Histol Histopath (1987) 2: 163-171

Histology and Histopathology

Immunocytochemical studies on prolactin cells in the adenohypophysis of the golden hamster

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Summary. Mammotrophs or prolactin (PRL) cells were identified in the adenohypophysis of adult golden hamsters by immunocytochemical techniques with a polyclonal anti-PRL, that was proved to be specific to PRL by the dot immunoblotting test. Postembedding immunostaining was performed on Araldite thin sections by immunoperoxidase and immunogold methods. PRL cells were classified into three types according to the different size of the secretory granules. The Type A cells were usually small and angular or oval in shape, and had secretory granules ranging in diameter from 100-230 nm, and showed poorly developed organelles. The Type B and C cells were larger and round or ovoid in shape, contained larger granules, 230-280 nm and 280-570 nm, respectively, and displayed well developed organelles. Immunoreactive PRL cells in the male pituitaries were far less numerous than in the nonpregnant female glands, and were mostly of the Type A and B, whereas in the female the Type C and B cells predominated. In pregnant females, Type C cells became activated and increased in number, while the other two types decreased in proportion. In lactating females, Type A and B cells significantly increased in number at the expense of the Type C cells; meanwhile, the exocytosis of secretory granules was frequently found in all types of PRL cells. The present findings suggest that Type C and B PRL cells, especially the former, are potent in producing and releasing PRL and highly responsive to various physiological stimuli, while Type A cells are probably relatively inert in synthetic activity.

Key words: Golden hamsters - Immunocytochemistry - Prolactin cells - Ultrastructure

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Introduction

Mammotrophs or prolactin (PRL) cells of the golden hamster have been identified ultrastructurally on the basis of the size and shape of the secretory granules in adult pituitaries from normal animals, including females, in different physiological states and in transplanted pituitaries (Dekker, 1968; Yang et al., 1981). Immunohistochemical identification of PRL cells has been performed in transplanted hamster pituitaries (Campbell et al., 1979) and in pituitaries of hamsters treated with cadmium chloride (Giord and Dubois, 1974, 1976). By immunoelectron microscopy Thompson and Trimble (1976) revealed that PRL cells in fattal and neonatal hamsters contain secretory granules smaller in diameter than those seen in adult animals. However, no heterogeneity of hamster PRL cells was mentionend in the above studies.

PRL cells in adult mammalian pituitaries have traditionally been characterized by containing rather large secretory granules as compared with those of other pituitary cells (Nakane, 1970; Girod, 1984). Recently, however, it has been noticed that there are subtypes of PRL cells which contain smaller granules in pituitaries of rats (Nogami and Yoshimura, 1980, 1982), mice (Harigaya et al., 1986), guinea pigs (Beauvillain et al., 1977) and swine (Dacheaux, 1980).

The present study was done to investigate the following problems by immunofluorescence and immunoelectron microscopic techniques with anti-PRL antiserum: (1) are there subtypes of PRL cells in the hamster pituitary, (2) are there any sex differences in morphology and population density of PRL cells, and (3) what are structural modulations of PRL cells in pregnant and lactating females? The results indicated that PRL cells in hamsters could be classified into three types; the population ratios of these types of PRL cells changed according to the sex and to the physiological state of the animal.

Materials and methods

Animals. Golden hamsters *Mesocricetus auratus* at the age of 8-10 weeks were used in this study. They consisted of the following: intact males, intact females at the diestrus stage, late pregnant females (on the 14 - 15th day of gestation) and late lactating females (left with their litters for 2 - 3 weeks).

Tissue preparation. Animals were anesthetized with Nembutal and sacrificed by decapitation. The pituitary gland was cut into pieces and fixed in 4% paraformaldehyde and 2% polyvinyl pyrrolidone in 0.1 M phosphate buffer, at pH 7.4, for 3 h. The tissue blocks were postifixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 30 min. They were dehydrated, infiltrated and embedded in Polybed-Araldite (Polysciences, Warrington, PA). Thin sections were mounted on formvar-coated nickel grids and processed for immunoelectron microscopy.

For immunofluorescence studies, the whole pituitary gland was fixed in 4% paraformaldehyde in phosphate buffered solution. After dehydration in a series of ethanols, it was infiltrated and embedded in paraffin.

Immunochemical reagents. Primary antibodies: polyclonal rabbit anti-human PRL (Accurate Laboratory, Westbury NY), and secondary antibodies: fluorescein isothiocyanate (FITC) or peroxidase goat anti-rabbit IgG (Cappel Laboratory, Cochranville, PA) and colloidal gold conjugated goat anti-rabbit IgG (Janssen Pharmaceutica, Belgium) were used. Six anterior pituitary hormones were obtained for the antibody specificity test; they were porcine growth hormone (GH), sheep PRL, porcine follicle stimulating hormone (FSH), horse luteinizing hormone (LH), porcine adrenocorticotropic hormone (ACTH) and bovine thyroid stimulating hormone (TSH). Except for TSH, which was purchased from Mann Research Laboratory, NY, the other five hormones were obtained from Sigma (St. Louis, MO). Normal goat serum and 3, 3'- diaminobenzidine (DAB) were purchased from Sigma. The reagent for HRP color development and nitrocellulose membranes was obtained from Bio-rad Laboratory (Richmond, CA).

