Changing Ultrastructures in the Estrous Cycle and Post-natal Development of Prolactin Cells in the Rat Anterior Pituitary as Studied by Immunogold Electron Microscopy

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Summary. Three types of prolactin (PRL) cells of the rat anterior pituitary were recognized by immunogold electron microscopy: Type I cells are characterized by irregularly shaped large secretory granules (500 nm in maximum diameter); Type II cells contain spherical granules of varying size (150-250 nm); and Type III cells are immature forms with a narrow cytoplasm and small round granules (100 nm). In the male adult pituitary, Type II cells occur most frequently (48%), Type I cells slightly less frequently (46%), while Type III cells are very rare (6%).

In the female gland, Type I cells exceed 90% of the total PRL cells, Type II cells make up 7% and Type III cells comprise 3% through the 4 day estrous cycle. Type I cells undergo marked changes in ultrastructure during the estrous cycle. This cell type shows ultrastructural signs of elevated secretory activity during the proestrus and estrus.

The postnatal development of PRL cells was also studied. At 8 days after birth, immunoreactive PRL cells are mostly Type III cells (more than 80%), Type II cells are much fewer (17%), and no Type I cells can be observed. At 21 days the female pituitary fully matures with regard to PRL cells. From the 33rd day, the male PRL cells show characteristics of the male pituitary. Transitional features changing from Type III into Type II or from Type II into Type I were observed. It is thus suggested that Type III cells are primitive immature cells which may give rise to mature, functionally active Type II or Type I cells. The Type III cells may frequently undergo mitosis.

Electron microscope studies have well documented the characteristics of the prolactin (PRL) cells of the rat anterior pituitary to contain large irregularly shaped or polymorphic granules ever since the first identification of this cell by HEDINGER and FARQUHAR (1975). Recent developments in immunocytochemistry have demonstrated, however, additional PRL-containing cells with round secretory granules either of various sizes ranging from 150 to 250 nm in diameter (NOGAMI and YOSHIMURA, 1980) or those of very small size about 100 nm in diameter (HARIGAYA et al., 1983). According to the order of discovery, the PRL cells with polymorphic granules were called Type I PRL cells, those containing round secretory granules with variable sizes were called Type II PRL cells, and those containing the smallest granules were called Type III PRL cells (KOYAMA, 1985; KOYAMA and KUROSUMI, 1986). It is well known that the pituitary PRL cells are strongly influenced by estrogen, and ultrastructural differences related to sex
have been reported (Nogami, 1984; Koyama, 1985). Ultrastructural changes accompanying the estrous cycle were reported by Poole et al. (1980a, b), but as they did not use immunocytochemical techniques their observations were limited to the classical PRL cells. The changes in the PRL cells during the estrous cycle thus remain to be studied immunocytochemically.

Though the PRL-immunoreactive cells of neonatal rats were reported to be entirely Type III cells (Koyama, 1985), in adult pituitaries, Type I is predominant in females, while Type II dominates in males. The developmental transition of PRL cells from the neonatal to the adult stage has been studied in mice (Harigaya and Hoshino, 1985), but no ontogenic studies of PRL cells performed on the rat pituitary are available. This paper thus aims at a demonstration of the ultrastructural changes in the PRL cells accompanying the estrous cycle and also the postnatal development of the PRL cells in the rat anterior pituitary.

MATERIALS AND METHODS

The pituitary glands from male and female adult rats (weighing 250-300 g) of the Wistar strain were taken after sacrifice by sudden decapitation without pharmacological anesthesia. The samples from female rats were taken at proestrus 12:00 and 18:30, estrus 3:00 and 12:00, metestrus 12:00 and diestrus 12:00. Young rats of both sexes at 8, 14, 21, 33 and 45 days after birth were also used.

The specimens of the anterior pituitary separated from the postero-intermediate lobe were cut into dice of about 1 mm³ and fixed in a mixture of equal amounts of 2% glutaraldehyde and 2% paraformaldehyde adjusted to pH 7.2 with 1/30 M phosphate buffer for 2 hr, rinsed with 1/15 M phosphate buffer containing 7% sucrose, and postfixed with 1% osmium tetroxide with 7% sucrose-added 1/15M phosphate for 1 hr. After dehydration with an ascending series of concentrations of ethanol, the specimens were embedded in a mixture of Epon 812 and Araldite. Ultrathin sections were made with a Porter-Blum type MT-2B ultramicrotome and mounted on nickel grids.

