

Effects of cigarette smoke exposure on the ultrastructure of the golden hamster parathyroid gland

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Summary. Cigarette smoking has been identified as one of the risk factors to induce osteoporosis. However, we find no study on the morphology of the parathyroid gland under smoking exposure. We studied the ultrastructure of the parathyroid gland, lung and femur of the golden hamster exposed to cigarette smoke. Four-week-old male hamsters were housed in a plastic case (48x31x30 cm) and were exposed to cigarette smoke for 12 weeks, 5 minutes exposure, 4 times a day, 4 days a week. There were no differences in serum calcium level and the whole bone mineral density between the control and the smoke-exposed groups. In the parathyroid gland of the smoke-exposed animals, the Golgi complexes associated with many prosecretory granules were well developed and many secretory granules were located near the plasma membrane. Large lipid-like inclusion bodies were observed in the alveolar macrophages of the smoke-exposed animals. The femur morphology showed a wider area of resorbing surface in the smoke-exposed group than in the control group. From these findings, it is conceivable that the secretory activity of the parathyroid gland was stimulated with cigarette smoke exposure.

Key words: Parathyroid gland, Smoking, Femur, Alveolar macrophage, Ultrastructure

Introduction

Several previous studies have indicated that cigarette smoking is found to be associated with a low bone mineral density (BMD) and an increased risk of osteoporosis (Slemenda et al., 1989; Hollenbach et al., 1993; Egger et al., 1996; Kiel et al., 1996; Vogel et al., 1997; Krall and Dawson-Hughes, 1999; Spangler, 1999). However, the mechanism that smoking increases the risk of osteoporosis is still not well clarified. Biochemical studies suggest that smoking reduces the sensitivity to

calcitonin (Hollo et al., 1979), intestinal calcium absorption (Krall and Dawson-Hughes, 1999) and the serum level of 25-hydroxyvitamin D (25OHD), 1,25-dihydroxyvitamin D (1,25(OH)₂D) and parathyroid hormone (PTH) (Brot et al., 1999). On the morphology of the parathyroid gland, however, no study on the effects of cigarette smoking is found. In the present study, we studied the ultrastructure of the golden hamster parathyroid gland to examine whether cigarette smoke exposure affects the parathyroid function. Besides the parathyroid gland, lung and femur were examined.

Materials and methods

Four-week-old male golden hamsters with an average body weight of 87 g were used in the present study. Animals were divided into two groups (control and experimental group) of 10 animals each. They were housed in stainless steel cages, five animals per cage, that were equipped with depressions where food pellets (CE-2, Clea Japan Inc) were placed. Smoke exposures were performed in a transparent plastic case (48x31x30 cm). The plastic case had two silicon tubes in the ceiling and each tube was equipped with a glass stopcock. A cigarette (Peace, Japan Tobacco Co.), whose filter was cut off, was attached to a silicon tube. The other tube was connected to a rubber bulb. By lighting the cigarette and puffing the rubber bulb, the cigarette smoke was pumped into the plastic case. The plastic case was filled with smoke from a cigarette in each smoking exposure. Experimental animals were exposed to cigarette smoke for 12 weeks, 5 minutes exposure, 4 times a day, 4 days a week. On the 85th day, the parathyroid gland, the lung and the right femur of each animal were removed under sodium pentobarbital anesthesia. The parathyroid glands and the lungs were immersed in a mixture of 2.5% glutaraldehyde and 2% OsO₄ in Millonig's buffer at pH 7.4 for an 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-800 electron microscope. Twenty micrographs at final magnifications

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of 22,000 were taken from different regions of the parathyroid glands of each animal from two groups. The areas of the cytoplasm, nuclei, Golgi complexes, lysosomes, lipid droplets and large vacuolar bodies and the numbers of the secretory granules were estimated with the aid of an image measuring system (Finetec). The right femurs were cut by razor, put into 5% NaClO for an hour and dehydrated through ascending concentrations of acetone, and dried by using a Hitachi Critical Point Dryer (HCP-2). After coating the specimens with gold palladium with a Hitachi Ion Sputter (E-1010), we examined them with a Hitachi H-3500 scanning electron microscope. Twenty micrographs at a magnification of 880 were taken from the endosteal surfaces of the right femoral diaphyses of each animal from two groups. The percentage areas of resorbing, forming and resting surfaces were estimated with the aid of an image measuring system (Finetec).

