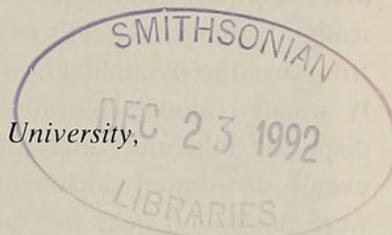


REVIEW

Heterogeneity and Development of Somatotrophs and Mammotrophs in the Rat

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ABSTRACT—Growth hormone (GH) and prolactin (PRL) are secreted from corresponding secretory cells, somatotrophs (GH cells) and mammotrophs (PRL cells) in the pituitary gland. A novel cell type, the mammosomatotroph (MS cell), which contains both GH and PRL in the same cell, has been found in many species including rats, mice and humans. In this article, we demonstrate the heterogeneity of GH cells and PRL cells at various levels, mainly in the rat, and discuss the developmental and functional significance of these heterogeneities. Age-related alteration in GH and PRL secretion is summarized, since it is one of the most important aspects of the developmental changes in pituitary glands. Heterogeneities of GH and PRL cells may be the outcome of various difference in the molecular variance of hormones, the intracellular age of hormones, the difference in receptors on the pituitary cells for hypothalamic regulatory hormones, the different mechanisms of intracellular signal transduction, and the location of pituitary cells in the gland, and may also reflect the maturation of pituitary cells. The possible relationship among GH cell, PRL cell and MS cells is discussed.

INTRODUCTION

Growth hormone (GH) and prolactin (PRL) are protein hormones that are produced in corresponding specific pituitary secretory cells, somatotrophs (GH cells) and mammotrophs (PRL cells) [1]. A significant amount of evidence has indicated that each cell population of GH cells and PRL cells consists of morphologically and functionally heterogeneous cells [2, 3]. Furthermore, variants of hormone molecules have been found. Such heterogeneity observed may reflect the functional difference or maturing process of pituitary cells and hormone molecules.

A novel pituitary cell, a mammosomatotroph (MS cell) or a somatomammothroph, which contained GH and PRL in the same cell, was found in several species. In this paper, the term mammosomatotroph is used to describe this secretory cell. The developmental and functional significance of

MS cells is not yet fully understood. It is noteworthy that GH and PRL genes are believed to be derived from a common ancestral gene [4, 5], and are regulated by a common transcription factor [6, 7]. Accordingly, MS cells are considered to be closely related to GH cells and PRL cells. Immuno-electron-microscopical studies showed subtypes of GH cells and PRL cells in rat pituitaries. MS cells in the rat closely resembled one of the subtypes of GH cells and PRL cells. This finding suggests that MS cells may be involved in the genesis of GH cells and PRL cells. In this paper, the morphological and functional heterogeneity of GH cells and PRL cells, and the relationship among the three pituitary cells, GH, PRL and MS cells, will be discussed using examples mainly from the rat. As one of the most important developmental aspects in pituitary glands, age-related alterations in GH and PRL secretion will be extensively studied in order to understand the significance of the heterogeneities of GH and PRL cells. The molecular heterogeneity of both hormones

will also be briefly considered.

I. SOMATOTROPHS

1. Identification of GH cells

Size, shape and distribution Immunocytochemically identified GH cells in rat pituitaries ranged from ovoid to pyramidal in shape, and were usually situated along sinusoids. They were evenly dispersed bilaterally and rostrocaudally, but unevenly distributed dorsoventrally [8]. GH cells were not found near the intermediate lobe, nor were they found in the anteroventral portion of the gland [9, 10].

Differentiation of GH cells The ontogeny of GH cells has been immunocytochemically studied in several laboratories. GH cells were first observed at 18 or 19 days of gestation in the rat (the day on which the vaginal plug was detected is designated as day 0 of gestation) [11–13]. Using a combination of the immunocytochemistry and the *in situ* hybridization method [14], GH immunoreactivity was detected from day 18 of gestation, but GH-mRNA positive cells were detected on day 19 of gestation. GH cells had substantially increased in number by day 19 of gestation. Pituitary specific transcription factor GHF-1 (Pit-1), which was responsible for activating GH and PRL genes, was detected in the anterior pituitaries on day 16 of gestation [15], although GH-mRNA and GH immunoreactivity were first expressed on day 18–19 of gestation as stated above. In mouse pituitaries, temporal and spatial correlation between the GHF-1 gene expression and GH gene expression was clearly observed [16]. GHF-1 transcript was first detected on day 13 of gestation, and had significantly increased by day 15 of gestation. GH-mRNA was first detected on day 15 of gestation. GH synthesis increased sharply between the 16th and 17th day of gestation [17]. GH cells were morphologically detected on day 16 of gestation [16].

2. Morphological heterogeneity of GH cells

Immuno-electron-microscopical studies have shown that there are three subtypes of GH cells in the rat [18]. GH cells were classified based on the

size of secretory granules (Fig. 1). The Type I GH cell contained large secretory granules (diameter, 250–350 nm, Fig. 2). The Type II GH cell contained large and small secretory granules (diameter, 100–150 nm, Fig. 3). The Type III GH cell contained small secretory granules (Fig. 4). Type I and II cells are polygonal or oval in shape, and medium in cell size. Type III cells are oval or sometimes irregular in shape, and small in size. The rough endoplasmic reticulum and the Golgi apparatus were moderately developed in Type I and II cells. Type I and II GH cells had been originally identified as somatotrophs. The relative proportion of each subtype of GH cells differed between male and female rats (Fig. 5), and changed with several hormonal treatments and age [19]. This will be discussed later.

3. Development of GH cells

Proliferation of pituitary cells The pituitary gland is an “expanding organ” (see Goss, [20]), and its development is characterized by the fact that cell differentiation is not incompatible with mitosis, and every pituitary cell probably has the capacity to divide during its life span [21]. Mitotic GH cells were immunocytochemically identified in several reports [22–25]. These observations clearly indicate that differentiated GH cells divide in a similar manner to other types of pituitary cells [26–30]. Pituitary cells proliferate using a mode of self-duplication, and the differentiated pituitary cells divide into the same two differentiated cells. However, another mode of proliferation cannot be ruled out. For example, undifferentiated “stem” cells, may divide and then one of the divided cells may terminally differentiate into a specific secretory cell.

Sexual difference in the percentage of GH cells

A sexual difference in the number of GH cells was found in rats [19, 31] and in mice [32]. Our study showed the percentage of GH cells in male and female rats at various ages (Table). The percentage of GH cells was higher in male rats than that in female rats, and decreased with age in both sexes. Similar findings were reported by a reverse hemolytic plaque assay [33]. On the contrary, Dada *et al.* [34] could not detect the sexual difference in the percentage of GH cells, although the

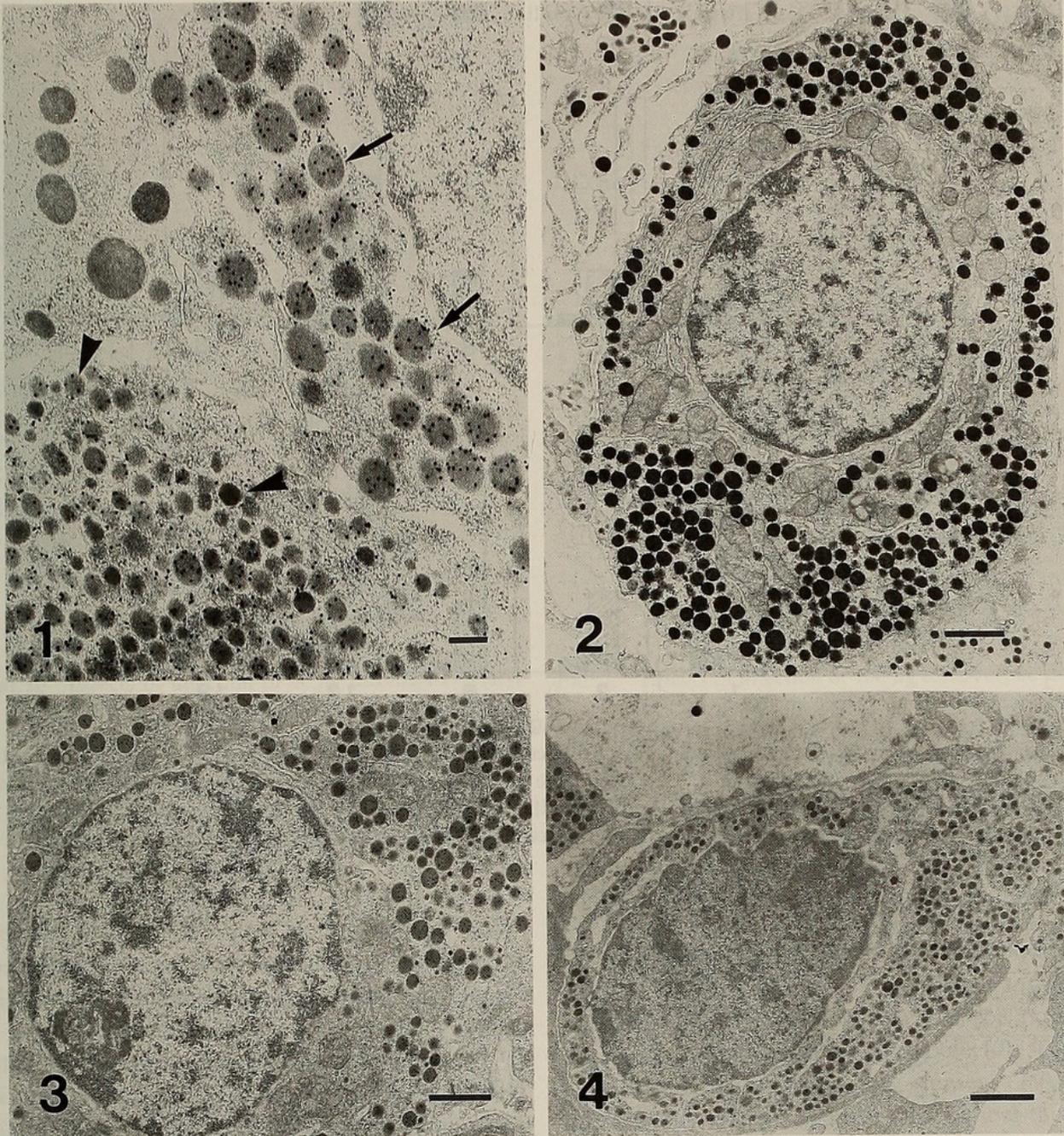


FIG. 1. Secretory granules immunocytochemically stained with rat GH antiserum by the protein A gold colloid method. Large secretory granules with a diameter of 250–350 nm (arrows) and small secretory granules with a diameter of 100–150 nm (arrowheads) are seen. Bar: 200 nm. (from Takahashi [19]).