Dot-immunobinding assay. This method was adopted from the Bio-rad instruction for immunoblotting. Nitrocellulose strips were immersed in Tris-buffered saline (TBS: 20mM Tris, 500 mM NaCl, pH 7.5) for 5-10 min and allowed to air dry. Solutions containing different hormones were made in 50mM carbonate buffer (pH 9.6) at 1 mg/ml protein concentration. Two µl each of the different protein solutions were spotted on three strips of the nitrocellulose membrane; proteins were fixed on the membrane by formaldehyde vapor (Larsson, 1981). One strip was stained with 1% amido black solution for visualization of the protein localization on the membrane strip. The other two strips were washed with TBS for 5 min and treated with BSA-TBS) (1% normal goat serum, 30% bovine serum albumin in TBS). One strip was reacted with anti-PRL (1:50 diluted in BSA-TBS) overnight; then, it was washed with TTBS (0.05% Tween 20 in TBS) for 30 min, reacted with peroxidase goat anti-rabbit-IgG (1:2500) for 2 h and developed in the peroxidase substrate solution. The control strip was incubated with the peroxidase-secondary antibody alone and then the substrate solution.

Immunofluorescence. Deparaffinized sections were hydrated and washed with PBS (phosphate buffered saline: 137mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8.0 mM Na_2HPO_4, pH 7.3). They were treated with 10% normal goat serum and reacted with 1:20 diluted anti-PRL at 37°C for 2 h. After washing with PBS, they were reacted with 1:20 diluted FITC conjugated goat anti-rabbit IgG for 2 h. After an extensive wash, sections were mounted in 2% n-propyl gallate, 60% glycerol in 0.1 M phosphate buffer, pH 8.0 (Giloh and Sadat, 1982). Observations were made using a Leitz Orthoplan microscope equipped with epifluorescent illumination.

Immunoelectron microscopy Grids for immunostaining were etched with 10% H_2O_2 for 10 min, washed with PBS and treated with 100% normal goat serum for 30 min. They were reacted with 1:50 diluted anti-PRL at 4°C for 48 h. After several rinses in PBS, the grids were incubated with 1:100 diluted peroxidase goat anti-rabbit IgG or 1:20 diluted 10nm gold conjugated goat anti-rabbit IgG at 37°C for 2h . For immunoperoxidase staining, sections were further reacted in DAB substrate solution (5 mg DAB, 400 µl of 30% H_2O_2 in 40 ml 0.05 M Tris-HCl, ph 7.6) for 20 min. After several washes with Tris-HCl buffer and distilled water, the grids were stained with uranyl acetate and lead citrate, and examined with an electron microscope.

The immunogold method afforded a clear-cut image of the intracellular fine structure even in the immunoreactive cells, but it was not advantageous for taking electron micrographs at a low magnification, which were used for cell counting. For the latter purpose the immunoperoxidase preparations were convenient because of the high electron density of the immunoreactive cells readily distinguishable from the nonreactive ones at a low magnification.

Cell count for the three types of PRL cells. Single ultrathin sections were prepared from six different tissue blocks chosen from each group of three animals, and were stained by the immunoperoxidase method as described above; all grid squares from a section were photographed at magnification of 2000 diameters. Only cells sectioned through the nucleus were classified and counted on montages of enlarged electron micrographs (× 4000). The relative proportion of the PRL cell types was represented by percentages of the cell number, and the data were analyzed with Student's *t* test.

Results

Characterization of anti-PRL. The specificity of rabbit anti-human PRL was determined by dot-immunobinding assay (Fig. 1). Strip 1 shows six protein spots stained by amido black. Incubation only with the peroxidase secondary antibody and the substrate solution did not give any background reaction (strip 2). Anti-PRL was

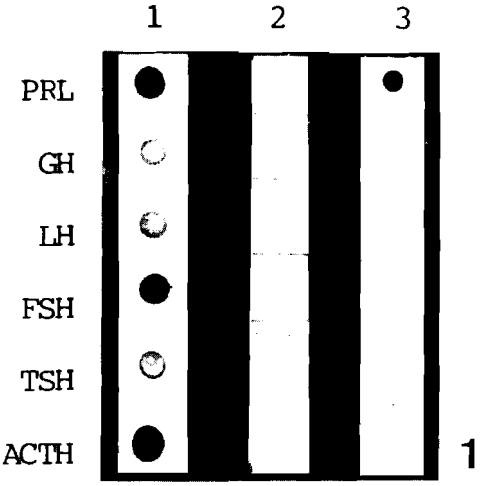
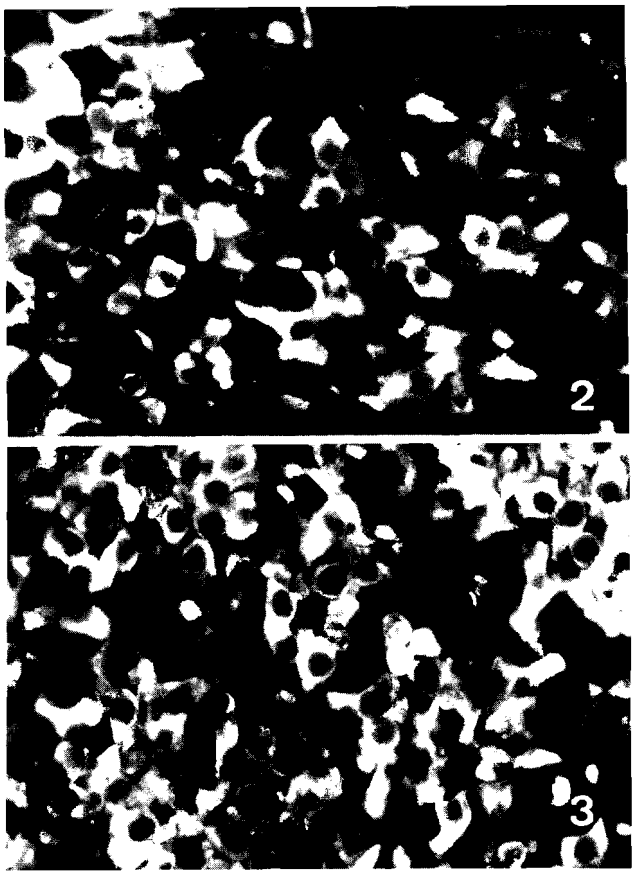


