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Ultrastructure of the parathyroid gland of the young golden hamster after short-term treatment with ethanol

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Summary. We studied the ultrastructure of the parathyroid gland of young golden hamsters after short-term treatment with ethanol (1.5 g/kg bw or 6.0 g/kg bw). We did not find any ultrastructural changes of the parathyroid gland after administration of 1.5 g/kg ethanol. In the hamsters, 3 hours after administration of 6.0 g/kg ethanol, the mean serum calcium concentration was significantly low as compared to that of the control animals. In the parathyroid gland 1 hour after administration of 6.0 g/kg ethanol, the Golgi complexes associated with a few prosecretory granules and the volume density occupied by the Golgi complexes decreased compared with that of the control animals. In the parathyroid glands 3 hours after administration of 6.0 g/kg ethanol, the Golgi complexes decreased as compared with those of the control animals, while the large vacuolar bodies increased. These findings suggest that the cellular activity of the parathyroid gland is suppressed after short-term treatment with ethanol. Intracellular lumen was found in the parathyroid chief cells 3 hours after administration of 6.0 g/kg ethanol, and the significance of this structure is discussed.

Key words: Parathyroid gland, Ultrastructure, Golden hamster, Ethanol, Intracellular lumen

Introduction

Several previous studies have indicated that ingestion of ethanol can induce hypocalcemia in some animals and in humans (Peng et al., 1972; Peng and Gitelman, 1974; Chanard et al., 1980; Krishnamra and Limlomwongse, 1983; Laitinen et al., 1991; Laitinen and Välimäki, 1991). The mechanism of ethanol-induced hypocalcemia is at present far from clear and considerable controversy exists as to the role of parathyroid hormone (PTH). Biochemical studies suggest that short-term treatment with ethanol causes an increase (Shah et al., 1978; Williams et al., 1978), a decrease (Magliola et al., 1986; Laitinen et al., 1991, 1992; García-Sanchez et al., 1995), or no change (Chanard et al., 1980; Krishnamra and Limlomwongse, 1983) in PTH secretion. In order to clarify the mechanism of ethanol-induced hypocalcemia, we have reported the ultrastructure of the parathyroid gland of the adult golden hamster after short-term treatment with ethanol (Chen et al., 1997). In the present study, the ultrastructure of the young hamster parathyroid gland after ethanol administration was studied.

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Materials and methods

Four- to 6-week-old male golden hamsters with an average body weight of 78 g were divided into 9 groups of 7 animals each. Ethanol was administered by gavage via an intragastric tube. A dose of 1.5 g/kg of 20% (v/v) and 6.0 g/kg of 50% (v/v) ethanol in distilled water or an equal amount of distilled water (12 ml/kg, control group) was administered. The parathyroid glands of all groups were removed under sodium pentobarbital anesthesia at 1, 3 and 5 hours, respectively, after administration. The glands were immersed in a mixture of 2.5% glutaraldehyde and 2% OsO_4 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-800 electron microscope. Twenty micrographs at final magnifications of 22,000 were taken from different regions of the parathyroid glands of each animal from the 9 groups. The areas of the cytoplasm, nuclei, cisternae of the granular endoplasmic reticulum, mitochondria, Golgi complexes, lysosomes, lipid droplets and large vacuolar bodies, and the number of secretory granules were estimated with the aid of an image measuring system

(Finetec). The blood ethanol concentrations were determined by gas chromatography, and the serum calcium concentrations were measured using a 940 Corning calcium analyzer.

All data are presented as mean±SEM. Statistical analysis was done using StatView J-4.5 (Abacus Concepts). Group mean values were compared by one-way analysis of variance (ANOVA) and Fisher's PLSD test for multiple comparisons as the post hoc test. A p value < 0.05 was considered statistically significant.