Two-ml samples of cardiac blood were withdrawn from the animals into heparinized syringes for analysis. Afterwards, all samples were centrifuged at 3,000 rpm for 10 minutes to obtain plasma fractions. The serum calcium levels were measured by the o-cresolphthalein complexone method (Connerty and Briggs, 1966). The bone mineral density (BMD) of the whole body was

measured by Dual Energy X-ray Absorptiometry (DXA) using Hologic QDR type 2000 on the same day of experiment before sacrifice.

All data are presented as mean \pm SEM. Statistical analysis was done using a Stat View J-4.5 (Abacus Concepts) on a Macintosh Computer. The significance of the differences between two groups was analyzed by unpaired Student's t test. A p value less than 0.05 was considered statistically significant.

Results

Body weights, BMD and serum calcium levels

The mean body weight, BMD and serum calcium levels of the control and smoke-exposed groups are shown in Table 1. In all subjects there was no significant difference between the control and the smoke-exposed groups regarding these values.

Electron microscopic findings

In the parathyroid glands of the control animals, the chief cells were oval or polygonal in shape, the plasma membranes of adjacent chief cells pursued a tortuous

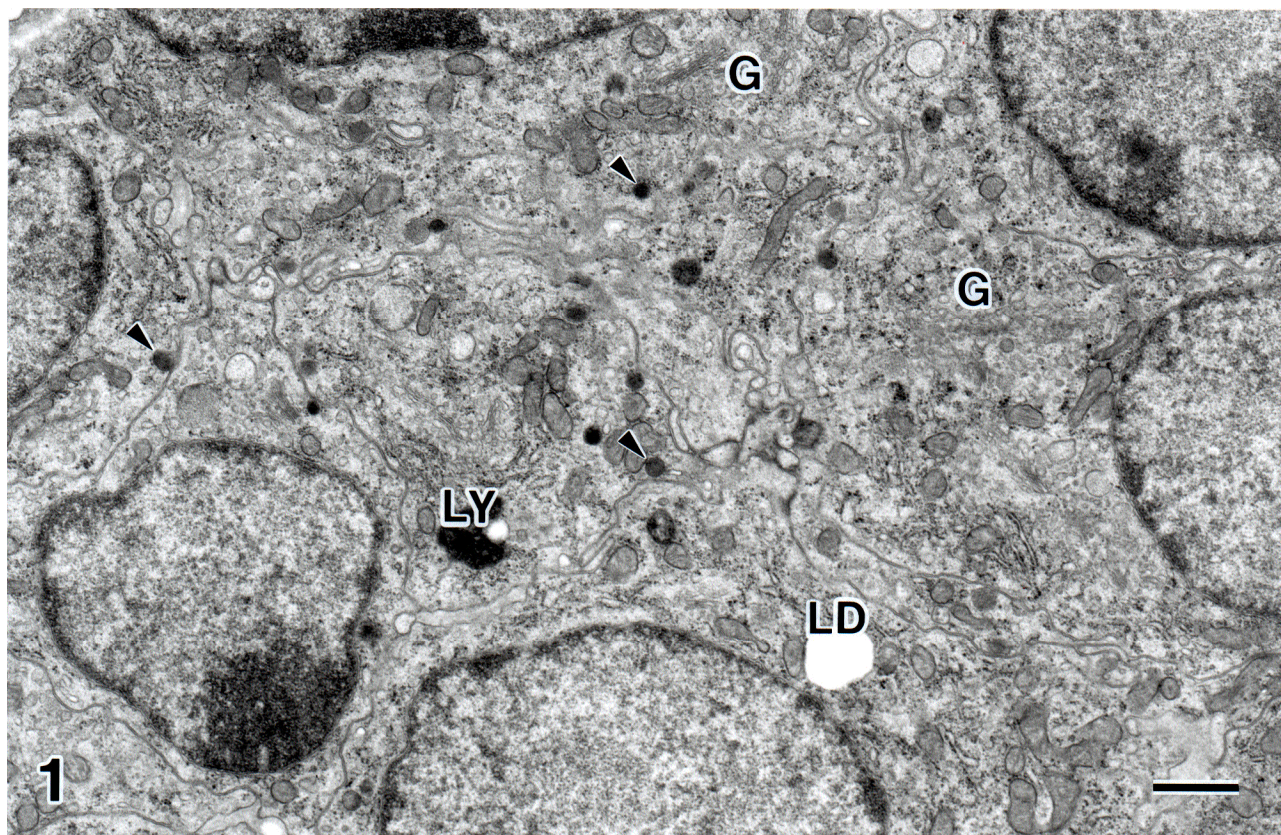


Fig. 1. Parathyroid chief cells of the control hamster. Golgi complexes (G) show moderate development. Secretory granules (arrowheads) are seen. LD: lipid droplet; LY: lysosome. Scale bar: 1 μ m.

Parathyroid of smoke-exposed hamster