Fig. 1. Specificity test of the rabbit anti-human PRL by dot immunobinding assay. Three strips of nitrocellulose membrane were spotted identically (2 μl per spot) with six hormones which were then fixed on the membrane by formaldehyde vapor. The strips were processed as describe in Materials and Methods. Strip 1 shows the protein spots stained with amido black. Strip 2 was incubated only with the peroxidase secondary antibody and then with the substrate; no background reaction is found. Strip 3 was incubated with anti-PRL primary antibody and then with the secondary one, showing that the anti-PRL reacted only with PRL but not with other hormones.



Figs. 2, 3. Sections of the pars distalis of a male (Fig. 2) and a diestrous female (Fig. 3) pituitary, showing immunofluorescence staining for PRL. Apparently more numerous and larger PRL cells are found in the female than in the male. × 1,700

shown to specifically react with PRL, but not with the other hormones (strip 3).

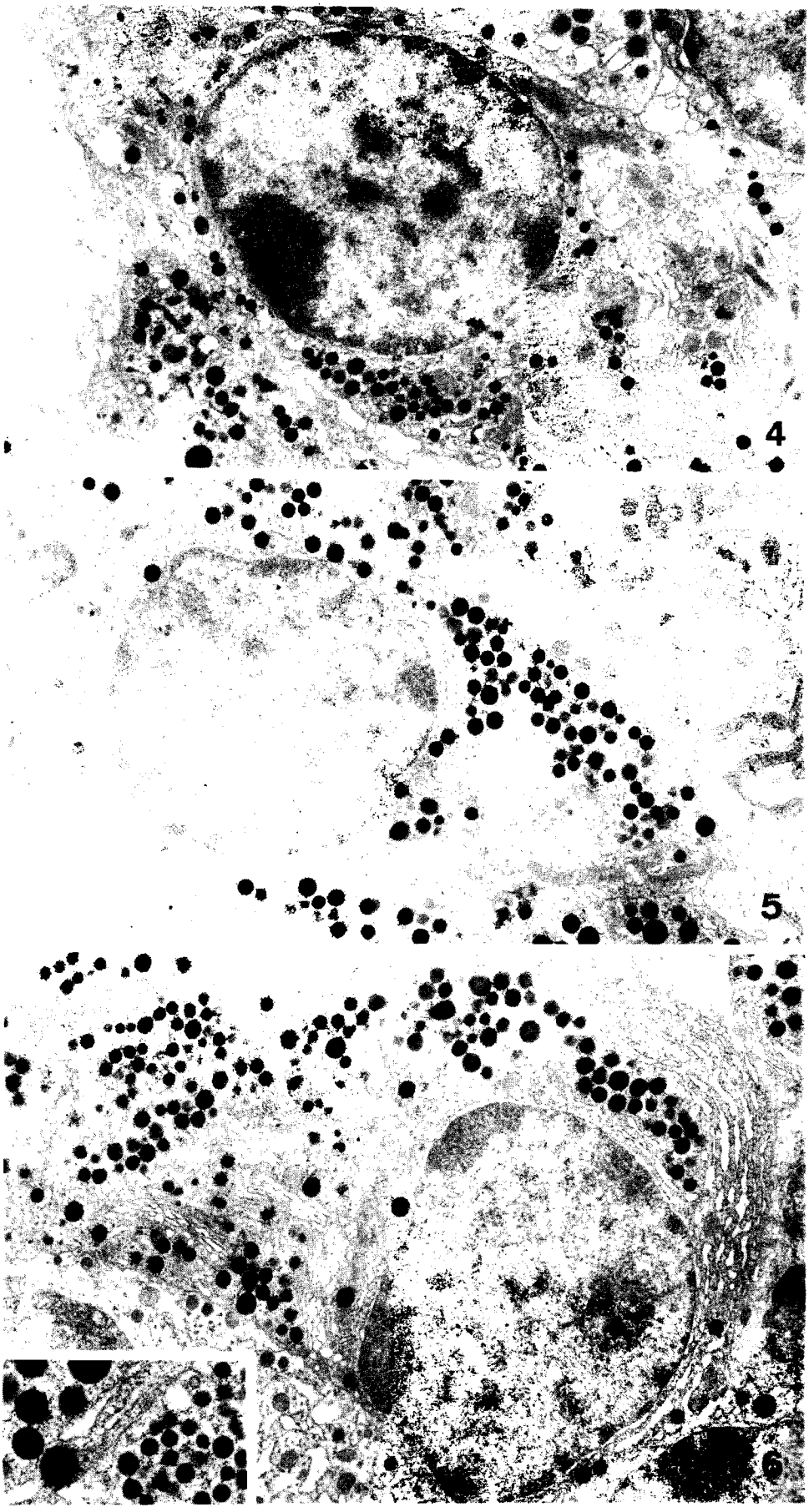
Immunofluoresence staining of PRL cells. Figures 2 and 3 show the immunostained PRL cells in male and female pituitaries, respectively. PRL cells in the females were large and oval in shape in contrast with the smaller, angular PRL cells in the males. There were apparently far more numerous PRL cells in females than in males.