FIG. 2. Type I GH cell in a 6-month-old female rat. Large secretory granules are seen throughout the cytoplasm. Bar: 1.0 μm . (from Takahashi [19]).

FIG. 3. Type II GH cell in a 6-month-old female rat. Large and small secretory granules are seen throughout the cytoplasm. Bar: 1.0 μm . (from Takahashi [19]).

FIG. 4. Type III GH cell in a 6-month-old female rat. Small secretory granules are seen throughout the cytoplasm. Bar: 1.0 μm . (from Takahashi [19]).

reason for this discrepancy is not clear.

Hormonal effects on GH cells Growth hormone-releasing hormone (GHRH) stimulates the

proliferation of GH cells [35]. Thus, hypothalamic peptides affect the proliferation of pituitary cells [30, 36]. GHF-1, a transcription factor of the GH

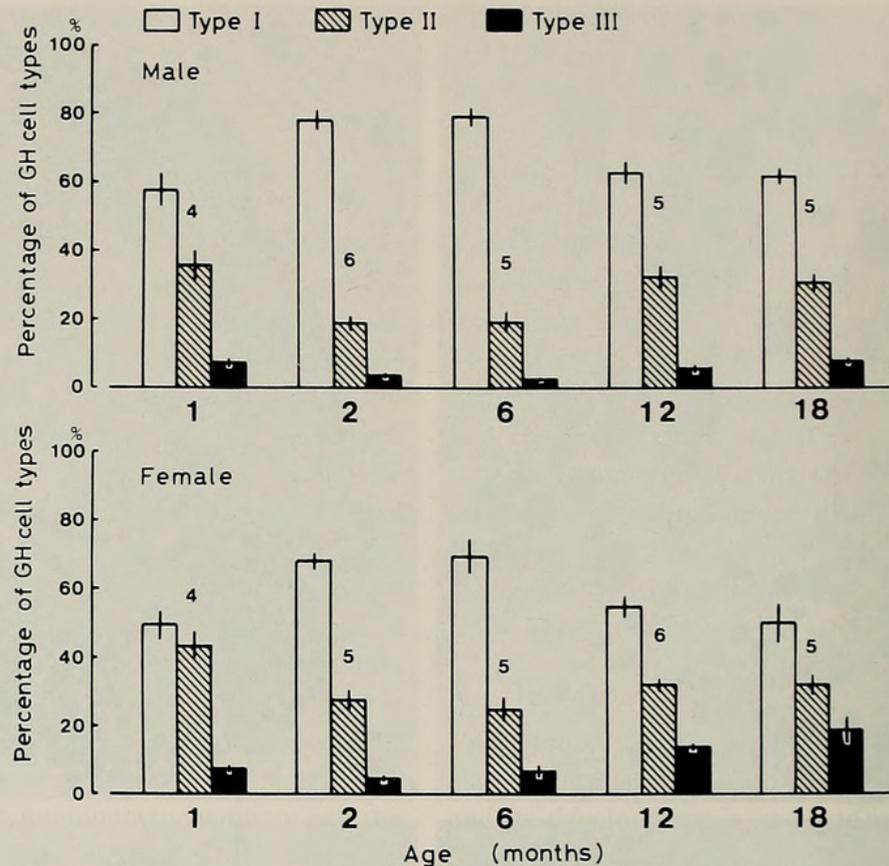


FIG. 5. Age-related changes in the percentages of GH cell types in male and female rats. The number above the columns depicts the number of rats. Bars depict the standard errors of means. The percentage of Type I cell increased, whereas the percentages of Type II and III cells decreased at 2 and 6 months of age. At 12 and 18 months of ages, the percentages of Type II and III cells increased. (from Takahashi [19]).

TABLE Percentage of GH cells and PRL cells in male and female rats

Sex	Age(months)	Percentage of cells (%) ²⁾	
		GH cell	PRL cell
Male	6	68.3 ± 2.2 ^{1)b}	15.5 ± 1.4
	12	59.7 ± 4.1 ^c	22.1 ± 1.6
	18	40.5 ± 4.2 ^{bc}	18.9 ± 3.0
Female	6	40.6 ± 2.0 ^b	34.0 ± 4.4 ^{ab}
	12	34.8 ± 4.1 ^a	48.7 ± 2.9 ^a
	18	23.4 ± 2.2 ^{ab}	52.1 ± 2.4 ^b

¹⁾ Mean ± S.E.

²⁾ In each age-group five rats were used for the determination of the percentages of GH cells and PRL cells.

Statistical significance was tested by ANOVA. When significant, the differences among age-groups of each sex were determined by Duncan's multiple range test. In each sex, there is a significant difference between the values for the age-groups with the same superscripts. a, $P < 0.05$; b, c, d, $P < 0.01$. (from Takahashi *et al.* [31]).

gene, may function in the proliferation of GH cells [37]. This suggests that stimulation of GH gene expression may stimulate the cell division of GH cells.

Estrogen decreases the percentage of GH cells, but androgen increases it [38, 39]. Estrogen increased the percentages of Type II and III GH cells (Fig. 6), and androgen increased the percentage of Type I cells and decreased the percentage of Type II cells (Fig. 7). As estrogen is known to inhibit GH secretion, and androgen is known to stimulate [39], it is concluded that the inhibitory factors for GH secretion decrease the percentage of GH cells, and the relative proportion of Type I cells, and, on the contrary, the stimulatory factors for GH secretion increase the percentage of GH cells and the relative proportion of Type I GH cells. T_3 is known to stimulate fetal somatotroph differentiation probably by a synergistic action with cortisol [40] and GH production [41]. T_3

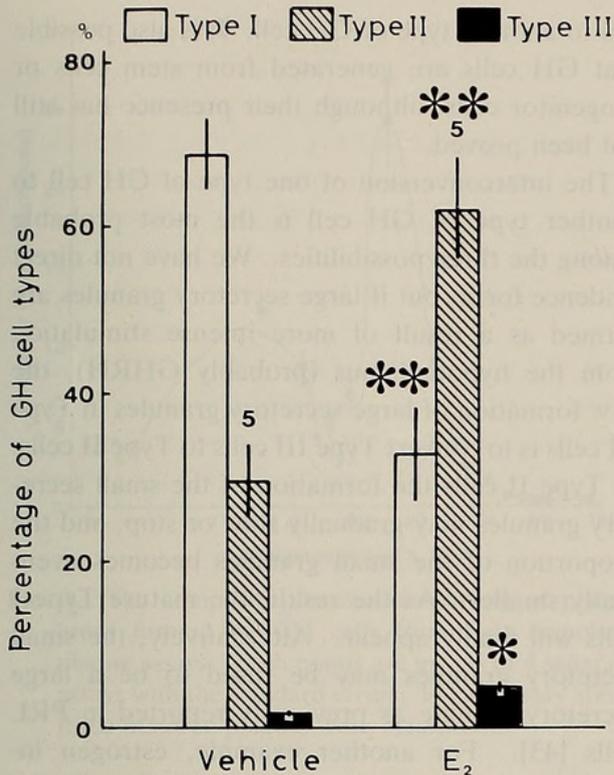


FIG. 6. Effects of injection of 50 μg estradiol-17 β (E₂) daily for 5 days on the percentage of GH cell types in male rats. In E₂-treated rats, the percentage of Type I cells decreased and the percentage of Type II and III cells increased. The number above the column depicts the number of rats. Bars depict the standard errors of means. * $P < 0.05$, ** $P < 0.01$ vs vehicle. (from Takahashi [19]).

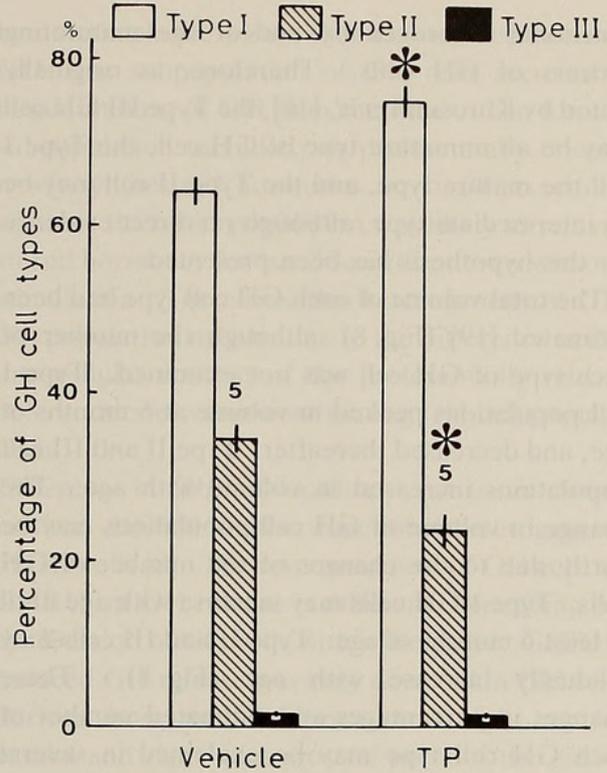


FIG. 7. Effects of injection of 100 μg testosterone propionate (TP) daily for 5 days on the percentages of GH cell types in male rats. In TP-treated rats, the percentage of Type I cells increased, but the percentage of Type II cells decreased. * $P < 0.05$ vs vehicle. (from Takahashi [19]).

treatment (10 $\mu\text{g}/100$ g BW daily twice for 5 days) significantly increased the percentage of Type III GH cells from the control level of $3.4 \pm 1.1\%$ ($n=6$) to $6.9 \pm 0.8\%$ ($n=6$). This increase in Type III cells may indicate the formation of immature GH cells (Type III) from undifferentiated cells, provided that the Type III GH cell is an immature type of GH cell. Thus, the relative proportion of GH cell subtypes changed, together with changes in GH secretion, indicating that three morphologically different GH cell types may have different secretory activities.

