compared with the control animals, the number and

diameter of lipid droplets in these cells of vitamin A treated-animals were significantly increased (Table 3). The volume density occupied by lipid droplets in these cells of vitamin A treated-animals was also markedly

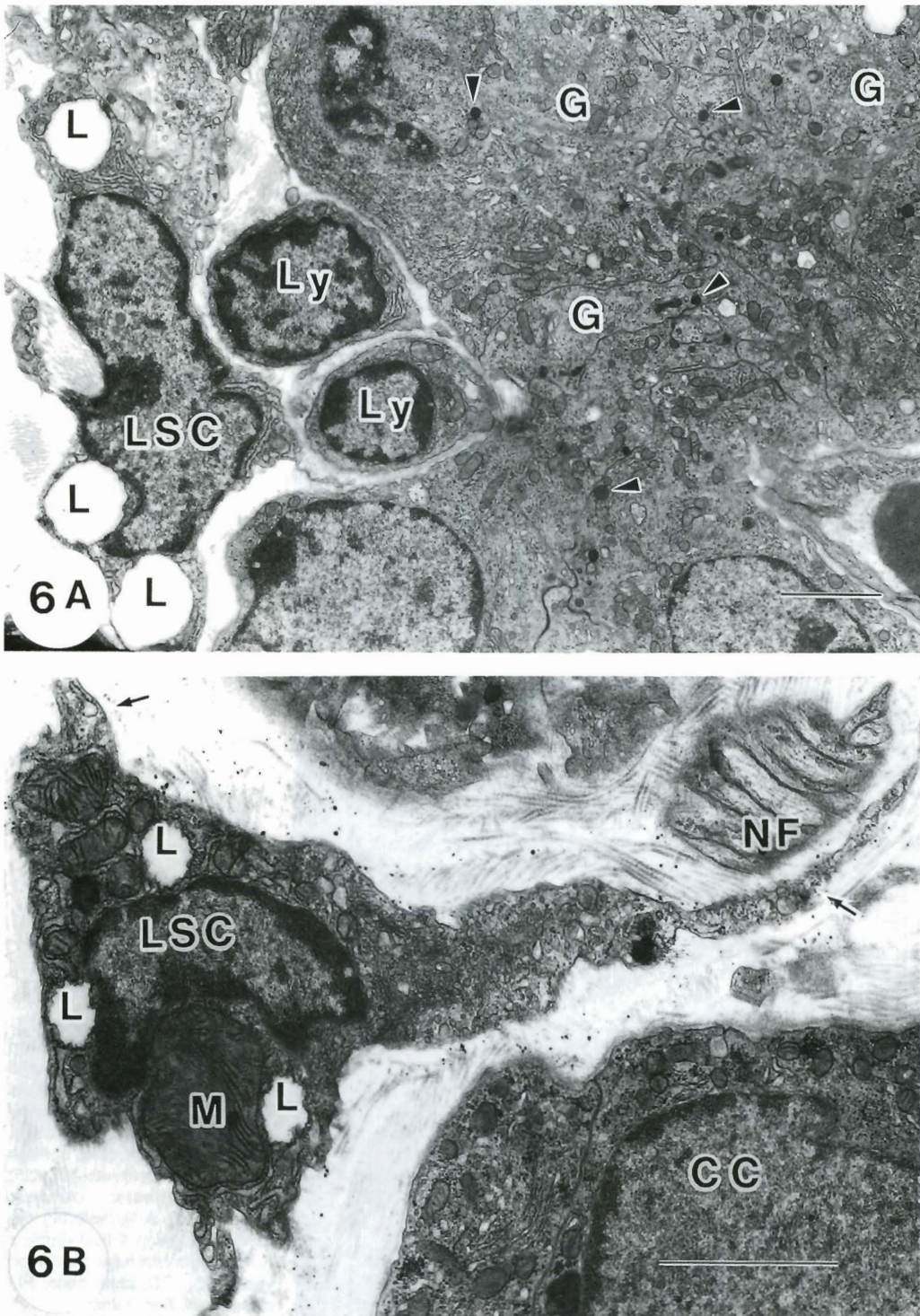


Fig. 6. Parathyroid gland of 3-month-old vitamin A-treated hamster. **A.** A lipid-storing cell (LSC) containing 3 large lipid droplets (L) is observed near 2 lymphocytes (Ly). Parathyroid chief cells contain well-developed Golgi complexes (G) and several secretory granules (arrowheads). **B.** A lipid-storing cell (LSC) contains relatively larger mitochondria (M), three lipid droplets (L) and long cytoplasmic extensions (arrows). CC: chief cells; NF: nerve fiber. Bar: 2 μ m.

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Table 3. Volume density of lipid droplets in lipid-storing cell (LD/LSC), the number (N.LD) and diameter (D.LD) of lipid droplets in lipid-storing cell and the number of lipid-storing cells (N.LSC).

AGE OF ANIMALS	PROTOCOL	LD/LSC	N.LD	D.LD (mm)	N. LSC
1 month	control	0.76±0.15	13.8±2.5	0.86±0.18	15
	experiment	10.87±1.38*	32.5±5.2*	1.13±0.27*	19
3 months	control	0.69±0.09	15.1±2.8	0.83±0.19	28
	experiment	11.91±1.74*	29.6±4.6*	1.05±0.23*	25

Values are mean±SEM. Volume density is presented as percentage of the cytoplasm. The number of lipid droplet is presented as per 100 μm^2 of the cytoplasm. *: $p < 0.05$

increased (Table 3).