Results

Blood ethanol and serum calcium concentrations

The mean blood ethanol (mg/ml) and serum calcium concentrations (mg/100 ml) of the control and ethanol-treated groups are shown in Table 1. The blood ethanol concentration of the animals at 1 and 3 hours after administration of 6.0 g/kg ethanol was significantly high ($p<0.05$) as compared with that of the control animals, with the peak level of 3.3 mg/ml 1 hour after administration. The serum calcium concentration of hamsters 3 hours after administration of 6.0g/kg ethanol was significantly decreased ($p<0.05$). This decrease was inversely correlated with the increase in blood ethanol concentration ($r = -0.75$).

Table 1. Blood ethanol (mg/ml) and serum calcium (mg/100 ml) concentrations (mean±SEM).

TIME (h)	PROTOCOL	ETHANOL	CALCIUM
1	Control	<0.10	10.35±0.25
	1.5g/kg ethanol	0.50±0.10	10.29±0.22
	6.0g/kg ethanol	3.30±0.51*	10.20±0.24
3	Control	<0.10	11.31±0.21
	1.5g/kg ethanol	0.15±0.05	10.99±0.15
	6.0g/kg ethanol	1.90±0.44*	9.96±0.14*
5	Control	<0.10	10.83±0.15
	1.5g/kg ethanol	<0.10	10.75±0.09
	6.0g/kg ethanol	0.13±0.03	10.45±0.15

*: $p<0.05$ vs control and 1.5 g/kg ethanol groups.