Development of GH cells Perinatal development of GH cell subtypes was reported by Kurosumi and Tosaka [42], and postnatal changes (from immature ages through to aged ones) were studied by Takahashi [19]. The Type III GH cell was the predominant type of GH cell during the prenatal period, and the percentages of Type I and II cells

gradually increased by the term. After birth, the Type I GH cell became the predominant type. Such morphological changes in GH cell populations have been extensively described [19]. Type I cells, containing large secretory granules (250–350 nm in diameter) predominated throughout the life (Fig. 5). The proportion of Type I cells was highest at 6 months of age. The proportion of Type II and of Type III cells decreased from 1 month to 6 months of age, but increased thereafter. This suggests that when GH secretion is more active (age-related changes in GH secretion will be discussed later), the proportion of Type I cell increases, and when GH secretion is less active, the proportion of Type II and III cells increases. GH cell-populations, morphologically classified, changed in accordance with GH secretory activity. Thus, it is highly probable that morphological heterogeneity of GH cells reflects

functional heterogeneity and/or the maturing process of GH cells. Therefore, as originally stated by Kurosumi *et al.* [18], the Type III GH cell may be an immature type of GH cell, the Type I cell the mature type, and the Type II cell may be an intermediate type, although no direct evidence for this hypothesis has been presented.

The total volume of each GH cell type had been estimated [19] (Fig. 8), although the number of each type of GH cell was not examined. Type I cell populations peaked in volume at 6 months of age, and decreased thereafter. Type II and III cell populations increased in volume with age. The change in volume of GH cell populations may be partly due to the changes of the number of GH cells. Type I GH cells may increase with age until at least 6 months of age. Type II and III cells may gradually increase with age (Fig. 8). These changes in percentages and estimated number of each GH cell type may be explained in several ways. One is that the increase in the percentage and number of GH cells is caused either by the proliferation of a specific type of GH cells, and/or the cell death of other specific types of GH cells. Another way is the conversion of one type of GH

cell to another type of GH cell. It is also possible that GH cells are generated from stem cells or progenitor cells, although their presence has still not been proved.

The interconversion of one type of GH cell to another type of GH cell is the most probable among the three possibilities. We have not direct evidence for it, but if large secretory granules are formed as a result of more intense stimulation from the hypothalamus (probably GHRH), the new formation of large secretory granules in Type III cells is to convert Type III cells to Type II cells. In Type II cells the formation of the small secretory granules may gradually slow or stop, and the proportion of the small granules becomes eventually smaller. As the result, the mature Type I cells will finally appear. Alternatively, the small secretory granules may be fused to be a large secretory granule as previously reported in PRL cells [43]. For another example, estrogen increased the percentage and number of Type II GH cells (Fig. 6), and it had already been verified that the proliferation of GH cells is not stimulated by such estrogen treatment [44]. Therefore, estrogen appears to cause the conversion of Type I cells to Type II cells. The small secretory granules may be newly formed in the Golgi apparatus, or the large secretory granules may be disintegrated to the small granules under estrogen treatment. The transition from the Type I cell to the Type II cell is more probable than the proliferation or genesis of the Type II cell.

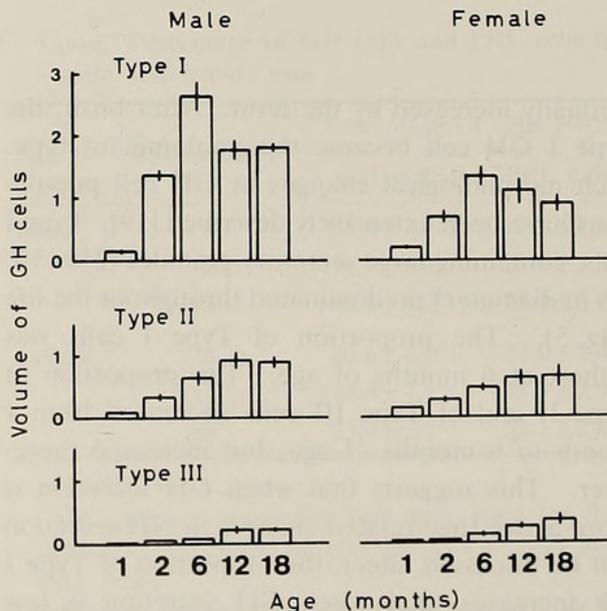


FIG. 8. Estimated total volumes of each GH cell type. Volumes were expressed in arbitrary units. Age-related differences were detected in each GH cell type of both sexes (male, for each cell type: $P < 0.01$; female, Type I: $P < 0.01$, Type II, III: $P < 0.05$). (from Takahashi [19]).

4. Functional heterogeneity of GH cells

Heterogeneity in GH synthesis Uptake of [^3H]-leucine into dissociated GH cells was studied using electron microscopic autoradiography. Only half of the GH cells were heavily labelled, indicating that GH synthetic ability differed among GH cell populations [45]. Furthermore, dissociated pituitary cells were separated into two subpopulations of GH cells by density gradient centrifugation. One of the two GH-cell subpopulations, which is less dense (the light fraction), produce more GH than the other [46].

Heterogeneity in GH release GH release from dissociated individual GH cells was analyzed by a reverse hemolytic plaque assay. Figure 9 shows

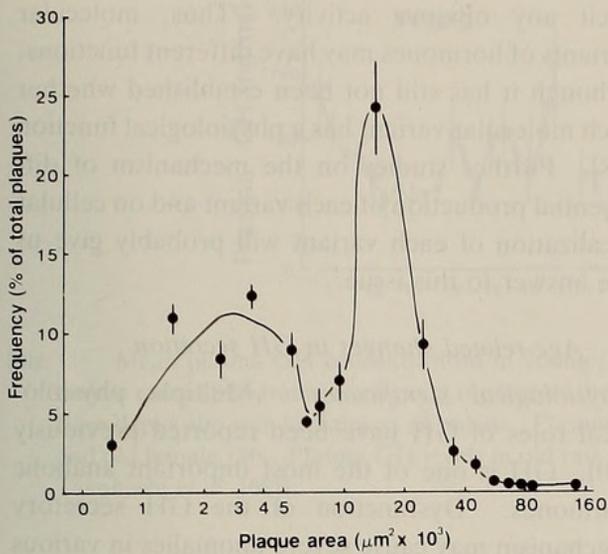


FIG. 9. The composite distribution pattern of plaque areas formed by GH cells from four hemolytic plaque assays. Each points are means of 4 separate assays with the standard errors. In each assay about three hundred plaques were measured.

the bimodal frequency pattern of the plaque areas formed by GH cells of adult female rats. One subpopulation formed the larger hemolytic plaques than the other. As the plaque area is proportional to the amount of hormones secreted, this result indicates that one group of GH cells secretes more GH than the other. Similar result has been already reported by Frawley and Neill [47], although the bimodal distribution of the plaque area was detected only in GHRH-treated pituitaries. The subpopulation of Type III GH cells was smaller than the other two subpopulations of Type I and II cells. Consequently, contribution of Type III GH cells in the reverse hemolytic plaque assay is quite small, and can be neglected. In young female pituitaries, the relative proportion of Type II cell-populations to Type I cell-populations was 41%. It is probable that the two subpopulation, morphologically divided, may correspond to the two subpopulation detected in the reverse hemolytic plaque assay. Further study is needed to clarify this correlation.

5. Possible mechanisms of functional heterogeneity

Preferential release of newly-synthesized hormones Chen *et al.* [48] found, using the reverse

hemolytic plaque assay, that in basal secretion of GH, one subpopulation of GH cells secreted a larger amount of GH than the other. The former subpopulation was likely to preferentially release newly-synthesized hormones for basal secretion, whereas the other population was likely to release stored hormones for basal secretion, even without stimulation. Therefore, this heterogeneity of GH cell population may be due to the difference in the intracellular content of the preferentially releasable hormone component among GH cell populations.

Difference in GH cell-location A tissue-slicing method clarified another functional heterogeneity of GH cells [8]. The responsiveness of GH cells to GHRH on GH release is different, depending on the location of GH cells within the gland. GHRH-induced GH release was only detected in GH cells derived from the left dorsorostral, right ventrocaudal and right ventrorostral parts of the gland. This location-dependent functional heterogeneity of GH cells may be due to the differential blood supply, the different concentrations of hypothalamic hormones in different portal vessels, or the effect of cell-to-cell communication (the paracrine effect).

Differences in intracellular signal transduction

Localization of protein kinase-C (PK-C) subtypes in the pituitary gland was studied immunocytochemically [49]. Not all of the pituitary cells contained PK-C. As for GH cells, only 9% of all GH cells contained PK-C. This finding suggests that GH cell populations can be divided into two subpopulations by the difference in the PK-C system. The function of PK-C in pituitary glands is not fully understood, although numerous physiological functions of PK-C are known [50]. As PK-C plays a key part in intracellular signal transduction, the GH cells which are deficient in PK-C may be controlled by other signal transduction mechanisms, or may not have some functions (for example, as PK-C is known to be involved in cell proliferation in some types of cells, PK-C deficient cells may not have the ability to proliferate).