Discussion

The morphology of the mammalian parathyroid glands has been studied extensively (Roth and Schiller, 1976; Isono et al., 1990; Parfitt, 1994; Wild and Setoguti, 1995). Water-clear cells have been reported in the parathyroid parenchyma of the rabbit and hamster (Emura et al., 1990, 1992). Recently, we found that macrophages existed among the parenchyma and the interstitial tissues of the hamster parathyroid glands (Chen et al., 1999). The hamster parathyroid gland showed different morphological features from neonatal to senile periods (Isono et al., 1990). In 1-month-old hamster, the parathyroid chief cells contained numerous lipid droplets. The composition of the lipid droplets is not fully understood yet.

The most interesting result in this study was the occurrence of a special cell type which contained several lipid droplets within the cytoplasm. The morphology of this cell was similar to that of lipid-storing cells in other organs (Yamada and Hirose, 1976; Kusumoto and Fujita, 1977; Hirose and Yamada, 1978; Yamamoto et al., 1978; Watari et al., 1989; Nagy et al., 1997). So we called it parathyroid lipid-storing cell. This is the first demonstration of parathyroid lipid-storing cells in golden hamsters of different ages. The present study showed that lipid-storing cells existed in parathyroid glands of the young, adult and senile hamsters. We did not find any lipid-storing cells in neonatal parathyroid gland. The number of parathyroid lipid-storing cells seemed to be increased with age. The cells were observed in the interstitial tissues. They possessed a few small lipid droplets and cytoplasmic projections. Some of them were surrounded by collagen fibers. There were no direct contacts between lipid-storing cell and parathyroid chief cell. Accordingly we consider that parathyroid lipid-storing cells originate from mesenchymal cells, not parenchymal cells as in other organs (Yamamoto et al., 1978; Watari et al., 1989).

After vitamin A administration, the number of lipid-storing cells was similar to that of the control animals, while the number and the diameter of lipid droplets in

these cells markedly increased. Vitamin A was detected in lipid droplets of lipid-storing cells using fluorescence microscopy and gold impregnation method (Kusumoto and Fujita, 1977; Hirose and Yamada, 1978; Yamamoto et al., 1978; Nagy et al., 1997). It is possible that the lipid droplets of the lipid-storing cells have a capacity to take up and store vitamin A as reported in other organs (Kusumoto and Fujita, 1977; Hirose and Yamada, 1978; Yamamoto et al., 1978). Though functional significance of lipid-storing cells is quite obscure, the only clear fact is that they incorporate vitamin A within the lipid droplets. They might either be a storage site of the vitamin or a detoxication site for excess vitamin A following exogenous administration (Hirose and Yamada, 1978; Watari et al., 1989). In the present study, we found that parathyroid lipid-storing cells sometimes occurred in proximity to macrophages, mast cells, lymphocytes and nerve fiber. Such a phenomenon might reflect mutual cooperation as reported earlier (Watari et al., 1989; Chen et al., 1999). It is not unlikely, therefore, that the parathyroid macrophages, lymphocytes, mast cell and nerve fiber could signal some information towards the lipid-storing cells. Thus, the lipid-storing cells could perform some special functions to affect parathyroid chief cells.

Considerable controversy exists as to the effect of vitamin A on the secretion of parathyroid hormone (PTH). Morphological and biochemical studies suggest that vitamin A causes an increase (Chertow et al., 1975, 1977), a decrease (Frankel et al., 1986), or no change (Hough et al., 1988) in PTH secretion. It was reported that Vitamin A stimulated PTH secretion in bovine parathyroid gland slices *in vitro* and in human volunteers *in vivo* (Chertow et al., 1975, 1977). This effect was dose- and time-dependent (Chertow et al., 1975, 1977). Frankel et al. (1986), however, indicated that the serum PTH levels significantly decreased in excess vitamin A-intoxicated rats. In contrast to these findings, Hough et al. (1988) found that excess vitamin A did not affect PTH secretion in rats. The present study showed no marked morphological or morphometrical changes in parathyroid chief cells after vitamin A administration. It was postulated that vitamin A did not affect the cellular activity of the hamster parathyroid gland. Since there is no convenient and reliable way of measuring hamster PTH, we have no data comparing serum PTH levels between control and vitamin A-treated hamsters at present. The apparent differences among these studies may be explained by the different dose of vitamin A and different animals used. It is well known that toxic substances administered to animals give rise to injury to particular organs and tissues including parathyroid glands. When animals received a relatively not so high dose of vitamin A, parathyroid lipid-storing cells could take up and store a certain amount of vitamin A in the lipid droplets. The chief cells were not affected by vitamin A. Excess vitamin A given to animals, probably beyond the capacity of parathyroid lipid-storing cells, affected the parathyroid chief cells and PTH secretion as

Frankel et al. reported (1986). So the development of vitamin A toxicity seems to be dose-dependent.

In conclusion, hamster parathyroid glands have cells which might be capable of vitamin A incorporation into the lipid droplets. These cells are morphologically similar to lipid-storing cells in other organs and can be included in the category. Though nothing is elucidated directly concerning any effects of these cells on the function of parathyroid gland, they can be classified as one of its cellular components.

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