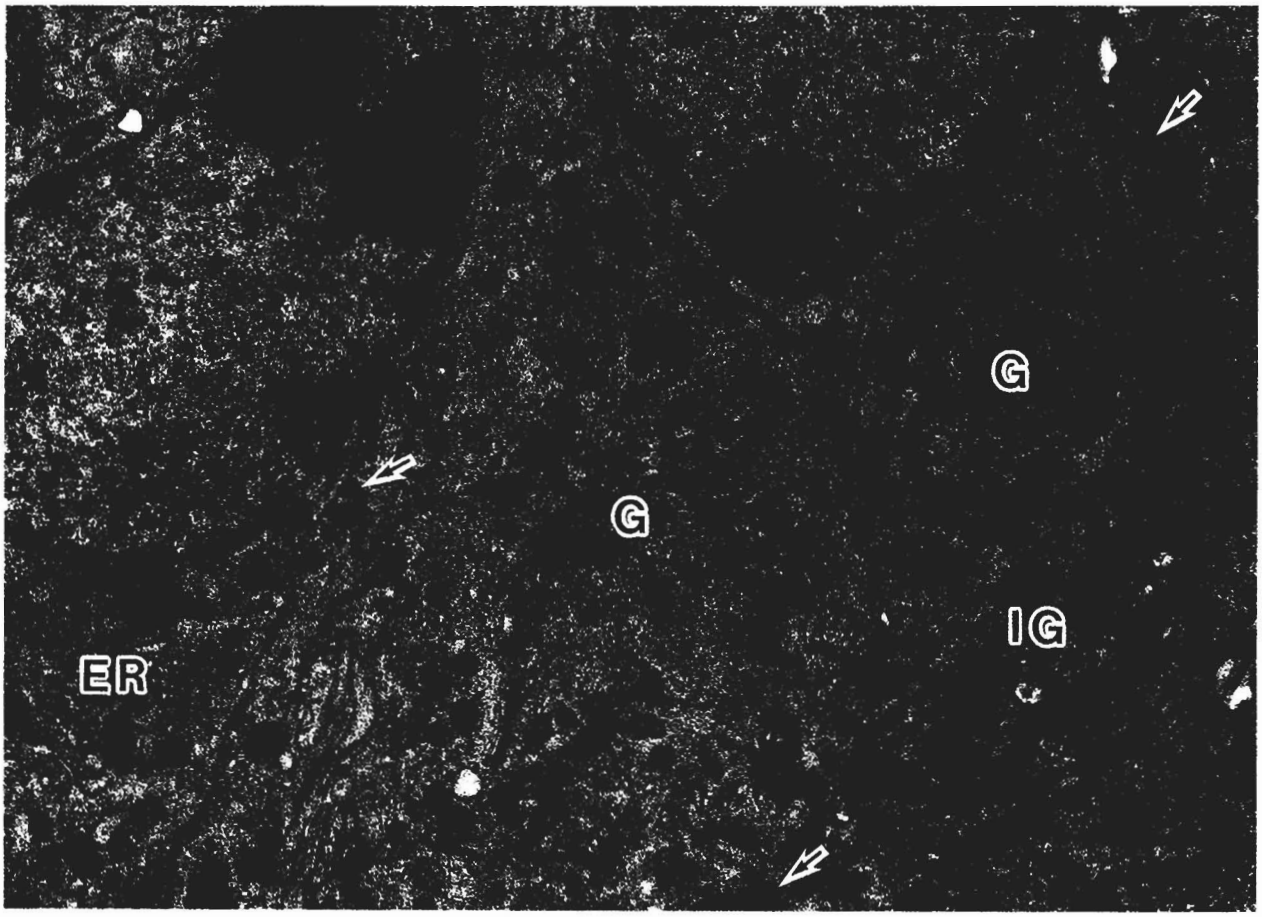

Fig. 1. Parathyroid chief cells of a control golden hamster. Relatively well-developed Golgi complexes (G), occasional interdigitation (IG) and some secretory granules (arrowheads) are observed. ER: cisternae of the granular endoplasmic reticulum. x 13,000

Fine structure of the parathyroid gland

Control group

In the parathyroid glands of the hamsters 1, 3 and 5 hours after administration of distilled water, the chief cells were oval or polygonal in shape. The plasma membranes of adjacent cells pursued a tortuous course with occasional interdigitations (Fig. 1). The intercellular spaces were generally narrow, and occasional enlargements contained floccular material. The cytoplasm was scattered diffusely with free ribosomes and mitochondria. Cisternae of the granular endoplasmic reticulum were randomly distributed or sometimes arranged in parallel arrays. Most Golgi complexes were relatively well developed and associated with some prosecretory granules (Fig. 1). Secretory granules, 150-300 nm in diameter filled with a finely particulate material, were scattered in the Golgi area as well as in the peripheral cytoplasm (Fig. 1). Large secretory granules, 350-600 nm in diameter, showed lower electron density than the secretory granules. Large vacuolar bodies, 350-750 nm in diameter, contained floccular material or vesicles. Lysosomes and lipid droplets were sometimes seen in the cytoplasm. Transitional forms between large secretory granules and large vacuolar bodies were present.

Ethanol-treated group

The morphology of the parathyroid glands of the hamsters 1, 3 and 5 hours after administration of 1.5 g/kg ethanol resembled that of the control animals.

In the parathyroid gland of the hamsters 1 hour after administration of 6.0 g/kg ethanol, many chief cells had rich ribosomes, and poorly-developed Golgi complexes associated with a few prosecretory granules. Randomly-distributed cisternae of the granular endoplasmic reticulum were often arranged in parallel arrays. Secretory granules were scattered in the cytoplasm.

In the parathyroid glands of the hamsters 3 hours after administration of 6.0 g/kg ethanol, many chief cells had rich ribosomes, and poorly-developed Golgi complexes associated with a few prosecretory granules (Figs. 2, 3). Large vacuolar bodies were observed more frequently than in the control (Fig. 2). Secretory granules