6. Molecular heterogeneity of GH

Multiple molecular forms of GH are found in the rat pituitary gland [51–54]. Farrington and

Hymer [54], for example, demonstrated that 11 variants of rat GH exist, ranging in molecular weight from 11 kDa to 88 kDa (Fig. 10). Some of them were dimeric and glycosylated (24 K, Bollen-gier *et al.*, [53]). These variants might be related to the functional heterogeneity of GH hormones, and the morphological heterogeneity of secretory granules. Multiple forms of GH molecules were also well known in human, bovine and ovine pituitaries [55, 56]. The most extensive studies were carried out on human GH variants. Human pituitary extracts contained at least half a dozen peptides. The major GH components in pituitary extract are 20 kDa, 22 kDa (the major component), and 45 kDa (dimer) [57, 58]. Using transgenic mice, 22 kDa, 20 kDa and 5 kDa variants of human GH were evaluated [59]. Both 22 kDa and 20 kDa forms stimulated linear body growth and liver hypertrophy. The linear growth mediated by the 22 kDa variant did not correlate with an increase in blood IGF-1 level. The 5 kDa variant did not

elicit any obvious activity. Thus, molecular variants of hormones may have different functions, although it has still not been established whether each molecular variant has a physiological function [58]. Further studies on the mechanism of differential production of each variant and on cellular localization of each variant will probably give us the answer to this issue.

7. Age-related changes in GH secretion

Physiological significance Multiple physiological roles of GH have been reported previously [60]. GH is one of the most important anabolic hormones. Dysfunction of the GH secretory mechanism may cause severe anomalies in various body functions. Sonntag *et al.* [61] found a decrease in protein synthesis, and GH administration reversed this. Takahashi and Meites [62] also reported the alterations in liver GH receptors with age, and GH administration in old rats reversed the age-related changes. The decreased GH secretion resulted in a low plasma somatomedin-C level [62]. Therefore, it is important to study the age-related changes in GH secretion.

Changes in morphology of GH cells with age

Morphological changes of GH cells have already been explained in the section *Development of GH cells*. DNA content of GH cell-populations, which indicate the number of GH cells, was estimated from pituitary DNA content data and the percentage of GH cells. The estimated DNA content constituting the GH-cell population did not change at 6, 12 and 18 months of age in male rats (16.1 ± 2.4 , 18.9 ± 2.1 and $13.9 \pm 1.8 \mu\text{g}$, respectively), but increased during this period in female rats (11.3 ± 1.6 , 18.4 ± 2.1 and $18.1 \pm 0.4 \mu\text{g}$, respectively).

Changes in GH release with age GH is released in a pulsatile fashion. In male rats, the pulse interval is 3–4 hours, and in female rats it is about 70 minutes [63, 64]. The pulse amplitude is higher in male rats than in female rats. GH secretory patterns in male rats continued to remain unchanged throughout the day and night, but switched to a rapid, highly pulsatile pattern at night in female rats [65]. There was no clear relationship between the GH secretory pattern and the phase of estrous cycle [65]. These sexual differences in GH secretion are clearly discussed in the review by

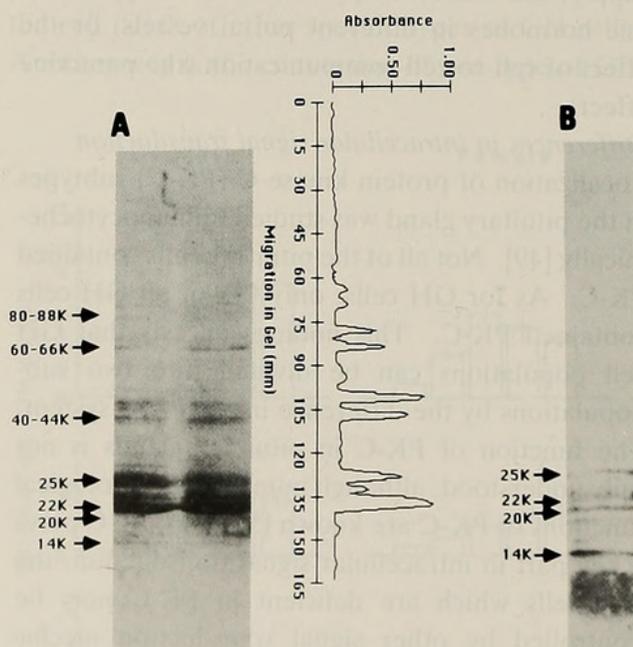


FIG. 10. Representative Western blots of rat pituitary GH contained in extracts electrophoresed under nonreducing (A) or reducing (B) conditions (see, Farrington and Hymer [54]). The reflectance optical density tracking of blot in A is shown in the middle. (from Farrington, M. and Hymer, W. C., *Growth hormone aggregates in the rat adenohypophysis*, *Endocrinology*, **126**: 1630–1638, 1990; © The Endocrine Society, with written permission).

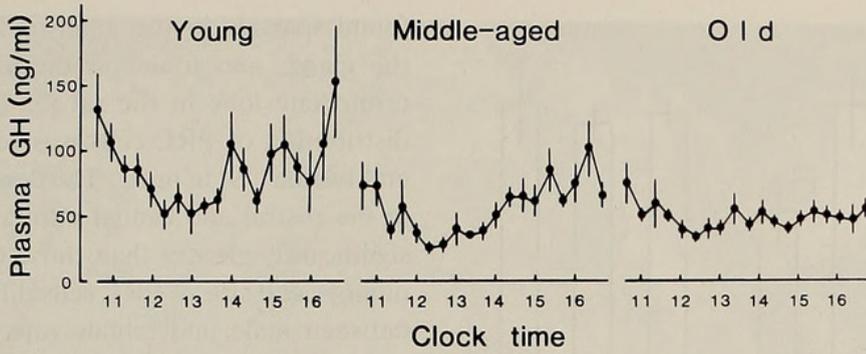


FIG. 11. Mean plasma GH concentrations in young (5 months), middle-aged (11 months) and old (25–29 months) female rats of SD strain. Each point represents the mean of GH levels in 17 young, 6 middle-aged and 13 old rats. Bars depict the standard errors of means. Plasma GH levels in young females were higher than in middle-aged and old female rats. Plasma GH levels in old rats tended to be somewhat lower than in middle-aged rats. (from Takahashi *et al.* [68]).

Jansson *et al.* [66]. In the rat, GH secretion diminished in both sexes with age [67, 68] (Fig. 11). The pulse intervals did not change with age, but the pulse amplitudes were significantly lower in old rats than in young rats. GHRH responsiveness to GH release was reduced in old rats *in vivo* [69, 70], although Weherenberg *et al.* [71] reported the opposite result that there were no age-related changes in the responsiveness to GHRH. A recent study showed, using a hemolytic plaque assay, that GH release from individual GH cells was less in old female rats compared to young females (Takahashi, unpublished observation). The mean plaque area produced by GH cells was significantly lower in 20–21 month-old females ($7.7 \pm 2.6 \times 10^3 \mu\text{m}^2$) than that in 3–4 month old young females ($14.9 \pm 2.4 \times 10^3 \mu\text{m}^2$). Responsiveness to GHRH was reduced in old female rats than in young rats. Accordingly, the diminished GH secretion in old rats is due partly to the reduced secretion of GH from individual GH cells.

Changes in GH synthesis with age Pituitary GH content decreased with aging [19]. GH synthesis in young, middle-aged and old rats was studied at the GH-mRNA level [31]. GH concentration per single GH cell was significantly lower in old rats than in young rats (Fig. 12). Also, GH-mRNA concentration per single GH cell was significantly lower in old female rats (Fig. 13). Thus, GH synthesis diminished with age at the transcription level of the GH gene. These results were in good agreement with a recent *in situ* hybridization study [72]. This decrease may be due partly to the

reduced release of hypothalamic GHRH in old rats [73–74], and the reduced binding sites of GHRH in old rats [75]. An uncoupling between the

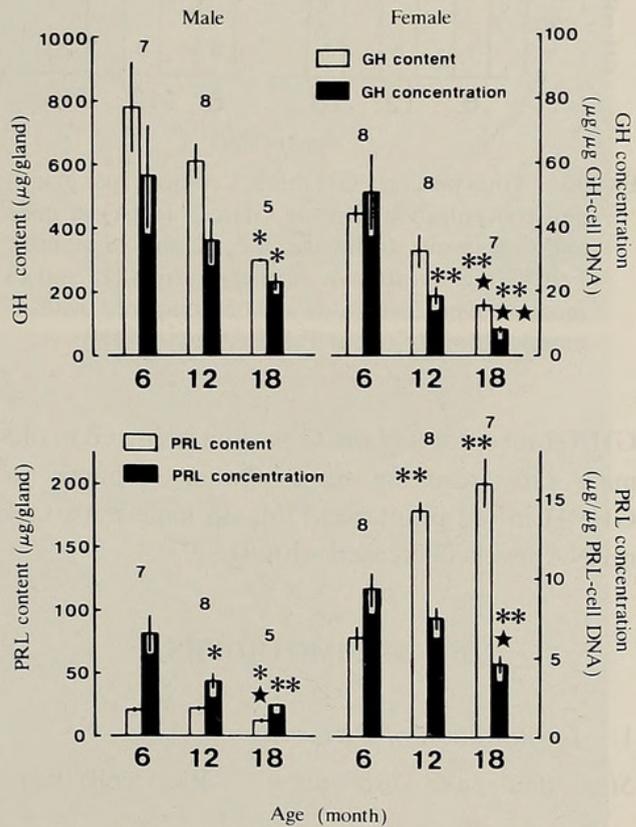


FIG. 12. Pituitary GH and PRL content (per gland) and concentration (per μg GH-cell DNA or PRL-cell DNA) in male and female rats at 6, 12 and 18 month of age. Female rats at estrus or persistent estrus (middle-aged and old) were used. * $P < 0.05$, ** $P < 0.01$ compared with 6-month-old rats. ★ $P < 0.05$, ★★ $P < 0.01$ compared with 12-month-old rats. (from Takahashi *et al.* [31]).