Types of PRL cells. PRL cells were identified by their immunopositive secretory granules as revealed by ultrastructural immunocytochemistry which included both immunoperoxidase and immunogold methods. The mature secretory granules of PRL cells in both sexes displayed a great variation in size. For the convenience of description, we classified PRL cells into three types on the basis of the size of secretory granules. The Type A cells contained secretory granules of the smallest size ranging in diameter from 100-230 nm (Fig. 4). They were ovoid or angular in shape with a large nucleo-cytoplasmic ratio; the cytoplasmic organelles were usually poorly developed: a few short profiles of the rough endoplasmic reticulum (rER) scattering in the cytoplasm. Secretory granules in the Type B and C cells were 230-280 nm and 280-570 nm in diameter, respectively (Figs. 5, 6). PRL cells of these types were larger than the Type A cells, and usually round or oval in shape often with well-developed organelles, i.e., stacks of cisternae of rER and large Golgi complexes. In the trans face of well-developed Golgi complexes immature secretory granules were frequently found; they greatly varied in size and were often irregular in shape as shown in Figure 7, apparently resulting from fusion of small granules. The mature granules were spherical, but never irregular in shape.

PRL cells in adult males (Fig. 9). PRL cells consisted of about 30 per cent of the whole nucleated cells in the male pituitaries. The majority of them were the Type A (45% of the total PRL cells), and B (46%); large granule-containing Type C cells were scarce (Fig. 8).

PRL cells in diestrous females (Fig. 10). In the nonpregnant females PRL cells comprised of about 57 per cent of the total pituitary cells. More than half of the PRL cells were Type C cells (58% of the total PRL cells), and only a small fraction (3%) were Type A cells (Figs.8). The predominant Type C and B cells in this group of animals often showed a wider range in the diameter of granules, that is, granules of the large and the medium-sized categories coexisted in a variable proportion in the same cells. This finding may suggest that there are intermediate forms between Type C and Type B cells. In the present study, however, such intermediate types of PRL cells were not noticed in the pituitaries of other groups of hamsters.

PRL cells in pregnant hamsters (Fig. 11). PRL cells amounted to about 54 per cent of the total pituitary cells. Type C cells predominated over the other two types in the late pregnancy (Fig. 8). Type B cells consisted of about 6% of the total PRL cells, while Type A cells were not found at all. The Type C cells in this group of hamsters were apparently hypertrophic as compared with those in the nonpregnant females. Besides



Figs. 4-6. Immunoelectron microscopic demonstration of PRL by colloidal gold-antibody method in Type A (Fig. 4), B (Fig. 5) and C (Fig. 6) cells, showing small secretory granules in Type A, medium-sized ones in Type B, and large ones in Type C. The development of cytoplasmatic organelles is greatest in Type C and lowest in Type A. A moderately developed Golgi apparatus shown in Fig. 4 is uncommon for Type A cells. × 9,000. Inset: High magnification of portions of Type A and C cells, clearly showing small and large secretory granules decorated with 10-m gold particles. × 18,000

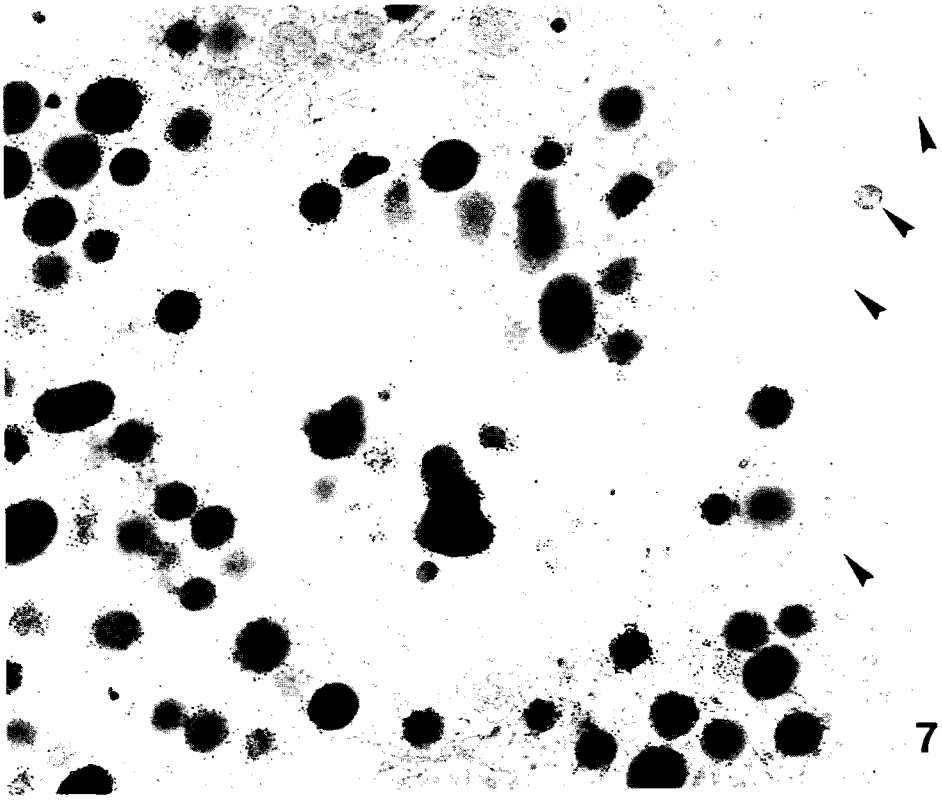


Fig. 7. High magnification view of the Golgi area in a Type C cell from a diestrous female, stained by immunogold method for PRL. Immature secretory granules of varying size and shape are all studded with gold particles. Arrowheads indicate nonreactive secretory granules in an adjacent cell. × 22,000