All of the ultrathin sections were processed for immunocytochemical reactions as follows: 1) the sections were treated with 10% hydrogen peroxide for 10 min; 2) rinsed with distilled water and phosphate buffered saline (PBS); 3) treated with 1:20 diluted normal goat serum for 1-2 hr; 4) incubated with rabbit anti-rat PRL serum supplied from NIADDK diluted to 1:400 or 1:500 overnight; 5) rinsed with PBS; 6) incubated with goat anti-rabbit IgG serum raised in our institute, diluted to 1:200 and labeled with 6 or 12 nm colloidal gold particles for 3-4 hr; 7) rinsed with PBS and distilled water, and dried; 8) stained with uranyl acetate and lead citrate for 8 min each, and washed with distilled water and dried.

The above processed specimens were observed and photographed with a transmission electron microscope, JEM 1200EX at 80 kV accelerating potential. The micrographs taken at magnifications of 6,000-30,000 times were further enlarged photographically as desired.
RESULTS

A. Adult male PRL cells

In the male rat pituitaries, the three types of PRL cells could rather easily be found as compared with the female pituitary.

1. Type I PRL cell

Type I PRL cells comprise about 46% of the total PRL cells in the male anterior pituitary, and are characterized by the large size (about 500 nm along the maximum diameter) and irregular shape of their secretory granules. In the male pituitary, Type I cells are relatively few in number, and are not so active as the same type of PRL cells in the female pituitary. The outline of the cell is crescent-shaped, polyhedral or spindle-like. The nuclei are round or oval and located eccentrically. Secretory granules are mostly round, through some oval or irregularly shaped granules are always

Fig. 1. Type I PRL cell of an adult male rat. The slightly deformed shape of secretory granules is characteristic of this type. The development of RER is inferior to that of the female PRL cells. Cisternae of RER are moderately dilated. The Golgi apparatus (G) contains small immature granules. Stout rod-like mitochondria (M) are gathered near the nucleus. Granule release by exocytosis is indicated by an arrowhead. The inset shows an exocytotic image (arrowhead) in a high magnification. Granules react to anti-PRL labeled by gold particles. ×18,000, inset: ×57,000
contained (Fig. 1). The granules are mostly arranged along the cell periphery, and are fewer in number than those of Type I PRL cells of the female rats at any stage of the estrous cycle.

Most mitochondria are stout rods accumulated near the nucleus. The Golgi apparatus is well developed, and dilated Golgi saccules often contain small secretory granules which are immunocytochemically positive to PRL. The rough endoplasmic reticulum (RER) is moderately developed. Most cells of this type in the male pituitary contain dilated cisternae of RER. In some dark appearing PRL cells, however, tightly packed parallel lamellae of RER can be observed, though such dark cells are relatively rare. Some of the peripherally arranged secretory granules are extruded into the intercellular space by exocytosis (Fig. 1).

2. Type II PRL cell

The Type II PRL cells are both most numerous (about 48%) and most active in the anterior pituitary of the male rat. They contain spherical secretory granules ranging widely from 75 to 250 nm in diameter. The number of granules is usually superior to

![Fig. 2. Type II PRL cell of an adult male rat. The cell is irregularly formed. The cytoplasm contains slightly dilated RER and a relatively well developed Golgi apparatus (G). Secretory granules reacting to anti-PRL labeled with gold particles are peripherally located, some of which are expelled to the extracellular space by the exocytosis (arrowheads). The inset show the immunoreactivity of round secretory granules to the PRL antibody and variation in size of granules of this cell. ×12,000, inset: ×38,000](image)
the Type I PRL cells of the male. Exocytosis of these secretory granules is also frequently observed (Fig. 2). The outline of the cell is polygonal or elongate. The nuclei are oval, spindle-like or kidney-shaped. Shallow invaginations on the nuclear surface make its outline slightly irregular.