course with complex interdigitations (Fig. 1). The intercellular spaces were generally narrow and included floccular or finely particulate materials. The nucleus of the chief cells had an oval or polygonal shape with occasional indentations. The mitochondria were dispersed throughout the cytoplasm, and most Golgi

complexes showed moderate development (Fig. 1). The cisternae of the granular endoplasmic reticulum were sometimes arranged in parallel arrays or randomly distributed in the cytoplasm. Occasional lipid droplets, lysosomes and large vacuolar bodies were observed in the cytoplasm.

In the parathyroid glands of the smoke-exposed animals, the chief cells were similar in shape to those of controls (Fig. 2). However, Golgi complexes were frequently encountered in the cytoplasm (Fig. 2). Well-developed Golgi complexes were provided with numerous prosecretory granules, and many of secretory granules were located near the plasma membrane (Fig. 3).

The volume density of the cell components in the parathyroid gland is shown in Table 2. In the smoke-

Table 1. Body weight (g), BMD (g/cm^2) of the whole body and serum calcium level (mg/dl).

	BODY WEIGHT	BMD	CALCIUM
Control	149.71 \pm 5.05	0.113 \pm 0.002	11.18 \pm 0.32
Smoke-exposed	145.61 \pm 3.40	0.112 \pm 0.004	11.32 \pm 0.30

Values are shown as mean \pm SEM.

Table 2. Volume percentage of the Golgi complex (G), lysosome (LY), lipid droplet (LD) and large vacuolar body (VB) to the cytoplasm and number of the secretory granules (SG) per 100 μm^2 of the cytoplasm.

	G	LY	LD	VB	SG
Control	2.79 \pm 0.10	0.40 \pm 0.04	1.02 \pm 0.18	0.46 \pm 0.07	13.74 \pm 0.48
Smoke-exposed	5.79 \pm 0.34*	0.42 \pm 0.02	0.68 \pm 0.10	0.64 \pm 0.08	18.44 \pm 0.78*

Values are shown as mean \pm SEM. *: $p < 0.05$.