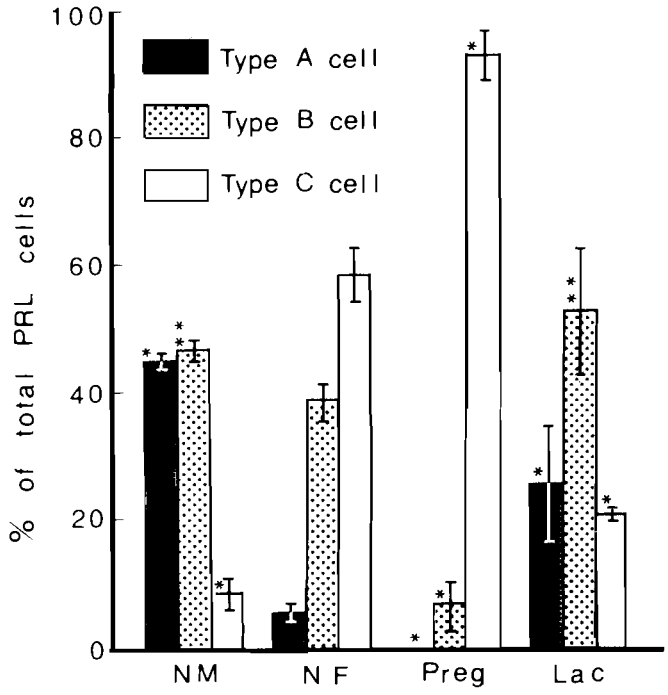
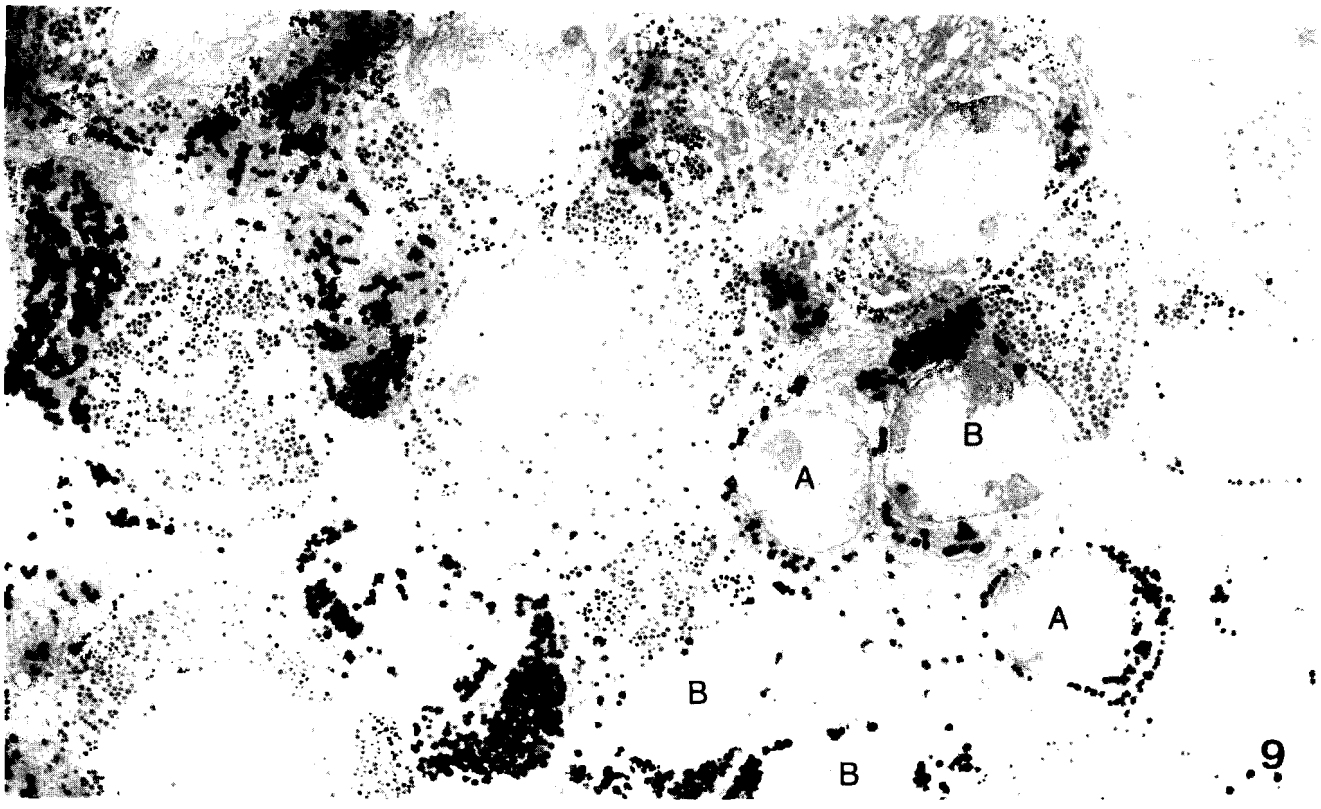
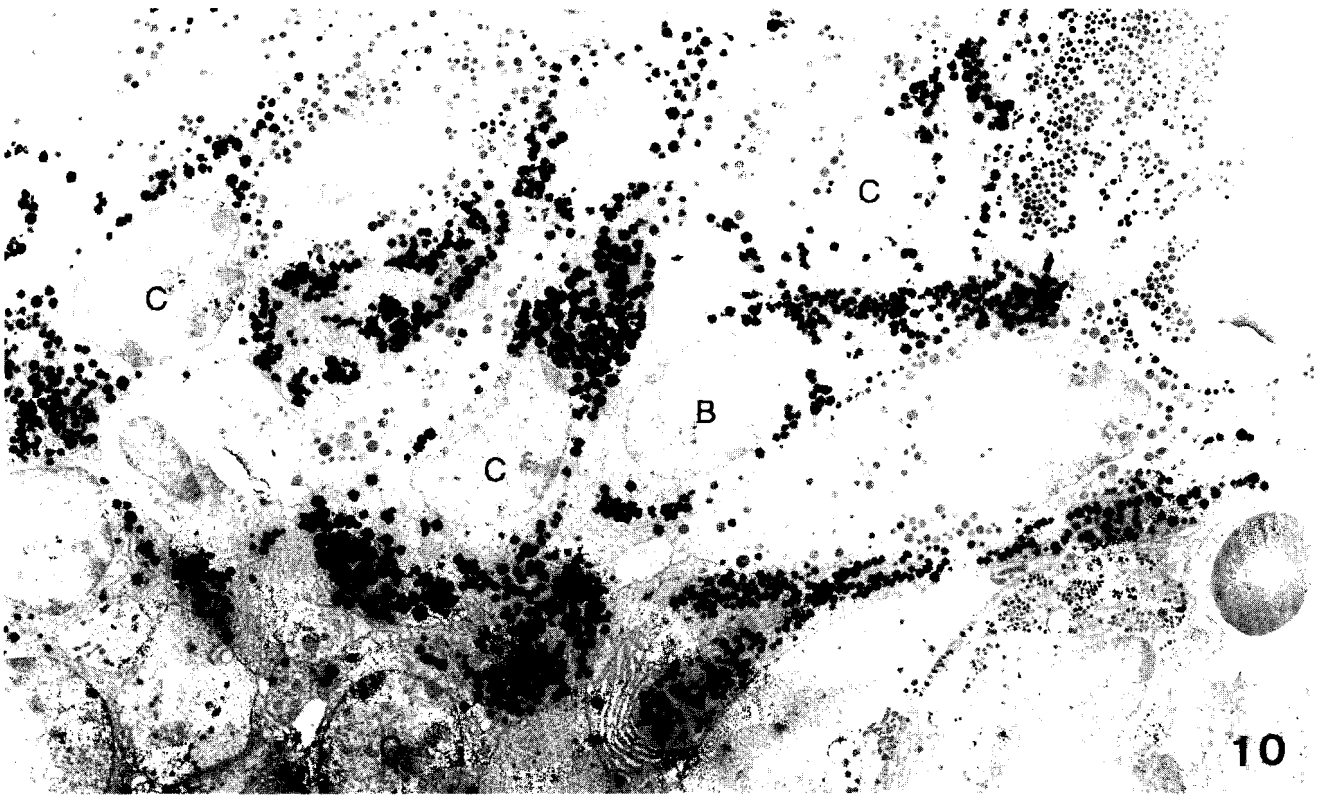