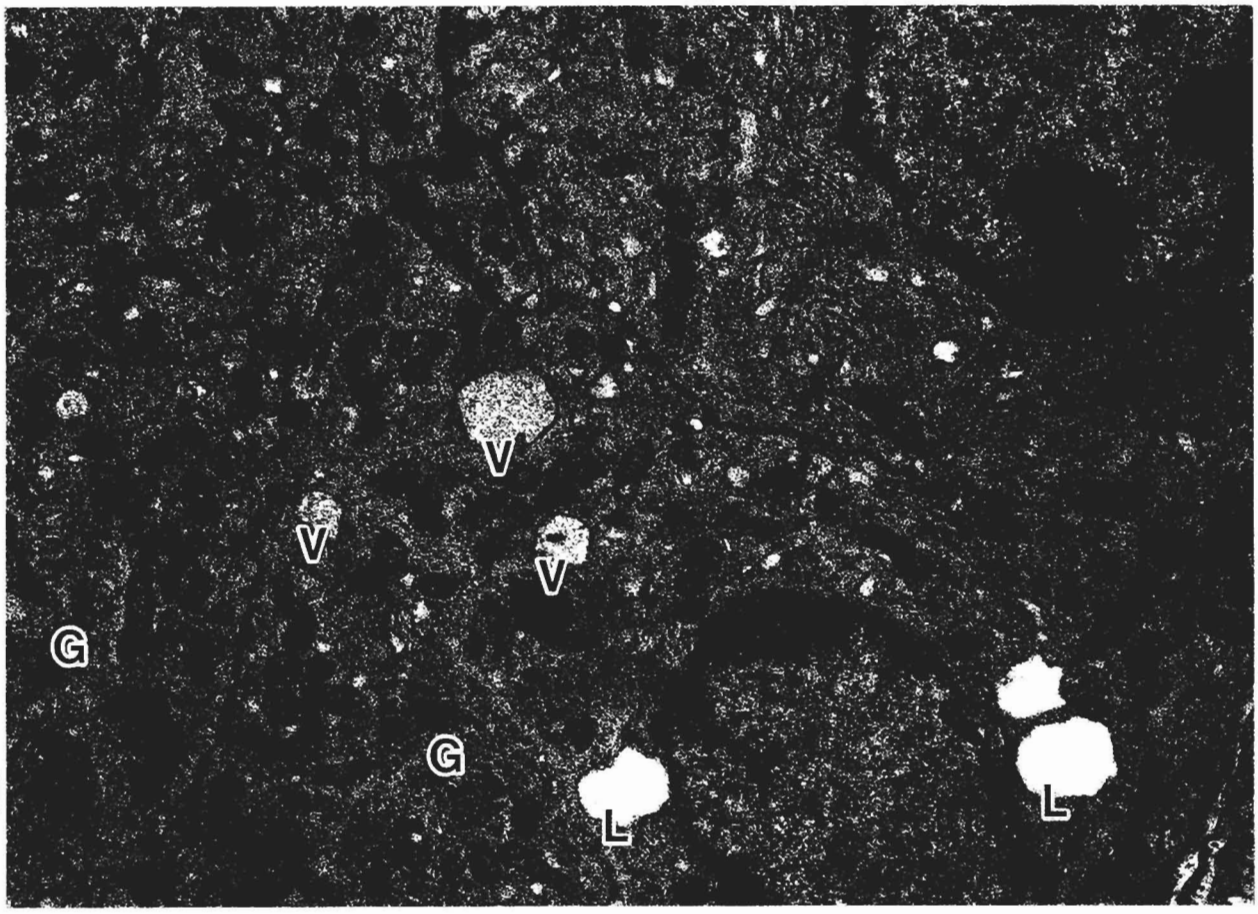

Fig. 2. Parathyroid chief cells of the golden hamster 3 hours after administration of 6.0 g/kg ethanol. Poorly-developed Golgi complexes (G), numerous large vacuolar bodies (V) and lipid droplets (L) are shown. x 13,000

were occasionally observed in the peripheral cytoplasm. Large secretory granules and lysosomes were observed in the cytoplasm.