Cisternae of the RER are randomly distributed and moderately dilated (Fig. 2, 3). Mitochondria are relatively large and mostly distributed around the nucleus. The Golgi apparatus is moderately developed and usually observed near the nucleus. Small immature secretory granules are sometimes observed in the Golgi area. Lysosomes containing secretory granules positively reacted with anti-PRL are also observed in the Type II PRL cells of the male rat.

3. Type III PRL cell

Type III cells occur abundantly in the anterior pituitary of young rats, being about 83% of the total PRL cells in the anterior pituitary at 8 days after birth. It is therefore conjectured that this is the immature type of PRL cell (Koyama, 1985). In the male pituitary, however, Type III cells can be recognized even in the adult age, though the occurrence is very low as few as about 6% of the total PRL cells. The morphological characteristics of the Type III cell are the poverty and smallness of its secretory granules and the small amount of cytoplasm with less development of cell organelles. The secretory granules are few in number and arranged along the cell periphery. They are round in shape and measure about 70–140 nm in diameter. The amount of cytoplasm is relatively small as compared with the size of the nucleus, making the nucleocytoplasmic ratio large. This characteristic strongly suggests its immaturity. Because the cytoplasm is so much less in amount, the size of the cell body is also small. The outline of the cell is polygonal or spindle-shaped and the nucleus is also polygonal or oval (Fig. 4).
The development of the RER and Golgi apparatus is rather poor. A few collapsed cisternae of RER are scattered in the peripheral part of the cytoplasm; in many cases the Golgi apparatus cannot be found (Fig. 4). An assembly of a few vesicles suggesting a primitive Golgi apparatus may rarely be observed. Mitochondria are not so poor in development. They are stout rod-like bodies scattered around the nucleus. These features are almost the same as those seen in the Types I or II PRL cells described above. Exocytosis has never been observed in this type.

B. Adult female PRL cells at different stages of the estrous cycle
Throughout the entire estrous cycle, the Type I PRL cells are the most prominent in the female pituitary as they comprise about 91% of the total PRL cells, compared with only about 7% for Type II cells and a low 3% for Type III cells. The ultrastructural changes of Type I PRL cells accompanying the estrous cycle could be clearly observed, although Type II cells were occasionally found and no systemic observation with regard to the estrous cycle could be performed. Type III cells were very few and only exceptionally observed.
1. Diestrus

The Type I PRL cells at the diestrus 12:00 are small in size and polygonal in body shape. The nuclei are oval, kidney-shaped, rounded triangular or polygonal with shallow indentations. The nucleoplasm is almost homogeneous with a slight accumulation of dark heterochromatin along the inner aspect of the nuclear envelope. Only a few PRL cells of the Type I contain well developed nucleoli. Pleomorphic secretory granules are mostly large in size, namely about 600 nm along the maximum diameter. They are abundant in number and accumulated in the cytoplasm along the cell periphery (Fig. 5).

Exocytosis of the granules is only rarely featured. The greater part of the cytoplasm is occupied by RER, whose cisternae are almost entirely dilated. Many of them are vesicular in appearance (Fig. 5), but a few are slightly dilated lamellar or whole-like cisternae. These indicate that the newly synthesized substance is accumulated within the cavity of RER. The Golgi apparatus is not extensive and the saccules are relatively dilated, containing a rather clear substance. Features of the formation of immature secretory granules are rather uncommon. Lysosomes containing an im-

Fig. 5. Type I PRL cell at the diestrus of the adult female rat. The cell body is polyhedral and the nucleus is roughly triangular with shallow indentations. Large oval or irregular-shaped secretory granules are gathered in the peripheral cytoplasm. A few small irregular granules are seen near the Golgi apparatus (G). Cisternae of RER are all dilated and randomly scattered throughout the cytoplasm. ×14,000
munoreactive secretory granule suggesting crinophagy are sometimes observed. Mitochondria are moderately developed showing a small rod-like shape.

2. Proestrus
Samples were taken twice during the proestrus day, namely at 12:00 and 18:00. At the early proestrus, Type I PRL cells contain highly developed RER, whose cisternae are markedly dilated. A few cells contain parallel lamellae of RER (Fig. 6). The size of the cell is mostly large and the shape is relatively irregular, polyhedral or slightly elongated.