Fig. 8. Histogram of different population ratios of three types of PRL cells in adult male (NM), diestrous female (NF), late pregnant (Preg) and late lactating (Lac) females. Values are mean ± SE. The change in population is particularly evident in Type A and C cells. *: $p < 0.01$ vs NF **: $p < 0.05$ vs NF.





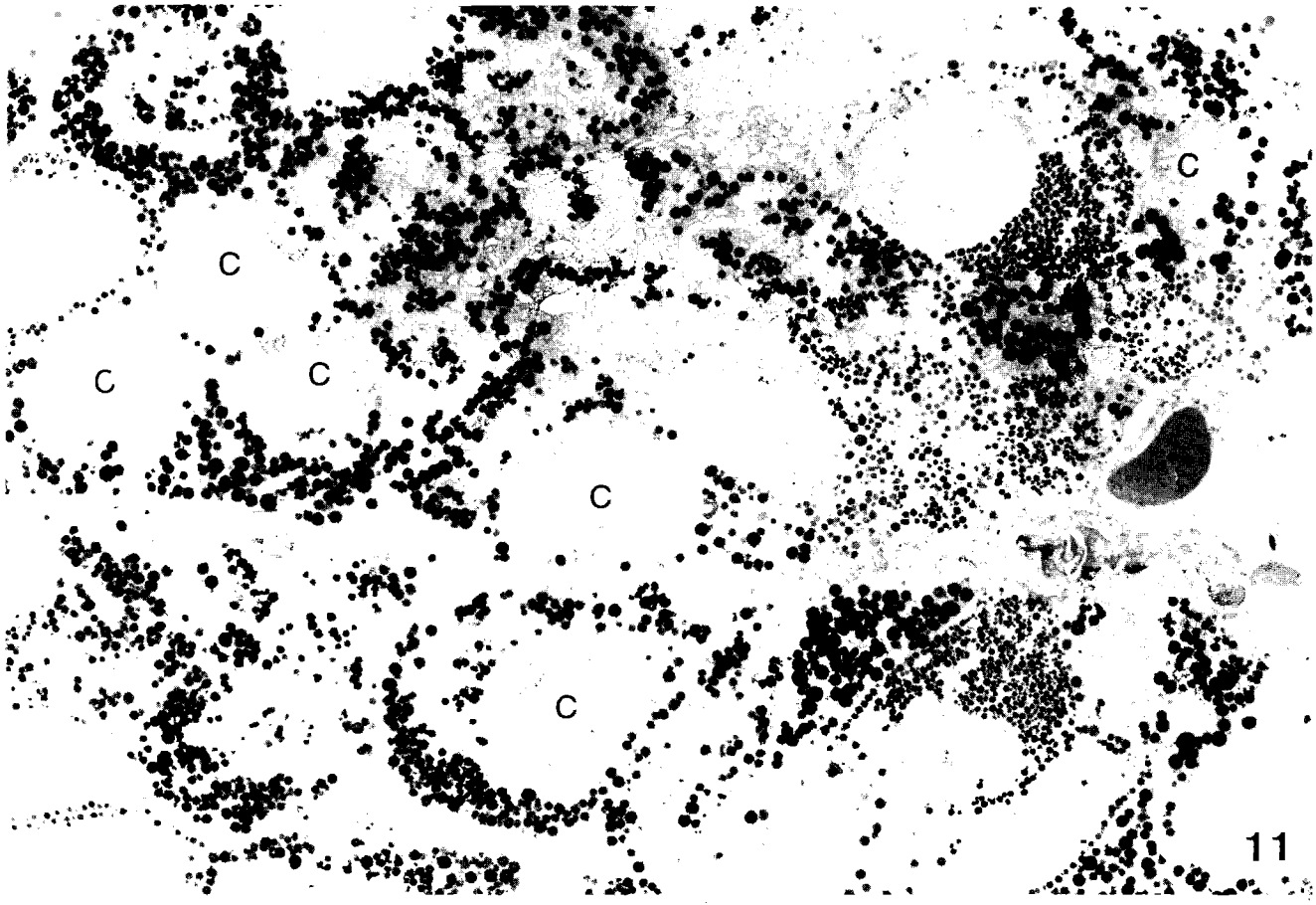
Figs. 9-12. Immunocytochemical localization of PRL in sections of the anterior pituitary by immunoperoxidase method. Different types of PRL cells are indicated on their nuclei. × 4,000.