Intracellular lumina were found in the parathyroid chief cells 3 hours after administration of 6.0 g/kg ethanol (Figs. 3, 4). They had a roughly spherical or oval shape, with an average diameter of 1.0-1.8 μm. Intracellular lumina were surrounded by the single membrane bearing some microvilli (Figs. 3, 4). The content showed a low density and was filled with floccular or finely particulate material (Figs. 3, 4). Secretory granules were sometimes observed in the periphery of the intracellular lumen and the membrane of the granules was fused with that of the lumen (Fig. 4). Using serial sections we could not find any direct communication between the intracellular lumina and the intercellular spaces. The morphology of the parathyroid glands of the hamsters 5 hours after administration resembled that of the control

Table 2. Volume density of the Golgi complex (G), lysosome (Ly), lipid droplet (LD) and large vacuolar body (VB). Volume densities are presented as percentage of cytoplasm, and number of secretory granules (SG) per 100 μm^2 in the cytoplasm.

TIME (h)	PROTOCOL	G	Ly	LD	VB	SG
	Control	5.73±0.29	0.75±0.06	0.95±0.43	0.49±0.04	2.80±0.29
1	1.5g /kg ethanol	6.02±0.37	0.76±0.03	0.39±0.10	0.50±0.10	2.32±0.35
	6.0g /kg ethanol	4.89±0.14*	0.74±0.03	1.01±0.40	0.49±0.09	2.71±0.35
	Control	7.57±0.24	0.38±0.03	0.33±0.04	0.39±0.03	5.12±0.27
3	1.5g /kg ethanol	7.64±0.18	0.44±0.03	0.35±0.04	0.44±0.03	5.24±0.32
	6.0g /kg ethanol	5.54±0.15*	0.38± 0.01	0.39±0.04	0.53±0.03*	4.55±0.19
	Control	7.19±0.22	0.42±0.02	0.44±0.04	0.45±0.03	4.89±0.21
5	1.5g /kg ethanol	7.23±0.25	0.41±0.02	0.46±0.05	0.40±0.02	5.07±0.25
	6.0g /kg ethanol	6.67±0.20	0.44±0.01	0.54±0.02	0.46±0.02	4.49±0.16

Values are shown in mean±SEM. *: $p<0.05$ vs control and 1.5 g/kg ethanol groups.

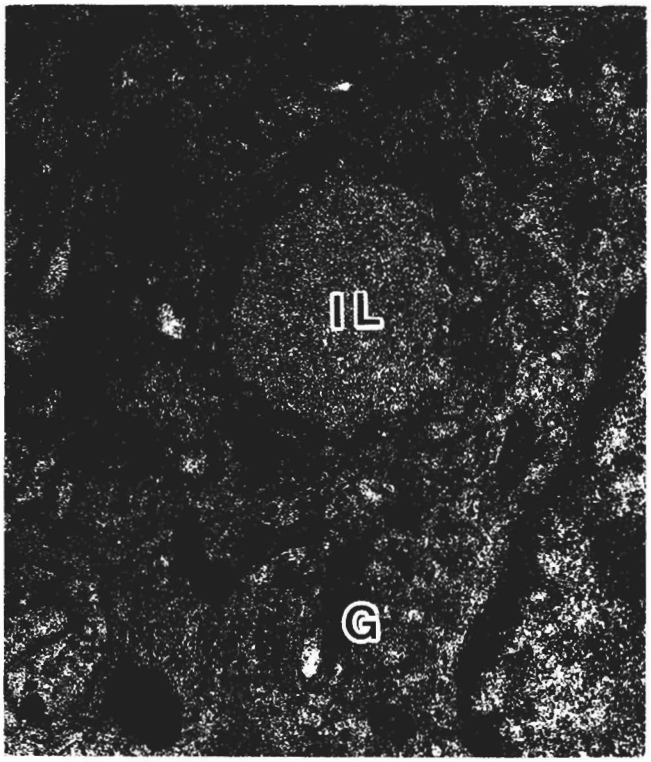

Fig. 3. Parathyroid chief cells of the golden hamster 3 hours after administration of 6.0 g/kg ethanol. Poorly-developed Golgi complex (G) and an intracellular lumen (IL) are observed. x 22,000