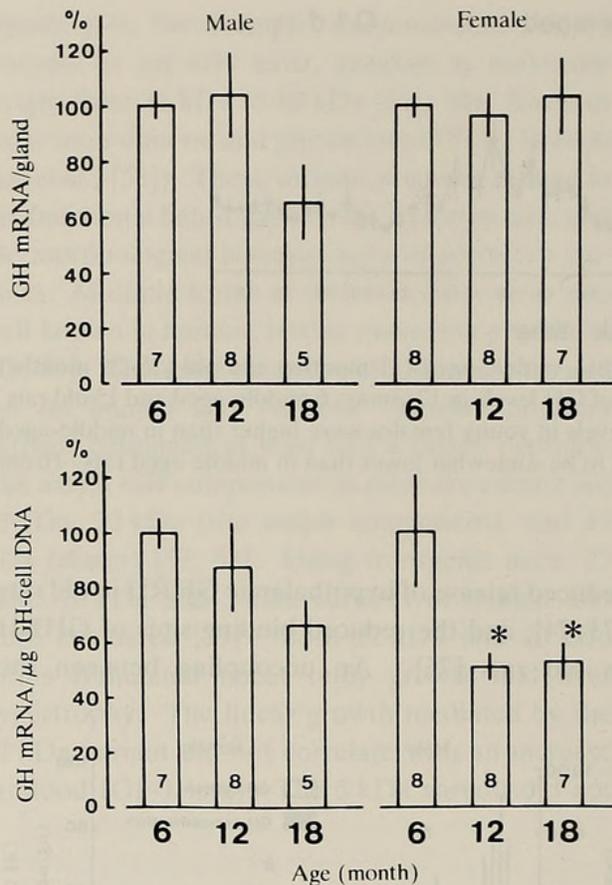


FIG. 13. Total pituitary GH mRNA content (per gland) and concentration (per μ g GH-cell DNA) in male and female rats at the age of 6, 12 and 18 months. Female rats at estrus or persistent estrus (12 and 18 months) were used. * $P < 0.05$ compared with 6-month-old rats. (from Takahashi *et al.* [31]).

GHRH receptor and the G protein occurred in old male rats, resulting in a weaker response to GHRH in old pituitaries [76]. In male mice GH mRNA levels decreased with age [77].

II. MAMMOTROPHS

1. Identification of PRL cells

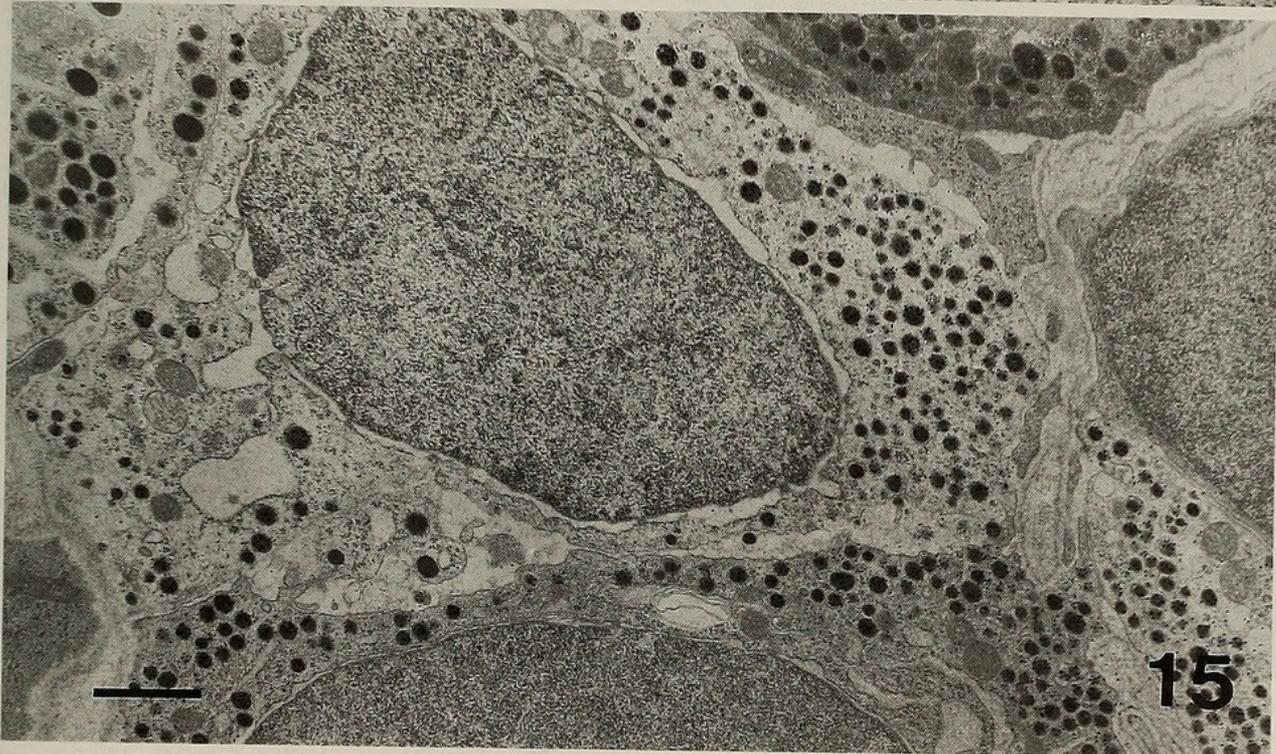
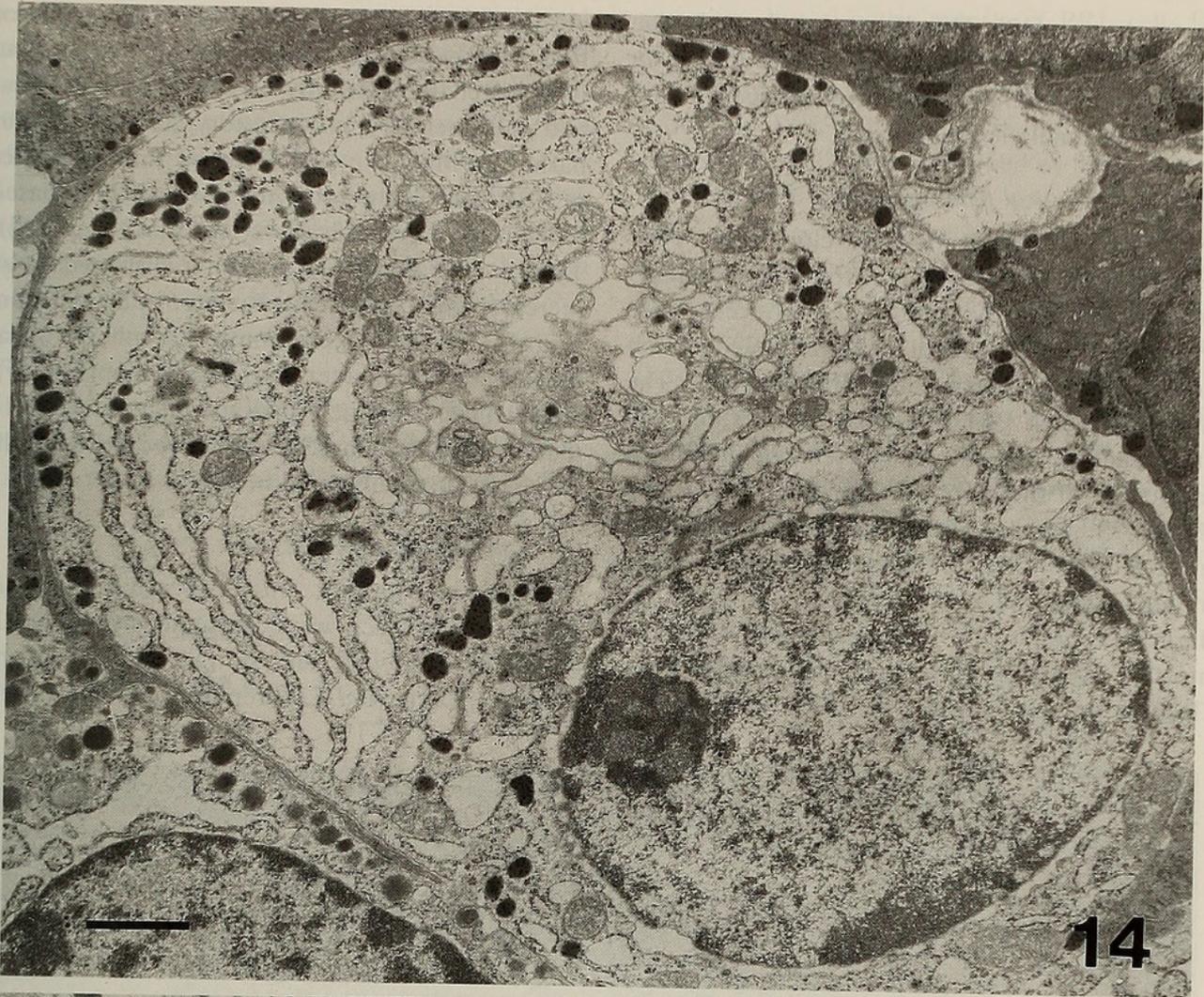
Size, shape and distribution PRL cells were

found sparsely in the anterior-ventral portion of the gland, and found in the areas near the intermediate lobe in the rat [9, 10, 78]. Regional distribution of PRL cells was reported by Sasaki and Iwama [79] in mice. The densities of PRL cells in the rostral and caudal pituitaries of mice were significantly greater than those of GH cells. The number and size of PRL cells differed significantly between male and female rats. PRL cells were polygonal, elongated and frequently cup-shaped and surrounded by large oval gonadotrophic cells [80].