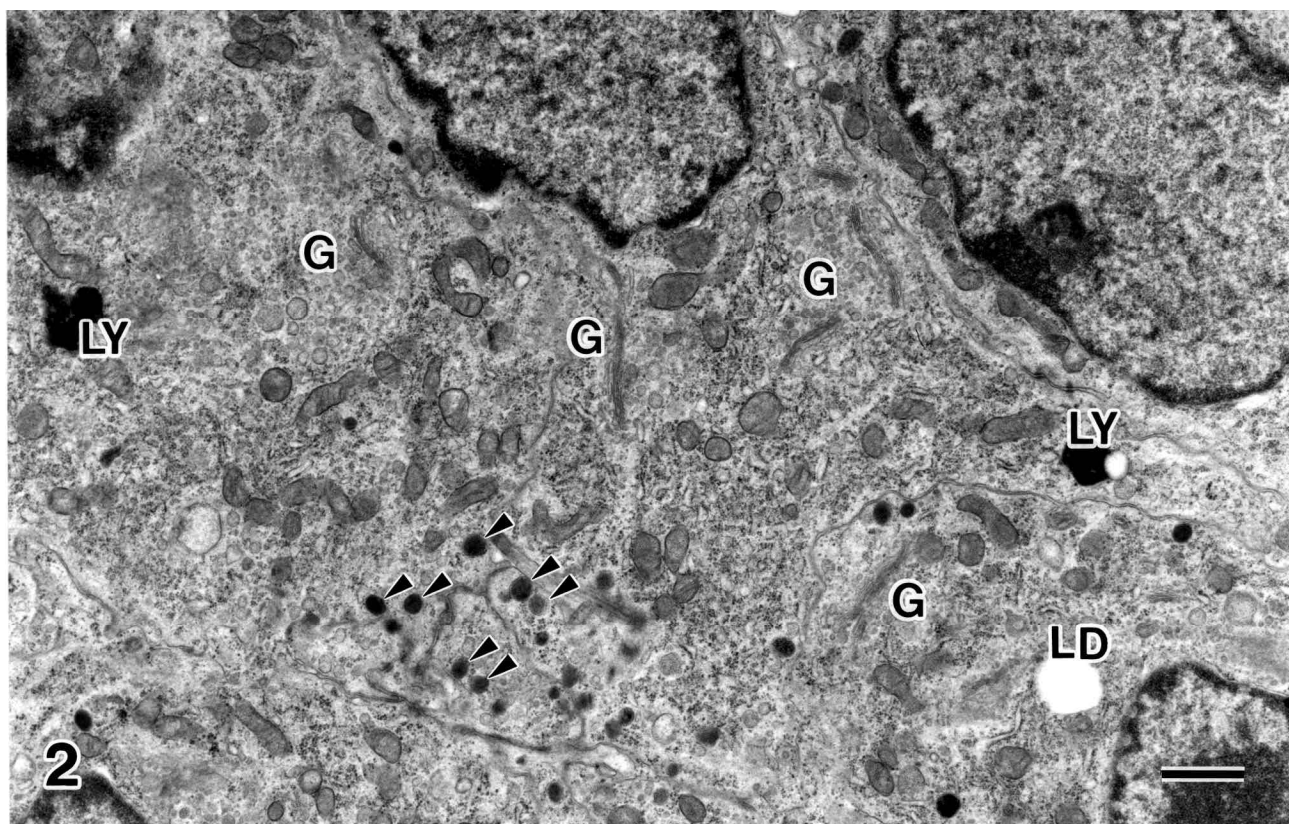


Fig. 2. Parathyroid chief cells of the smoke-exposed hamster. Golgi complexes (G) are frequently encountered. Many secretory granules (arrowheads) are observed in the peripheral region of the cell. LD: lipid droplet; LY: lysosome. Scale bar: 1 μm .

exposed group, a higher volume density occupied by the Golgi complexes ($p < 0.05$) and more secretory granules ($p < 0.05$) were observed in the cytoplasm of the parathyroid chief cells as compared with those of the control group. With regard to the lysosomes, lipid droplets and large vacuolar bodies, there was no significant difference between the control and the smoke-exposed groups.

Regarding the lung, we focused on the alveolar macrophages to examine the effects of cigarette smoke exposure. We found many alveolar macrophages in the lung of the smoke-exposed animals. Alveolar macrophages from the control animals contained a single nucleus and included some lysosomal bodies (Fig. 4). In alveolar macrophages from the smoke-exposed animals,

many large lipid-like inclusion bodies were observed. These inclusions appeared to occupy the major portion of the cytoplasm (Fig. 5).

Endosteal surfaces of the femoral diaphyses in the control and smoke-exposed animals were observed with a scanning electron microscope (Figs. 6, 7). Endosteal surfaces seen in the present study were similar to those described by others (Boyde and Hobdell, 1969; Wink, 1982). Observed surface type was classified as follows:

Resorbing surface shows depressions or pits with bright scalloped edges and represents bone matrix that had undergone osteoclastic resorption.