In the late proestrus, the cell shape becomes much more elongated, often sending out long slender processes, or embracing another cell of a different type. Such peculiar forms of the PRL cell look like a signet ring, sickle or demilune. The RER is markedly different between the early proestrus and late proestrus, that is, the RER of the Type I PRL cells at the late proestrus show the characteristic arrangement of parallel lamellae. Only a few cells contain vesicular dilated cisternae.

The Golgi apparatus is relatively well developed at both times of proestrus. The formation of new immature secretory granules continues actively in both early and late proestrus (Fig. 6). They appear in the cavities of small vesicular saccules of the
Golgi apparatus, mostly of its trans side, and their shapes are either round or irregular. In some PRL cells of both Types I and II, membrane structures called rigid lamellae or GERL of Novikoff are observed, and some immature secretory granules may be seen in the GERL or its related vesicles. Immature granules tend to show remarkably irregular shapes, which are not comparable to any forms of mature secretory granules.

The average size of the secretory granules of Type I PRL cells at the proestrus is larger than that at the diestrus. Large, mature granules are accumulated predominantly along the cell periphery (Fig. 7). Exocytotic images of granules are often found in specimens of both early and late proestrus. The cells containing well developed lamellar RER tend to more frequently show features of exocytosis than the cells containing dilated vesicular RER.

In this period of the estrous cycle, the Type II PRL cell also looks very active. The RER of the Type II cell is also well developed and dilated considerably (Fig. 8). Exocytotic figures are also encountered. Lysosomes containing secretory granules suggestive of crinophagy are sometimes observed. Mitochondria are rod-like or strand-like and often gathered near the nucleus. The eccentrically located nuclei are mostly oval in shape, but occasionally spherical or kidney-shaped with irregularly

![Fig. 7. Type I PRL cell at the late proestrus. Mature secretory granules are round or oval in shape and arranged along the cell periphery. Some small immature granules are mixed. RER is mostly lamellated and not so markedly dilated. Mitochondria (M) are rod-like or oval and distributed around the nucleus. The inset shows the positive immunogold reaction for PRL in the secretory granules. ×11,000, inset: ×32,000](image)
3. Estrus

Two points in time of the estrus stage were used for gathering samples from the anterior pituitary, i.e., 3:00 and 12:00. In the early estrus, almost all the PRL-immunoreactive pituitary cells found are Type I, which show a very active secretory function. The size of the cell body is relatively large, being polygonal, oval or spherical in shape. The nucleus is usually located in an eccentric position. The outlines of the nuclei are mostly oval, but some other exceptional cases such as kidney-shaped or slightly indented outlines may be observed. Most nuclei contain rather homogeneous nucleoplasm, but in a few cases heterochromatin clumps are noticeable. The development of the nucleolus is most prominent at this stage of the estrous cycle.
The RER is best developed and consists of moderately dilated parallel lamellae either straight or whorled (Fig. 9, 10). Very few cells classified as Type I PRL cells contain vesicular cisternae of RER. Large lipid droplets are often observed among the well developed RER in the Type I PRL cells at this stage of the estrous cycle (Fig. 10). The Golgi apparatus forms a semicircular or circular area near the nucleus circumscribed by the stacks of parallel arranged saccules. Mitochondria are also abundant within and around the Golgi area. The formation of immature secretory granules is seen inside the Golgi area, and some are contained within the Golgi saccules. The secretory granules are not so abundant as compared with the former periods of the estrous cycle, and the average size of the mature granules is also somewhat smaller. The immature secretory granules are often highly irregular in shape, but very small granules which might be considered as least mature are rather round. It is presumed that the immature round granules formed in the Golgi saccules or in the GERL may be fused into granules which are of irregular shape. The mature granules accumulated in
the wide cytoplasmic area other than the Golgi area are again round or oval: the irregularity of the granule shape becomes unremarkable.