Differentiation of PRL cells There are several reports about the first appearance of PRL cells in rat pituitaries, and these are somewhat contradictory. Sétáló and Nakane [11] found PRL cells on day 16 of gestation. Chatelain *et al.* [12] reported that PRL cells were detected on day 21 of gestation, and Watanabe and Daikoku [13] reported that PRL cells were first detected postnatally. Nogami *et al.* [14] observed immunocytochemically and by *in situ* hybridization that PRL was detected on day 18–19 of gestation, and PRL-mRNA was also detected on day 18–19 of gestation. PRL gene expression during the neonatal period had been studied [81]. PRL genes were expressed by at least 3 days of age, but the translation of the PRL message was, interestingly, reported to be blocked by the lack of association of the PRL message with ribosomes. In the mouse pituitary, immunoreactive PRL cells were detected at birth, but PRL cells might possibly appear in fetal pituitary glands [82]. In mice pituitaries an *in situ* hybridization with a PRL riboprobe, actually showed a few PRL cells 15.5 days after conception, and the number of PRL cells decreased 16.5 and 17.5 days after conception, although GH cells remarkably increased in number during this period [16]. In another study, PRL synthesis in mice was first detected at 8 days of age by a two-dimensional

FIG. 14. Type I PRL cell in an adult female rat. The cell is elongated and a round nucleus is located slightly eccentrically. The rough endoplasmic reticulum and the Golgi apparatus are well developed. Large round or irregularly shaped secretory granules are located in the peripheral cytoplasm. Bar = 1.0 μ m. (from Takahashi and Miyatake [88]).

FIG. 15. Type II PRL cell in an adult female rat. Type II cells contain round secretory granules with a diameter of 150–250 nm. The number of secretory granules is larger than that in Type I cells. Bar = 1.0 μ m. (from Takahashi and Miyatake [88]).



electrophoresis [17]. This discrepancy is partly due to the difference in the sensitivity of assays used (*in situ* hybridization, immunocytochemistry, and two-dimensional electrophoresis).

2. Morphological heterogeneity of PRL cells

Sato [80] studied postnatal development of PRL cells in the rat, and suggested from an immunocytochemical study that the oval PRL cells were premature, the polygonal ones mature, and the cup-shaped ones particularly differentiated. The ultrastructure of PRL cells had been extensively studied and three types of PRL cells, mainly based on the size of the secretory granules, were found [83–85]. Smets *et al.* [78] subdivided rat PRL cells into two types, one containing large polymorphic granules, and the other small round granules. Harigaya *et al.* [86] also classified mouse PRL cells into three types by immuno-electron-microscopy. Electron microscopically, the PRL mRNA was

localized in the rat pituitary, and two types of PRL-synthesizing cells were identified [87]. One type was characterized by large secretory granules, and the other by small secretory granules. Takahashi and Miyatake [88] observed three subtypes of PRL cells in the rat, and classified them based on Kurosumi's classification (Kurosumi *et al.* [89]). Type I cells contained irregularly shaped large secretory granules with a diameter of 300–700 nm (Fig. 14). Type II cells contained spherical granules with a diameter of 150–250 nm (Fig. 15). Type III cell contained small round granules with a diameter of 100 nm (Fig. 16). Type I PRL cells had been originally identified as mammatrophs.

3. Development of PRL cells

Sex difference in PRL cells Sex differences in PRL cell number were immunocytochemically found in mice [32] and in rats [90]. On the contrary, Dada *et al.* [34] reported that sex differ-

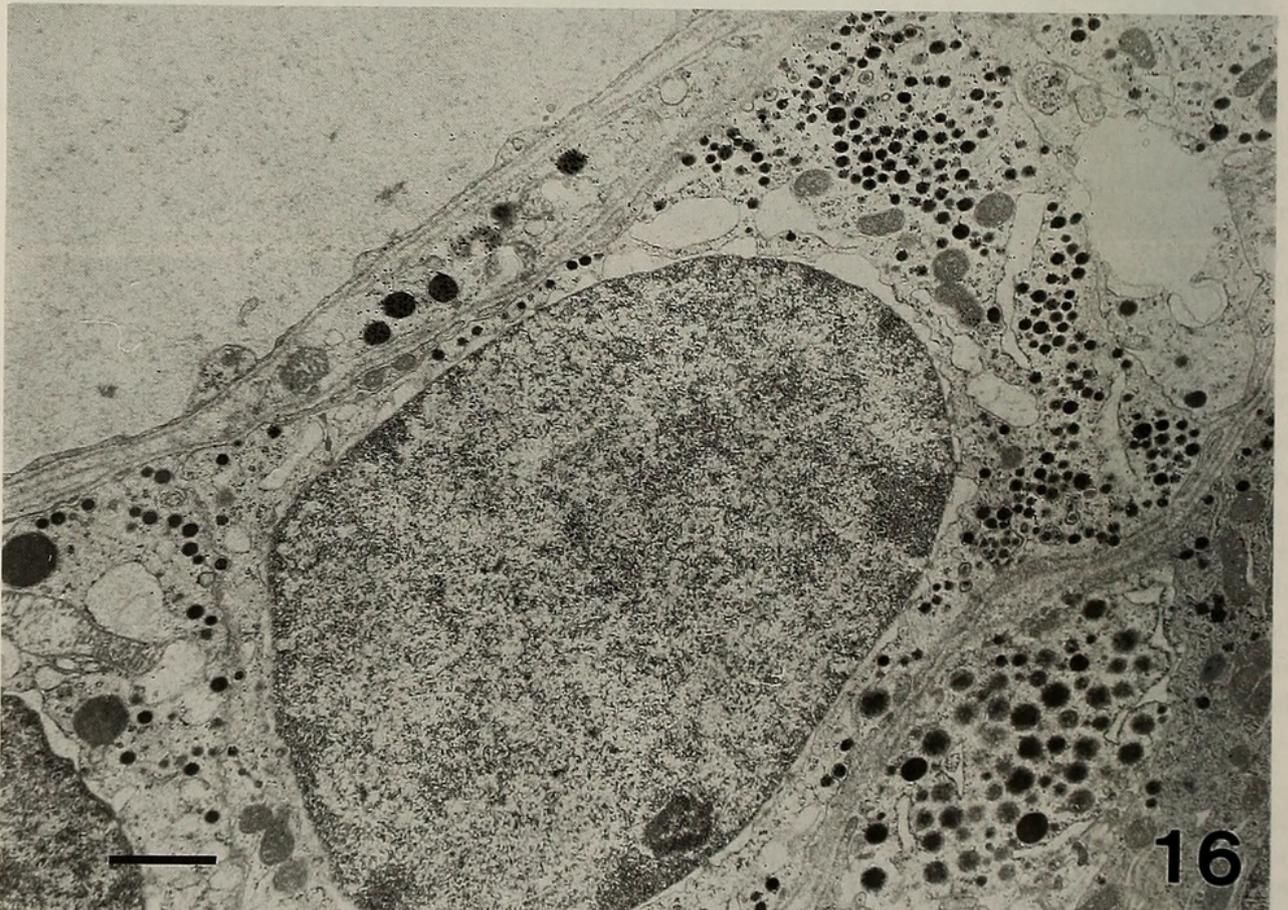


FIG. 16. Type III PRL cell in an adult female rat. The cell is characterized by the small amount of cytoplasm containing small secretory granules with a diameter of about 100 nm. Cell organelles are less developed. Bar = 1.0 μ m. (from Takahashi and Miyatake [88]).

ences were not detected in adult rats. Using the reverse hemolytic plaque assay, postnatal development of PRL cells was studied, and it was clearly shown that the percentage of PRL-secreting cells did not differ between male and female rats at immature ages, but significantly increased in adult female rats as previously reported [33, 91]. These sex differences in the percentage and the number of PRL cells were caused by the difference in estrogen level [90, 92-94].

Proliferation of PRL cells The proliferation of PRL cells is stimulated by estrogen [29], and is closely correlated with PRL secretion. Bromocriptine, a dopamine agonist, inhibited not only PRL

secretion, but also the mitosis of PRL cells [44, 95]. The difference in the number of PRL cells may partly result from the difference in the mitotic activity of PRL cells. The sex difference in the number of PRL cells is explained by the difference in the mitotic activity of PRL cell. Actually, the mitotic activity of PRL cells in estrous female rats was significantly higher than that in male rats [29].

Development of PRL cells Estrogen increased the percentage of Type I PRL cells, and decreased the percentages of Type II and III cells (Fig. 17). On the contrary, ovariectomy and bromocriptine decreased the percentage of Type I PRL cells and increased the percentages of the other two types (Fig. 18). Thus, the relative proportion of PRL cells changed in accordance with the change in

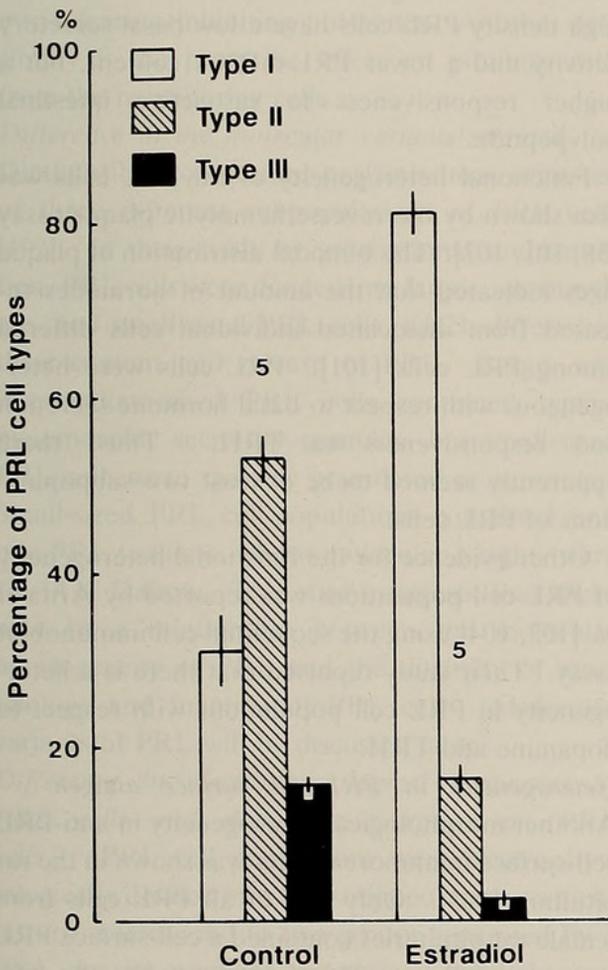


FIG. 17. Effects of injection of 50 μ g estradiol-17 β (E_2) daily for 5 days on the percentage of PRL cell types in male rats. In E_2 -treated rats, the percentage of Type I cells increased and the percentage of Type II and III cells decreased. The number above the column depicts the number of rats. Bars depict the standard errors of means. Estrogen affected the relative proportion of each subtype of PRL cells ($P < 0.01$). (from Takahashi and Miyatake [88]).