Forming surface is composed of many nodules. It means a stage of mineralization or calcification of the collagen matrix.

Resting surface is composed of completely mineralized collagen fibers or bundles of collagen fibers.

In both groups, these three types of bone surfaces were detected and the resting surface was dominant (Figs. 6, 7). In the smoke-exposed group, we recognized more resorbing surfaces than forming ones (Fig. 7). The percentage area occupied by each of the bone surfaces is shown in Table 3. The percentage of the resorbing surface was significantly higher ($p < 0.05$) and the resting surface lower ($p < 0.05$) in the smoke-exposed group than those of the control group. There was no significant

Table 3. Percentage area occupied by the bone surface types on the endosteal surfaces of femoral diaphyses.

	BONE SURFACE TYPES		
	Resorbing surface	Forming surface	Resting surface
Control	8.71±0.81	10.68±0.71	80.61±1.20
Smoke-exposed	25.81±3.56*	10.50±0.73	63.68±3.32*

Values are shown as mean±SEM. *: $p < 0.05$.

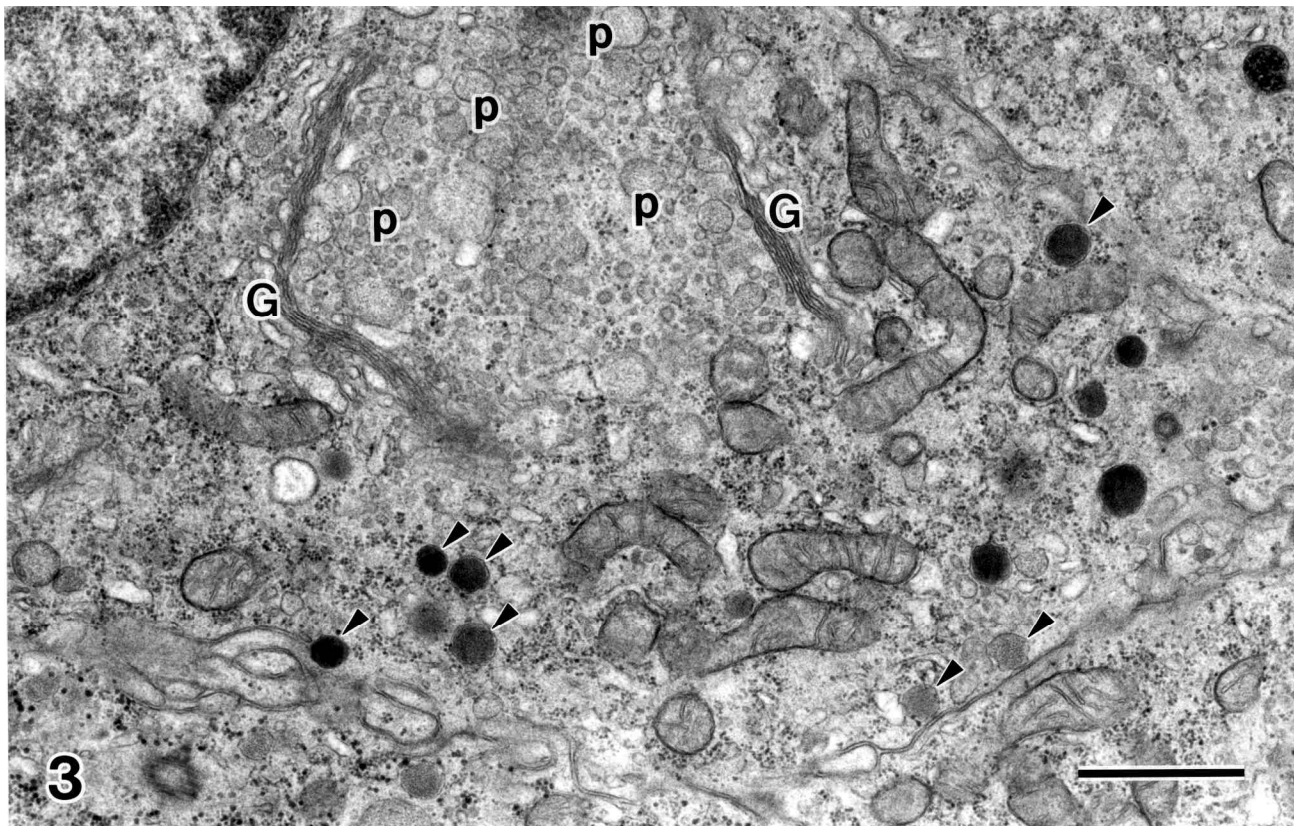


Fig. 3. Parathyroid chief cells of the smoke-exposed hamster. Well-developed Golgi complexes (G) are associated with many prosecretory granules (p). Many secretory granules (arrowheads) are located near the plasma membrane. Scale bar: 1 μ m.

difference regarding the forming surface between the control and the smoke-exposed groups.

Discussion

Although the changes of bone metabolism and PTH level in smokers have been mentioned, no morphological reports of the parathyroid gland are found. The present study demonstrated that chief cells in the parathyroid gland of the smoke-exposed animals contained well-developed Golgi complexes. Many of the secretory granules were located near the plasma membrane. These morphological features are fairly consistent with the findings which indicate an elevated activity of the parathyroid gland (Wild and Becker, 1980; Chen et al., 1990; Isono et al., 1990; Shoumura et al., 1990), and suggest that smoking exposure stimulates the synthesis and release of the PTH.