Crinophagy is also often observed in the estrus stage: lysosomes or multivesicular bodies are found to contain secretory granules which are positive to immunocytochemistry for PRL (Fig. 11a). The exocytosis of secretory granules is very frequently recognized in the early estrus stage. At a time within this period, several granules extruded into the intercellular space by exocytosis are observed around a single Type I PRL cell. Two or three granules have been seen in a single pit opened to the extracellular space, which corresponds to "multigranular exocytosis" of Fujita et al. (1983). Small pinocytotic (endocytotic) pits are sometimes associated with granule extrusion, and are probably related to the membrane retrieval and recycling (Fig. 11b).

At the late estrus 12:00, Type I PRL cells are still active and possess a relatively large amount of cytoplasm. The shape of the cell is rather irregular, though some are
oval, conical or triangular with rounded corners. The nuclei are mostly spherical but localize in an eccentric position. The nuclear outline is mostly smooth; although shallow depressions are sometimes observed, no deep invaginations were found. The nucleoplasm appears homogeneous in many cases, and perinuclear chromatin clumps are very rarely seen. The nucleoli are moderately developed and situated either at the center of the nucleus or attached to the inner aspect of the nuclear envelope.

The cytoplasm is filled with vesiculated cisternae of RER. Most PRL cells (Type I) in this stage contain dilated cisternae of vesicular or vacuolar forms, but some cells contain lamellated parallel cisternae, either straight or whorl-like, which are also moderately dilated (Fig. 12). At the center of the whorl of concentric lamellae, a few vesicles with smooth membrane and secretory granules are accumulated (Fig. 13). Some PRL cells with dark cytoplasm contain very flat cisternae of RER.

The Golgi apparatus is moderately developed both in the clear cells with dilated RER and in the dark cells with flat RER. As a few immature secretory granules can be seen in the Golgi area, the formation of new granules may be carried out during late estrus, though this activity is less than at early estrus. The number of secretory granules are slightly decreased as compared with the early estrus. The shape of mature secretory granules situated in the peripheral cytoplasm is very irregular and often elongated (Fig. 12). This is in clear contrast to the round or oval mature granules found at the early estrus. Granule extrusion by exocytosis is seen at the late estrus, but it is not so frequent as compared with the early estrus. Multivesicular bodies containing secretory granules are occasionally recognized. Large lipid droplets found in the PRL cells at the early estrus stage could not be observed at this stage.
Fig. 12. Type I PRL cells at the late estrus stage. The RER is dilated, forming either lamellar or somewhat vesicular cisternae. Secretory granules are accumulated at the central area of the cell. They are irregular in shape. ×9,000

Fig. 13. A part of a Type I PRL cell. Secretory granules are arranged along the surface plasma membrane, and are mostly irregular or elongate in shape. The RER is a concentric lamellae whose center is a cytoplasmic island containing smooth surfaced vesicles and secretory granules (arrow). The cisternae of such concentric lamellae contain a transparent substance. ×13,000
4. **Metestrus**

Material was taken at metestrus 12:00. The cell size of the Type I PRL cells decreased slightly. Here the outline of the cell body is either spherical, oval or polygonal, and a few cells are slightly elongated. The nuclei are mostly spherical, although a nucleus with a deep invagination was observed. They are eccentrically situated in the cell. The nucleoplasm is mostly homogeneous, and the nucleoli are moderately developed (Fig. 14).

The cytoplasm contains well developed RER, whose cisternae are vesicularly by diluted in many cases. The parallel lamellar arrangement of RER is rather rare in this stage. The secretory granules are slightly increased in number, and most mature granules are round or oval (Fig. 14). Only a few granules, especially immature ones, are irregularly shaped. Therefore, differentiation between Types I and II is difficult. Such a spherization of secretory granules is more conspicuous as compared to the Type I PRL cells at the diestrus and other stages. Exocytotic images are sometimes, but not so frequently, seen.

Mitochondria are mostly oval or spherical and found near the nucleus. The Golgi
apparatus is not extensive; granule formation at the Golgi area was observed in rare cases. Special inclusions such as lysosomes, multivesicular bodies or lipid droplets were not recognized in this stage.