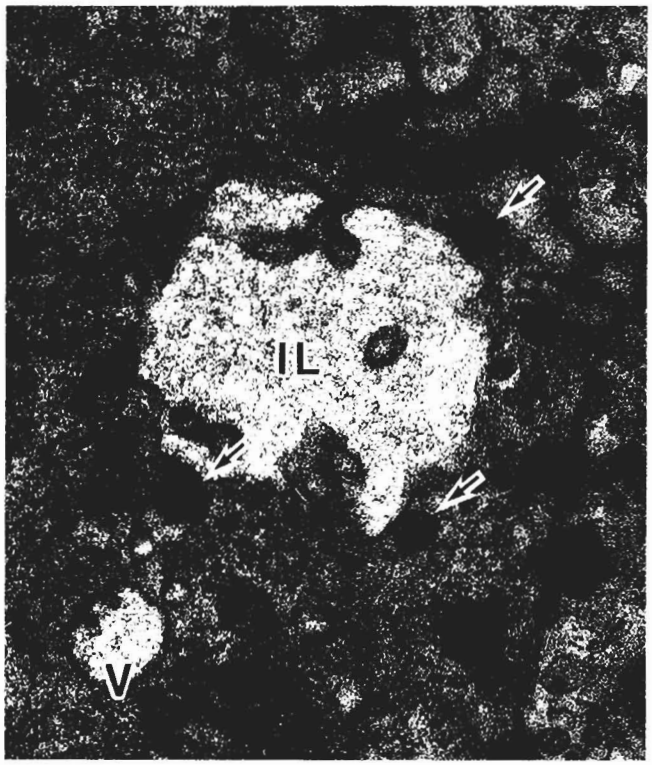

Fig. 4. Parathyroid chief cells of the golden hamster 3 hours after administration of 6.0 g/kg ethanol. Secretory granules (arrowheads) are located in the periphery of the intracellular lumen (IL). V: large vacuolar body. x 36,000

animals.

Stereological analysis of the parathyroid gland

The results obtained from the control and ethanol-treated groups are shown in Table 2. In the parathyroid glands of the hamsters 1 and 3 hours after administration of 6.0 g/kg ethanol, the volume density occupied by the Golgi complexes was significantly decreased ($p<0.05$) as compared with that of the respective control groups. In the parathyroid gland 3 hours after administration of 6.0 g/kg ethanol, the volume density occupied by the large vacuolar bodies was significantly increased ($p<0.05$) as compared with that of the control and 1.5 g/kg ethanol groups. There was no significant difference between the controls and ethanol-treated groups with regard to lysosomes, lipid droplets and secretory granules.

Discussion

Our results show that the serum calcium concentration of the hamsters decreased significantly 3 hours after administration of 6.0 g/kg of ethanol. This result is in agreement with previous studies showing that ethanol can produce hypocalcemia in rats and dogs (Peng et al., 1972; Peng and Giltelman, 1974; Chanard et al., 1980; Krishnamra and Limlomwongse, 1983).

Shah et al. (1978) reported that ethanol caused significant elevation of the serum PTH concentrations in rats. The same research group also found dose-related increases in PTH secretion when bovine parathyroid slices were incubated with ethanol (Williams et al., 1978). In contrast to these findings, the in vivo and in vitro studies indicated that ethanol caused an inhibition of PTH release in animals and human (Magliola et al., 1986; Laitinen et al, 1991, 1992; García-Sanchez et al., 1995). Chanard et al. (1980) however, indicated that ethanol did not affect PTH secretion, but could prevent an increase in plasma PTH in spite of a significant decrease of plasma calcium induced by EDTA. In vitro studies also showed that ethanol could suppress the increase in PTH secretion caused by decreased calcium concentration in the culture medium. It was postulated that acute ethanol loading induced suppression of PTH secretion in the presence of hypocalcemia (Chanard et al., 1980).