Brot et al. (1999) described that smokers had a 22% decrease of serum PTH and no differences in serum-ionized calcium as compared with non-smokers. Landin-Wihelmsen et al. (1995) also mentioned that smoking decreased the serum level of intact PTH. In contrast to these findings, Ortego-Centeno et al. (1997) showed an increasing trend of serum PTH in young male smokers.

A similar increasing trend in serum PTH was also noted by Rapuri et al. (2000) in elderly female heavy smokers. These differences are unexplained, but it is necessary to make allowance for various factors such as estrogen level, calcium uptake in bone, vitamin D metabolism and human lifestyles. In the present study, though we had no evidence of PTH level, we suppose that smoking accelerates the synthesis and secretion of PTH from the morphological viewpoint.

It has been reported that smoking has an effect on bone metabolism (Hollenbach et al., 1993; Egger et al., 1996; Kiel et al., 1996; Vogel et al., 1997). However, the biological mechanisms between smoking and BMD are not well understood. Krall and Dawson-Hughes (1999) warned that smoking accelerates bone loss and less efficient calcium absorption. Brot et al. (1999) reported that smokers had significantly reduced levels of serum 25OHD, 1,25-(OH)₂D, and that this may contribute to the reported risk of osteoporosis among smokers. From these remarks, it is conceivable that serum calcium level is in inverse correlation with smoking. Khaw et al. (1992) described that the effects of low 25OHD levels on bone may be mediated through PTH. In the present study, there is no significant difference on mean serum calcium levels between the control and the smoke-