### Table 1. Frequency of occurrence of each type of PRL cells in the rat anterior pituitary

| Age (Days after birth) | 8 d | 14 d | 21 d | 33 d | 45 d | Adult |
|------------------------|-----|------|------|------|------|-------|
| **Male**               |     |      |      |      |      |       |
| Type I                 | 0%  | 4.3% | 8.7% | 22.2%| 38.6%| 46.8% |
| Type II                | 17.6| 41.3 | 36.8 | 60.0 | 54.3 | 48.0  |
| Type III               | 82.3| 54.3 | 54.3 | 17.7 | 7.0  | 6.4   |
| **Female**             |     |      |      |      |      |       |
| Type I                 | 0   | 4.0  | 56.1 | 67.6 | 77.9 | 91.1  |
| Type II                | 16.6| 36.0 | 34.1 | 26.4 | 16.9 | 7.5   |
| Type III               | 83.3| 60.6 | 9.7  | 5.9  | 5.0  | 2.8   |

**Fig. 15.** Type III PRL cell at the neonatal stage (female 8 days after birth). The nucleocytoplasmic ratio is large. Secretory granules about 100 nm in diameter are arranged along the cell surface. Cell organelles are very weakly developed, viz., a few mitochondria (M) and very poor RER are visible. The inset shows PRL-immunoreactive secretory granules arranged in a single row along both the nuclear and cell surfaces. ×18,000, inset: ×50,000
C. Postnatal development of PRL cells

The differentiation of PRL cells is rather late as compared with other cell types of the anterior pituitary (WATANABE and DAIKOKU, 1979). By the immunoelectron microscopic technique, PRL cells can be observed only after birth, though anterior pituitary cells engaged in the production of other hormones may appear in the late fetal stages.

At 8 days after birth, a few immunoreactive PRL cells were observed. Most of them were Type III cells with far fewer Type II cells included; no Type I cells were observed in either sex. There is no sexual difference in the cell population at the early postnatal stage. In both sexes Type III cells comprise more than 80% of the total PRL cells at 8 days after birth, but this decreases with aging (Table 1). Type II cells make up about 17% in both sexes at this stage. At 14 days after birth, when sexual differences could not yet be recognized, the percentage of occurrence for Type III decreased to 50–60%, whereas that of Type II cells increased to about 40%, and Type I cells appeared in about 4%.

At 21 days the sexual difference becomes readily noticeable, i.e., the tendency of occurrence for each cell type in the male was the same as those in the earlier stages, but in the female, the population rate for each type of PRL cells was similar to that in the adult pituitary.

The most frequent among the PRL cells is the Type I at 21 days and thereafter, while those of Type II and III cells decrease progressively with age (Table 1). The frequency of occurrence of Type I cells increases also in the male pituitary, but the rate does not exceed half of the total. From 33 days on, Type II cells are always the most frequent among the various types of PRL cells in the male anterior pituitary.

In early postnatal stage in the first 2 weeks after birth, PRL cells in the anterior pituitaries of both sexes are mostly Type III cells, which have been considered to be immature PRL cells. They are relatively small cells with a small amount of cytoplasm.

Fig. 16. Type III PRL cell of a male rat 14 days after birth. The cell is shaped as an elongated rod. Two nuclei (N) separated at a distance suggest the possibility that the cell appears just after mitotic nuclear division. Secretory granules are arranged along the cell surface. The Golgi apparatus (G) is situated in the cytoplasm between the two nuclei. The inset shows the positive immunoreaction of granules to PRL. ×16,000, inset: ×50,000
containing a few secretory granules about 100 nm in diameter arranged along the cell surface (Fig. 15). The cell organelles are poorly developed: a few spherical or short rod-like mitochondria are seen near the nucleus. The cisternae of RER are not dilated, and are scattered at random in a narrow cytoplasmic area. The Golgi apparatus is moderately developed in some Type III cells, but could not be found in many cells. Mitosis of this cell type is often encountered (Fig. 16). The cytoplasm is filled with free scattered ribosomes, and this feature as well as a high nucleocytoplasmic ratio indicate that Type III is the immature type of PRL cell, which may then convert into other types of PRL cells.