Fig. 9 Adult male. A small number of Type A and B cells. They are mostly angular in shape.

Fig. 10. Diestrous female. PRL cells are numerous and large as compared with those in male gland; Type C cells predominate.

Fig. 11. Late pregnant female. A profusion of hypertrophic Type C cells is noted.

Fig. 12. Female lactating for 2 weeks. Type A and B cells predominate, whereas Type C cells are greatly diminished. For the most part secretory granules are also decreased in number.



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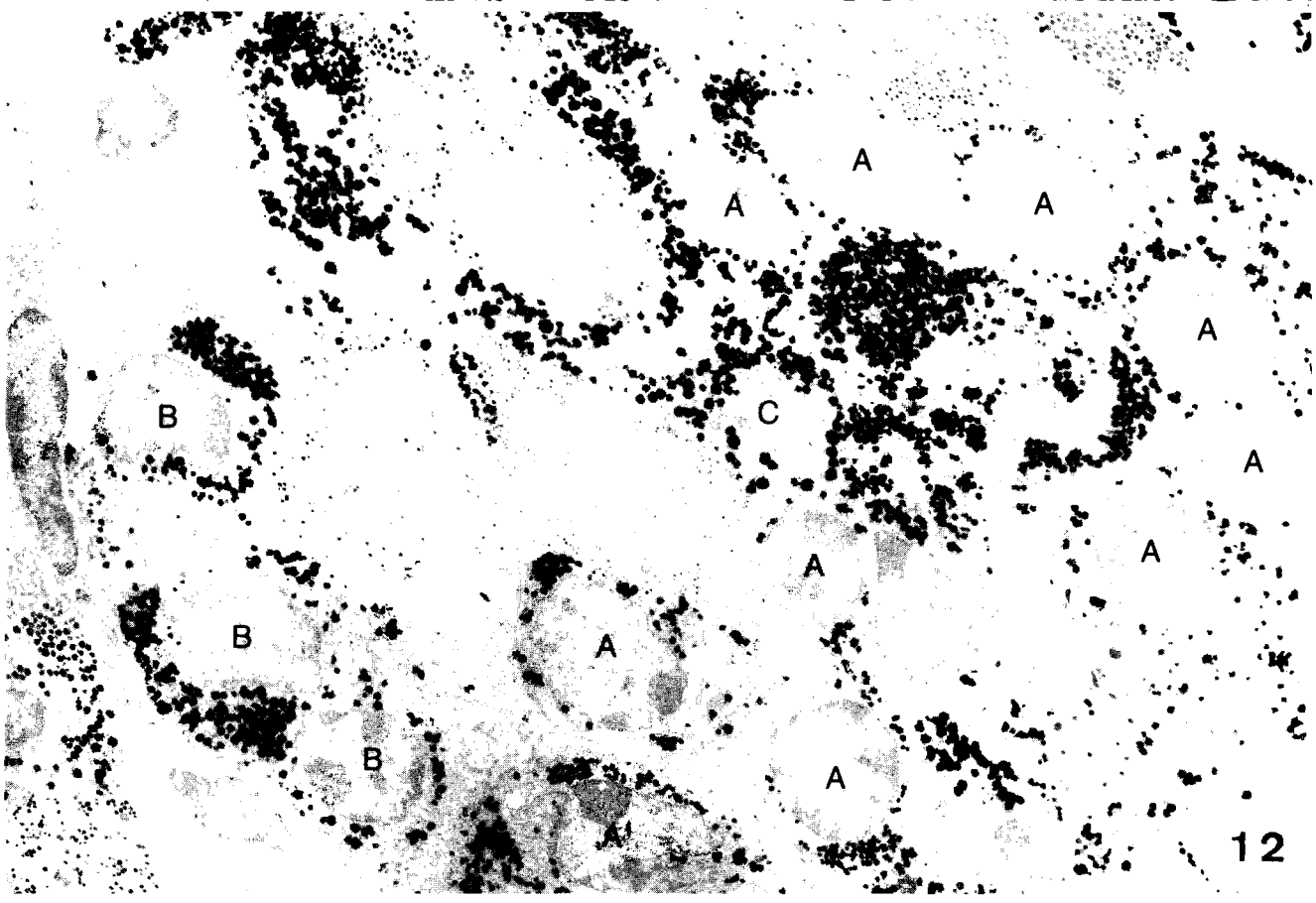