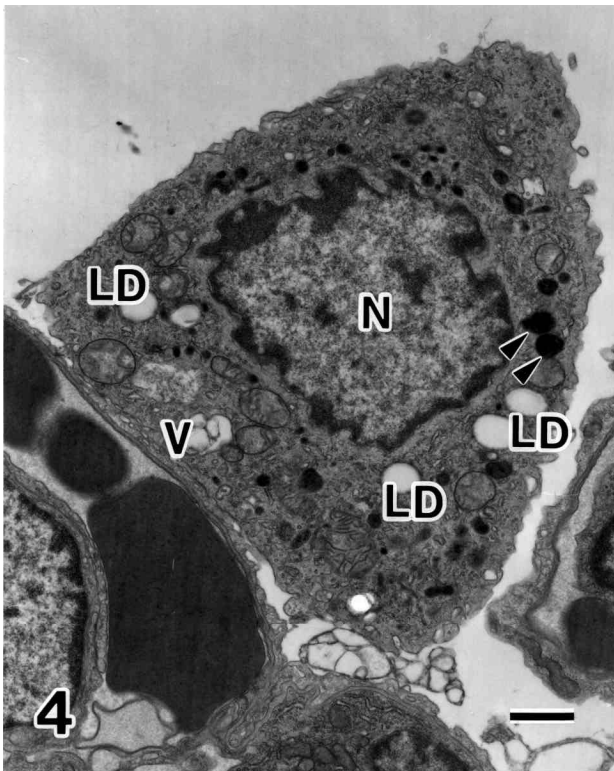


Fig. 4. Alveolar macrophage of the control hamster. Lysosomal bodies (arrowheads) and lipid droplets (LD) are visible in the cytoplasm. V: vacuolar body; N: nucleus. Scale bar: 1 μ m.

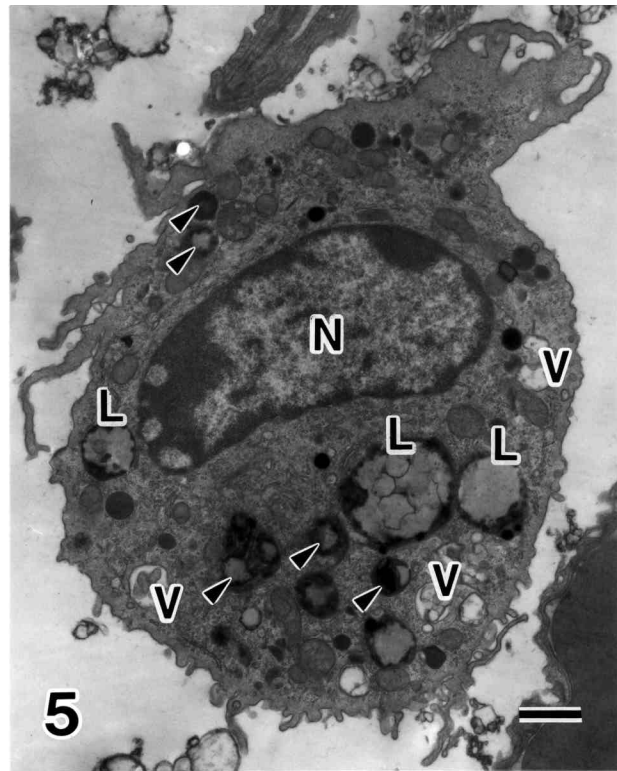


Fig. 5. Alveolar macrophage of the smoke-exposed hamster. The cytoplasm is packed with many large lipid-like inclusion bodies (L), lysosomal bodies (arrowheads) and vacuolar bodies (V). N: nucleus. Scale bar: 1 μ m.

exposed groups. It seems that serum calcium level under smoking exposure is maintained by stimulation of the parathyroid activity.

The alveolar macrophage may have an important role with regard to killing pathogens deposited in airways (Brain, 1988). In the present study, many lysosomal bodies were scattered in the cytoplasm of the alveolar macrophages of the smoke-exposed animals. This result agrees with previous studies (Pratt et al., 1971; Brody and Craighead, 1975; Harris and Gonzalez-Rothi, 1984) and suggests that more foreign materials were digested in the smoke-exposed lung. Wallace et al. (1992) mentioned that the smokers had a significantly increased number of alveolar macrophages per unit lung volume and per unit surface area. In the present study, large lipid-like inclusion bodies were observed in the alveolar macrophages from the smoke-exposed animals. This structure was not found in the alveolar macrophages of nonsmokers (Pratt et al., 1971). According to this, we think that smoking exposure has certain effects on alveolar macrophages.

From a statistical study, McDermott and Witte (1988) suggested that smoking does not directly affect bone loss, but may do so indirectly through its effects on other risk factors like age at menopause, body weight,

dietary habits, and possibly physical activity. Especially, it was supposed that osteoporotic changes in smokers were associated with lower estrogen levels (Spangler, 1999). Contrary to these reports, Egger et al. (1996) described that the relationship between cigarette smoking and BMD cannot be entirely explained by the altered estrogen status, diet, physical activity and alcohol consumption. In the present study, BMD of the whole animal body had no significant difference. However, wider resorbing surfaces of the femoral diaphyseal bone were observed in the smoke-exposed group than in the control group. Forming surfaces showed no difference between the control and the smoke-exposed groups. This result suggests that bone resorption in the smoke-exposed animals was much increased when compared with controls. Femoral bone resorption in the smoke-exposed animals might be stimulated by activated function of the parathyroid gland.