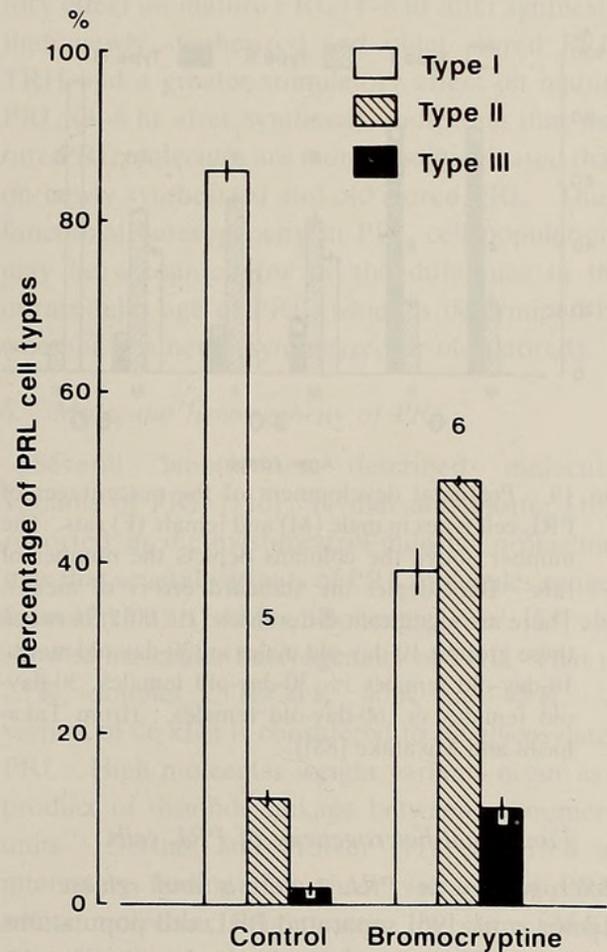


FIG. 18. Effects of bromocriptine treatment on the percentages of PRL cell types in adult female rats. The number above the column depicts the number of rats. Bars depict the standard errors of means. Bromocriptine affected the relative proportion of each subtype of PRL cells ($P < 0.01$). (from Takahashi and Miyatake [88]).

PRL secretion. The Type III PRL cell, containing small secretory granules, was predominantly present at immature ages (Fig. 19), and was small in size. Therefore, the Type III PRL cell is considered to be an immature type of PRL cell. Type I PRL cell, containing large irregularly-shaped secretory granules, constitutes most of the PRL cell population in adult female rats and is large in size. Thus, the Type I PRL cell is considered to be a mature type of PRL cell. The Type II PRL cell is considered to be an intermediate cell between the Type I and III cell [88, 89]. The change of secretory granules in size and shape is explained by the fusion and lysosomal degradation of preexisting secretory granules, which had previously been shown by Farquhar *et al.* [43].

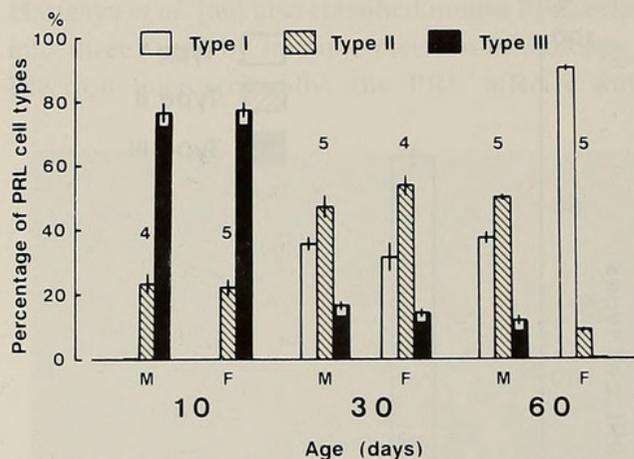


FIG. 19. Postnatal development of the percentages of PRL cell types in male (M) and female (F) rats. The number above the columns depicts the number of rats. Bars depict the standard errors of means. There are significant differences ($P < 0.01$) between these groups: 10-day-old males vs. 30-day-old males, 10-day-old females vs. 30-day-old females, 30-day-old females vs. 60-day-old females. (from Takahashi and Miyatake [88]).

4. Functional heterogeneity of PRL cells

Heterogeneity in PRL synthesis and release

Hymer *et al.* [96] separated PRL cell populations using the differences in unit gravity, that is, the difference in the cell shape and secretory granule content. This method revealed that the intracellular content of PRL differed among the separated PRL cell fractions, and the amount of PRL released during the culture period of 14 days was

positively correlated with the initial intracellular PRL content [97]. Swearingen [98] first found the heterogeneity in turnover of PRL in *in vivo* and *in vitro* studies. Walker and Farquhar [99] further clarified heterogeneity in PRL cells with respect to the PRL synthetic rate, which was autoradiographically visualized using the difference in the uptake of [^3H]-leucine in PRL cells. They also found a subpopulation of PRL cells which secreted preferentially newly synthesized PRL. Velkeniers *et al.* [100] separated PRL cell populations into high density and low density populations using the discontinuous Percoll gradient, and found that low density PRL cells have a high basal secretory activity and a higher PRL-mRNA content, and high density PRL cells have a low basal secretory activity and a lower PRL-mRNA content, but a higher responsiveness to vasoactive intestinal polypeptide.

Functional heterogeneity of rat PRL cells was also shown by the reverse hemolytic plaque assay [38, 101, 102]. The bimodal distribution of plaque sizes indicated that the amount of hormones released from dissociated individual cells differed among PRL cells [101]. PRL cells were heterogeneous with respect to basal hormone secretion and responsiveness to TRH. Thus, there apparently seemed to be at least two subpopulations of PRL cells.

Other evidence for the functional heterogeneity of PRL cell populations was reported by Arita *et al.* [103, 104] using the sequential cell immunoblot assay. Their study reported that there is a heterogeneity in PRL cell populations with respect to dopamine and TRH.

Heterogeneity in PRL-cell surface antigen

Another morphological heterogeneity in anti-PRL cell-surface immunoreactivity was shown in the rat pituitary [105]. Only half of all PRL cells from female rat pituitaries contained a cell-surface PRL immunoreactivity. This finding implies the presence of PRL receptors on the cell surface, or some of the released PRL is retained on the surface of these cells. From this finding, PRL cell populations may also be divided into at least two subpopulations. However, it is not easy to correlate this heterogeneity of PRL cell populations to the PRL cell types stated above.

5. Possible mechanism of functional heterogeneity of PRL cells

Difference in PRL cell-location A location-dependent functional heterogeneity in PRL cells was shown by the reverse hemolytic plaque assay [106], similar to the findings that have already been described in GH cells. In this study, PRL cells from the peripheral rim (outer zone) responded greatly to TRH, but only moderately to dopamine. PRL cells from the central region (inner zone) were affected slightly by TRH, but were markedly inhibited by dopamine. These regional differences in pituitary cells may be derived from the regional differences in the portal blood levels of hypothalamic releasing/inhibiting hormones. Another possibility is the paracrine effect on pituitary cells from the neighboring cells.

Difference in the molecular variants secreted

Diethylstilbestrol-induced prolactinomas consisted of three different subpopulations of PRL cells [102]. In their study by gravitational sedimentation, PRL cells were divided into large-, intermediate- and small-sized PRL cells, which differed in their content and release of PRL. Large- and intermediate-sized PRL cells contained typical pleiomorphic secretory granules, but small-sized PRL cells were sparsely granulated or agranular. Small-sized PRL cell-populations contained unique PRL variants, whose molecular weights were 10–14 K Dalton. This study suggests that there may be a relationship between the molecular heterogeneity of PRL and the diversity of morphology and function of PRL cells. Molecular variants of PRL will be discussed later.

Difference in electrophysiological properties of PRL cells and dopamine receptors on PRL cells

PRL cell populations were electrophysiologically divided into two subpopulations, which, in turn, correspond to two groups separated by a BSA density gradient separation, the light and heavy groups [107]. Most of PRL cells of the light fraction showed a type 1 response; dopamine induced a hyperpolarization of the membrane potential from the resting potential. The other PRL cells of the heavy fraction mostly do not respond to a dopamine (type 2 response), but when the membrane potential has been depolarized,

dopamine induces a repolarization. The expression of the two dopaminergic D2 receptors, D2₄₁₅ and D2₄₄₄, was studied and was found to be different in these two PRL cell populations [108]. The ratio D2₄₁₅/D2₄₄₄ was higher in the light fraction of PRL cells than in the heavy fraction. This result indicates that the two different responses to dopamine in PRL cells could be associated with the differential expression of two different D2 receptors. Such differences may eventually bring about a difference in PRL secretion, and/or even in the morphology of PRL cells.