The increase in volume of the cytoplasm, the development of cell organelles such as mitochondria and RER, the dilatation of its cisternae, and the appearance of large-sized spherical secretory granules may suggest the transformation of Type III into Type II cells (Fig. 17). Such developing PRL cells occasionally contain a few elongated or irregularly shaped secretory granules which are similar to those of Type I cells. Transformation from Type II to Type I is also suggested by the feature of relatively small PRL cells containing small and medium-sized round granules mixed with one or two elongated secretory granules (Fig. 18). Although Type I cells can be found in the early postnatal stage, though their incidence is very low, they possess relatively rich cytoplasm containing a well developed Golgi apparatus (Fig. 19), where...
the formation of new secretory granules occurs (Fig. 20). The size of the mature secretory granules is smaller than that in the Type I cells of adult animals, but some of them are characteristically elongated, viz., oval or rod-like granules are intermingled with spherical granules.

**DISCUSSION**

It has long been believed that the PRL cell in the rat anterior pituitary gland is characterised by irregularly shaped large secretory granules, ever since the morphological discovery of this cell type by Heding and Farquhar (1957). An additional type of PRL cell with spherical granules ranging from 130 to 200 nm was demonstrated by Nogami and Yoshimura (1980) using a comparison technique between a thick section stained immunohistochemically with anti-PRL serum for light microscopy and a thin adjacent section treated for routine electron microscopy.

In their second paper, Nogami and Yoshimura (1982) classified immunoreactive PRL cells of the rat anterior pituitary into three or four types, the former classification...
is based on the size of granules: 1) small spherical granules about 130-200 nm; 2) middle sized spherical and polymorphic granules about 250-300 nm; and 3) large irregularly shaped granules about 300-700 nm. Their fourth type is a cup-shaped PRL cell containing middle sized granules.

HARIGAYA et al. (1983) also noted three types of PRL cells in the mouse anterior pituitary: Type I containing small spherical granules about 100 nm in diameter; Type II containing medium sized spherical granules 150-200 nm; and Type III with large polymorphic granules with a maximum diameter of about 300 nm. Their fourth type is a cup-shaped PRL cell containing middle sized granules.

KOYAMA (1985) and KOYAMA and KUROSUMI (1986) called the classical type of PRL cell first described by HEDINGER and FARQUHAR (1957), Type I. The PRL cells containing medium sized spherical granules found by NOGAMI and YOSHIMURA (1980, 1982) were called Type II, and the PRL cell with the very small granules noted by HARIGAYA et al. (1983) called Type III, according to the chronological order of the discovery. The authors of this paper also use this classification by KOYAMA (1985).

Though the code numbers of PRL cell types of our classification are the reverse of those of HARIGAYA et al. (1983) and of NOGAMI (1984), our classification of PRL cells corresponds to the nomenclature for many other cell types producing various hor-

Fig. 19. Type I PRL cell found in the anterior pituitary of a male at 14 days after birth. The cytoplasm is relatively rich and contains many secretory granules, most of which are round, but a few are characteristically irregular in shape. Lamellar RER and a well developed Golgi apparatus (G) are shown. ×12,000
mones, such as GH cells and ACTH cells (Kurosumi, 1986; Kurosumi and Inoue, 1986).

The presence of sexual dimorphism in PRL cells and the dependency of PRL cells containing large polymorphic granules upon estrogen was reported both by Nogami (1984) and Koyama (1985). But before these studies by immunocytochemistry, the unusual development of female PRL cells was reported after prolonged administration of estrogen without using any immunocytochemical method (Watari and Tsukagoshi, 1969).

Only a couple of studies (Poole et al., 1980a, b) have been published concerning the ultrastructural morphology of rat PRL cells during the estrous cycle; these studies did not use immunocytochemical techniques but measured the PRL content in the serum and in the pituitary. Poole et al. (1980b) described the PRL cells at diestrus as being relatively small with a few poorly developed organelles. At proestrus, the PRL cells increased in size and contained more extensive RER and Golgi complexes. Mature granules in the cell periphery increased in number, and exocytosis was often observed. At estrus, extensive RER with thin lamellar forms accompanied by some vesicular type was observed. An abundance of large mature granules was contained. At metestrus, the Golgi complex and RER were more extensive and both mature and immature granules were increased in number.

The elevated activity of PRL cells at proestrus and estrus was morphologically proved by Poole et al. (1980b) before our present observation, but the high incidence of exocytosis at estrus detailed in this report was not described by the above authors.