In conclusion, we consider that smoking exposure increases the cellular activity of the parathyroid gland and stimulates the synthesis and release of PTH. Moreover, smoking exposure promotes bone resorption. Further research is needed to clarify the relationship between the parathyroid function and the morphological changes of bone induced by smoking.

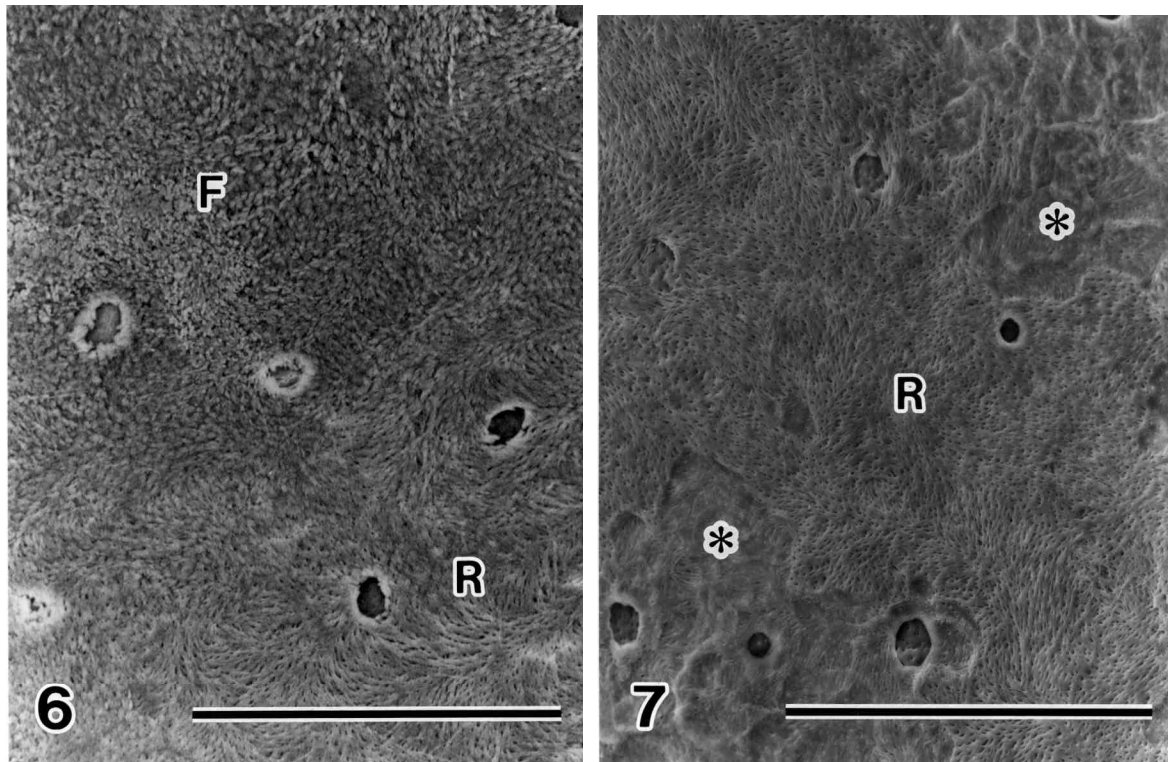


Fig. 6. Scanning electron micrograph of the endosteal surface of the femoral diaphyses of the control hamster. Dominant resting surface (R) is seen in the bottom of the figure. Occasional forming surface (F) occupies a rather large area in this figure. Scale bar: 100 µm.

Fig. 7. Scanning electron micrograph of the endosteal surface of the femoral diaphyses of the smoke-exposed hamster. Large resorbing surfaces (asterisks) spread among the resting surface (R). Scale bar: 100 µm.

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Accepted December 3, 2001