We recently investigated the effects of short-term treatment with ethanol on the ultrastructure of the adult hamster parathyroid gland (Chen et al., 1997). The results showed that the serum calcium concentration was significantly low at 3 and 5 hours after administration of 6.0 g/kg ethanol, that the Golgi complexes of the parathyroid chief cells significantly decreased 1 and 3 hours after administration, and that the lipid droplets and the large vacuolar bodies significantly increased 5 hours after administration. These findings suggested that the cellular activity of the adult hamster parathyroid gland was suppressed after short-term treatment with ethanol.

The present study demonstrates that the morphology of the parathyroid gland after administration of 1.5 g/kg ethanol resembled that of the control animals. It is supposed that ingestion of a low dose of ethanol did not affect the cellular activity of the parathyroid gland.

In the parathyroid glands of hamsters 1 and 3 hours after administration of 6.0 g/kg ethanol, chief cells had poorly-developed Golgi complex associated with a few prosecretory granules as compared with those of the control animals. In the parathyroid gland 3 hours after administration of 6.0 g/kg ethanol, chief cells included numerous large vacuolar bodies. These results are fairly consistent with the findings that indicate a decrease in functional activity of the parathyroid gland (Roth and Schiller, 1976; Isono et al., 1977, 1980, 1981, 1982, 1985, 1990; Wild and Becker, 1980; Wild et al., 1982; Hayashi et al., 1981; Emura et al., 1984, 1994, 1997; Iwasaki et al., 1987; Shoumura et al., 1988, 1989, 1990; Ishizaki et al., 1989; Chen et al., 1991, 1997). We consider that these changes, together with a decrease in serum calcium concentration, are induced by suppression of the synthesis of PTH after administration of ethanol.

In this study, the ultrastructure of the parathyroid glands 5 hours after administration of ethanol was very similar to that of the control animals. It is conceivable that the functional activity of the young hamster parathyroid gland had returned to normal 5 hours after administration. In the adult hamsters, the cellular activity of the parathyroid gland returned to normal 12 hours after administration. Accordingly, it seems that the functional recovery of the adult hamster parathyroid gland is slower than that of the young one.

We recently investigated the effects of different ages on large vacuolar bodies in the parathyroid glands of hamsters after short-term treatment with calcium (Emura et al., 1992), prostaglandin E_2 (Emura et al., 1994) or progesterone (Emura et al., 1995) and the effect of $CaCl_2$ or EDTA on large vacuolar bodies of the parathyroid glands in pregnant hamsters (Emura et al., 1997). The results suggested that large vacuolar bodies in the parathyroid gland of hamsters were increased with acute hypercalcemia and decreased with hypocalcemia induced by progesterone. The present study showed that in the parathyroid gland of hamsters 3 hours after administration of 6.0 g/kg ethanol, large vacuolar bodies were significantly increased when compared to those of the control animals. Hence, it is acceptable that the cellular activity of the parathyroid gland may be suppressed 3 hours after administration of 6.0 g/kg ethanol.

In the present work, intracellular lumina were observed in some of the parathyroid chief cells of hamsters 3 hours after administration of 6.0 g/kg ethanol. It was reported that these structures were found in porcine (Remy et al., 1977) and rat thyroid follicle cells (Ericson, 1979) and also in human parathyroid gland of primary hyperparathyroidism (Cinti et al., 1986). No such formations were reported in the parathyroid gland of any other animals.

The origin and physiological significance of the intracellular lumina are not clear as yet. It was supposed that the intracellular lumina are formed by intracellular fusion of exocytotic vesicles (Remy et al., 1977) or by an invagination of the plasma membranes, or directly from the membrane of the Golgi complex (Ericson, 1979). In the present study, it was found that there were no direct connections between intracellular lumina and the plasma membrane, and that some secretory granules located in the periphery of the lumen and the membranes of some granules were fused with those of the lumen. Although we did not apply any histochemical procedures, we suppose that intracellular lumen in the parathyroid chief cells of ethanol-treated hamsters is a part of an intracellular digestive system of the contents of the secretory granules. Additional investigations are required to clarify the origin of the intracellular lumina.

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