Concentrations of PRL in the serum and in the pituitary tissue have been reported by several authors as occurring during the estrous cycle of the rat. Taya and Igarashi (1973) reported twin peaks at late proestrus and early estrus as well as a relatively low peak at late metestrus in serum PRL levels of Holtzman rats. Butsher et al. (1974)...
showed two big peaks in PRL levels in the serum of Sprague-Dawley rats: a main peak at the proestrus afternoon, and a small sharp one at the late estrus afternoon. Combined studies of radioimmunoassay of PRL and ultrastructural morphology of PRL cells were performed by POOLE et al. (1980a, b), using rats of the Holtzman strain. They demonstrated three peaks similar to the report of TAYA and IGARASHI (1973), viz., PRL level in the serum sharply rises at the proestrus afternoon, with a lower rising at the estrus afternoon follows, and finally at the diestrus morning, the third rising occurs.

The morphologically demonstrable high activity of PRL cells as indicated by the development of the RER and Golgi apparatus at proestrus and estrus correspond to the high rate of secretion of PRL at the late proestrus and early estrus. The high incidence of exocytosis at the early and late proestrus effects a rise in the PRL serum level at the late proestrus. Very frequent occurrences of exocytosis in Type I PRL cells at the early estrus as seen by our observation may correspond to the high level of PRL concentration in the serum at the same stage of the estrous cycle as shown by POOLE et al. (1980b), though these authors did not describe exocytotic features at this stage.

No morphological finding of exocytosis has been correlated to the third peak of PRL serum level at the diestrus. POOLE et al. (1980b) also did not describe increased exocytotic images at this stage. As the level of the third peak is neither so high nor long lasting, exocytotic images accounting for that may be very rare at this stage. As an alternative possibility, there may be some other mechanism of hormone release in the PRL cells activated at this stage, such as that reported by OSAMURA et al. (1982).

The shapes of the secretory granules in Type I PRL cells also vary during the course of the estrous cycle. For example, most granules at the early estrus are round or oval, but those in the late estrus are irregular in shape. The granules again become less irregular at metestrus. Large lipid droplets found at the early estrus are also the only finding not reported in previously published papers on ultrastructural studies of anterior pituitaries. The significance of such findings can not be explained.

Some reports maintain that the first appearance of PRL cells in the rat anterior pituitary may occur at the late fetal stage (SETALO and NAKANE, 1972, 1976), but some other authors have argued that the only PRL cells appear after birth, though other cell types may have already appeared in the fetus (WATANABE and DAIKOKU, 1979). Because the number of immunoreactive PRL cells in the fetal rat anterior pituitary is very low, if at all, it is difficult to find PRL cells by electron microscopy in the fetal pituitary. This is the reason why no reports on the ultrastructure of fetal PRL cells are available. Only in the neonatal stage were the immunopositive PRL cells observed by electron microscopy (HEMMING et al., 1986). Almost all PRL cells found in the anterior pituitaries 8 days after birth were Type III cells, though a few Type II cells were found. No Type I PRL cell was found at that age. This finding is the same as that of HARIGAYA and HOSHINO (1985) in the C57BL mouse, but they reported that the sex difference in the frequency of occurrence of PRL cell types appeared from 5 weeks of age. In the rat, however, we found female characteristics in the rate of PRL cell types from 3 weeks after birth, while the male characteristics appeared one week later. It is not certain whether the difference in the results between the rat and mouse may be due to the difference of animal species or that in the observation techniques used. In the data from HARIGAYA and HOSHINO (1985), however, the cell numbers of Type I (their Type III) in the female slightly predominated over those in the male from 3 weeks; this predominance of Type I in female becomes remarkable after 5 weeks of age. The difference between their data and ours is not fundamental.

Both NOGAMI (1984) and KOYAMA (1985) demonstrated that PRL cells at the
neonatal stage are almost totally small spherical granule-containing cells. Studies on
the postnatal development of PRL cells performed in the mouse (HARIGAYA and
HOSHINO, 1985) and in the rat (the present observation) have both suggested the
transition of the immature type of PRL cells (Type III in our classification) into Type
II and Type I under the influence of maturation on the gonadal function, this being
especially more remarkable in female as the effects of estrogen.

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