12

prominent arrays of cisternae of the rER and well developed Golgi complexes, a large accumulation of secretory granules was also readily noticed in the cytoplasm.

PRL cells in lactating females (Fig. 12). About 47 per cent of the nucleated pituitary cells were constituted by PRL cells. The population of Type A and B cells tremendously increased at the expense of that of Type C cells (Fig. 8). Secretory granules in most of the PRL cells were small in number. Extrusion of secretory granules was frequently observed in all types of PRL cells; in some instances multipe granules were seen within a single caveola of the plasma membrane.

Discussion

In the present study hamster PRL cells were distinguished into three subtypes based on the size of secretory granules. Type A cells have small granules (100-230 nm) and poorly developed organelles, Type B and C cells, both displaying well developed organelles, possessed medium-sized granules (230-280 nm) and large granules (280-570 nm), respectively. We postulate that Type B and C cells represent active states of PRL cells, where as Type A cells represent an inert state of PRL cells on the basis of the following observations. Type C cells greatly increased in number in pregnant females. There is a positive correlation between abundance of Type C cells and high PRL content reported in the pituitaries of pregnant female hamsters (Kent, 1968). In lactating females, Type C cells significantly decreased in number, while Type A and B cells increased in proportion. Moreover, PRL cells of all types became degranulated, a result from active extrusion of secretory granules in response to the suckling stimulus. Our observation of the decreased number of Type C cells is consistent with the low PRL content shown in the pituitaries of lactating female hamsters (Kent, 1968). Thus, the number of Type C PRL cells appears to reflect the amount of PRL in the pituitary.

The presence of subtypes of PRL cells has been reported in the guinea pig (Beauvillain et al., 1977): aspect I cells with 180-250 nm granules and aspect II cells with 250-350 nm granules, the swine (Dacheux, 1980): one type containing 450-750 nm granules and the other 250-500 nm granules, and the rat (Nogami and Yoshimura, 1982): Type I cells with 130-200 nm granules, Type II cells with 250-300 nm granules and Type III cells with 300-700 nm granules. The secretory granules of PRL cells as a whole in the hamster are slightly smaller in size than those in the rat (Nogami and Yoshimura, 1982) and larger than in the guinea pig (Beauvillain et al., 1977) and the mouse (Harigaya et al., 1986). The shape of mature or large secretory granules in the hamster was spherical instead of polymorphic which is very common in the other rodents mentional above. Plolymorphism is restricted to the immature granules within the Golgi area in the hamster PRL cells.

The observation that PRL cells with large secretory granules contain well-developed organelles was consistently found in the guinea pig (Beauvillain et al., 1977), rat (Nogami and Yoshimura, 1982) and hamster (present study). Our result, showing a preferential dominance of PRL cells with large granules in female hamsters agreed well with a similar finding in female rats (Nogami, 1984).

The marked changes in the relative ratios of the three types of PRL cells found in the pregnant and lactating hamsters were not accompanied by significant changes in the proportion of total PRL cells to all pituitary cells. Therefore, it is plausible that one subtype might transform into another one. A hypothesis has been postulated by Nogami (1984) in rats for the interconversion of different types of PRL cells in response to estrogen stimulation. Further studies by autoradiography in conjunction with immunocytochemistry may contribute to prove the hypothesis.

In conclusion, this study demonstrated the presence of three subtypes of PRL cells in the anterior pituitary of golden hamsters. The population ratios of three subtypes in all PRL cells exhibited sex difference and fluctuated in response to the physiological alteration of PRL secretion in pituitaries. PRL cells with large or medium-sized granules (Type C and Type B) seem to be more potent in synthesis and secretion of PRL than the small granule-containing PRL cells (Type A).

Acknowledgments. This work was supported by grants from the National Science Council, Republic of China (75-0412-B-002-04 and 101).

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Accepted December 12, 1986