Differences in the intracellular age of PRL The intracellular age of PRL molecules in the pituitary cells may be another important factor for the functional heterogeneity of PRL cell populations [109]. Dopamine had a significantly lower inhibitory effect on mature PRL (4–8 hr after synthesis) than newly synthesized and older stored PRL. TRH had a greater stimulatory effect on mature PRL (4–8 hr after synthesis), indicating that mature PRL molecules are more readily released than on newly synthesized and old stored PRL. Thus, functional heterogeneity in PRL cell populations may be accounted for by the difference in the intracellular age of PRL, which is determined by whether it is newly synthesized or old (stored).

6. Molecular heterogeneity of PRL

Several laboratories described molecular variants of PRL [110]. Hymer and Motter [102] reported in diethylstilbestrol-induced prolactinomas that several variants of PRL molecules ranged from 12 kDa to 64 kDa. Bollengier *et al.* [53] also showed molecular heterogeneity of PRL. That is, 23 K, doublet 25 K–26 K, 40 K and 42 K. A variant of 26 kDa is considered to be glycosylated PRL. High molecular weight variants occur as a product of disulfide linkage between monomeric units. Oetting and Walker [111] reported an interesting finding that three variants of PRL, whose molecular weights were the same (24 K), were different in their net charge (isoform 1, least negatively charged isoform of PRL; isoform 2; isoform 3, most negatively charged isoform), and considered them to be synthesized in PRL cells. Isoform 2 was the predominant form inside the cell and isoform 1 was the predominant secreted form,

although all three isoforms were released.

Physiological significance of molecular heterogeneity Frawley *et al.* [112] indicated the possibility that each molecular variant of PRL differs in biological activity, and suggested that each molecular form may have specific target cells, and consequently, have specific physiological roles. A good example to demonstrate the possible physiological significance of molecular variants of PRL was recently reported in ram pituitaries. The study clearly showed that production of variant forms of PRL in ram pituitary glands varied seasonably [113]. In their study, the 23 K form is a primary hormone, and the 25 K form is a glycosylated form. High molecular weight-forms (more than 25 K), which are aggregated by a disulfide linkage between monomers, are significant in winter, and may be for storage. During the season when PRL secretion is active, high molecular forms disappeared. An explanation for this could be that synthesized hormones may be rapidly released into circulation, and are not stored in the cell. On the contrary, during the season when PRL secretion is low or inhibited, synthesized hormones are more likely to aggregate and to become the stored type. Thus, it is probable that changes in molecular forms of a hormone may be parallel to changes in the secretory activities of hormones.

7. Age-related changes in PRL secretion

Physiological significance A number of physiological actions of PRL have been reported [114]. The altered PRL secretion induces various diseases (eg. [115, 116]). Therefore, it is valuable to study age-related changes in PRL secretion.

Changes in morphology of PRL cells with age

Kawashima [117] reported, electron microscopically, the morphological changes in pituitary cells, particularly hypertrophy and hyperplasia of PRL cells in female rats, although an immunocytochemical identification had not been done. Age-related changes in immunocytochemically identified-PRL cells were reported by Takahashi and Kawashima [90]. The percentage of PRL cells significantly increased in female rats with age (Table). The total number of PRL cells had not been measured, but it had been estimated from the pituitary DNA content and the percentage of PRL

cells as described in GH cells. Actually, the DNA contents constituting the PRL-cell population at 6, 12 and 18 months were as follows; in male rats, $3.7 \pm 0.6 \mu\text{g}$ (7), 7.0 ± 0.8 (8) and 6.5 ± 0.8 (5), and in female rats, 9.5 ± 1.4 (8), 25.8 ± 2.9 (8) and 40.3 ± 2.4 (7). The DNA contents of PRL cells increased in both sexes with age, but more markedly in old female rats, suggesting the significant increase of PRL cells in number. This was confirmed by Chuknyiska *et al.* [118]. The increase in the number of PRL cells with aging was caused by the ovarian estrogen, the stimulatory factor for the proliferation of PRL cells [119]. Prepubertal ovariectomy prevented the increase of PRL cells in number in old female rats.

Age-related changes in PRL cell mitosis in the rat were observed (Fig. 20). Even in 2-year-old female rats mitotic pituitary cells were encountered. Immuno-electron-microscopical studies have been done in male rats [120]. The relative proportion of each type changes with age in male rats. One type of PRL cell, containing small round secretory granules (Type III cells in Kurosumi's classification [89]), increased in percentage, and on the contrary, another type of PRL cell with large irregularly-shaped secretory granules (Type I cells in Kurosumi's classification) decreased in percentage in old male rats.

Changes in PRL synthesis and secretion with age

PRL secretion increased with age, and the enhanced secretion of PRL is partly due to the dysfunction of the hypothalamic dopaminergic mechanism [121–123]. A reverse hemolytic plaque assay revealed that the amount of PRL released per cell decreased in old rats [124]. Pituitary PRL content significantly increased with age in female rats, but PRL concentration per PRL cell decreased [31] (Fig. 5). PRL mRNA levels per PRL cell decreased with age in both sexes (Fig. 21). PRL synthesis in each PRL cell decreased at the transcription level with age. However, since PRL cells significantly increased in number in old female pituitaries of the Wistar/Tw rats [90], the total amount of PRL significantly increased with age. Stewart *et al.* [125] recently reported no significant change in PRL mRNA concentrations (per measured amount of pituitary DNA) with age in female rats, but did report a significant increase

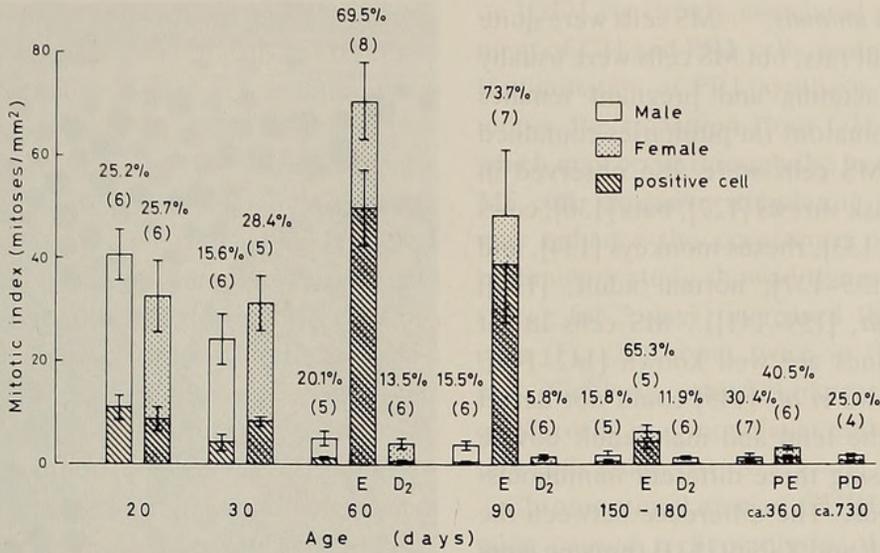
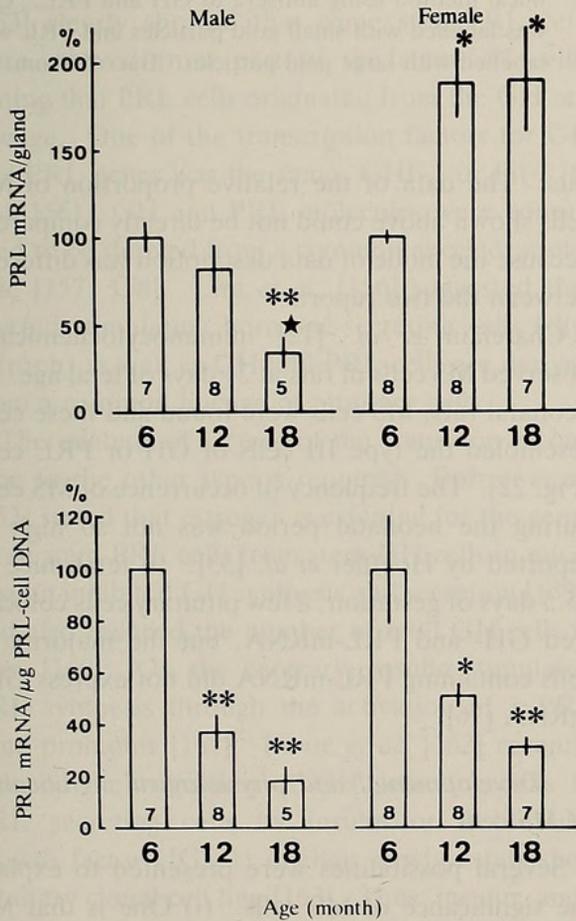


FIG. 20. Age-related changes in the mitotic indices of pituitary cells and PRL cells in male and female rats. The colchicine-arrested mitotic cells and immunocytochemically-identified PRL cells (positive cells) were observed. For the detail of the method, see Takahashi *et al.* [29]. Female rats at estrus (E), 2nd day of diestrus (D₂), persistent estrus (PE) and persistent diestrus (PD) were used. The percentage above the each column depicts the percentage of mitotic PRL cells in total mitotic pituitary cells. The number in parenthesis depicts the number of rats, and bars depict the standard errors of means.



in serum PRL level. We did not find any significant difference in PRL mRNA concentrations (per μg of pituitary cell DNA) in female rats, either (data not shown). Crew *et al.* [77] reported an age-related decrease in PRL mRNA in male mice.

III. MAMMOSOMATOTROPHS

1. Identification of MS cells

Mammomatotrophs (MS cells), which contained both GH and PRL in the same cell, were immuno-electron-microscopically described in intact adult rats [126-128]. MS cells were small in size and irregular in shape. Secretory granules, 50-150 nm in diameter, contained both hormones [126].

FIG. 21. Total pituitary PRL mRNA content (per gland) and concentration (per μg PRL-cell DNA) in male and female rats at the age of 6, 12 and 18 months. Female rats at estrus or persistent estrus (middle-aged and old) were used. * $P < 0.05$, ** $P < 0.01$ compared with 6-month-old rats. $P < 0.05$ compared with 12-month-old rats. (from Takahashi *et al.* [31]).

MS cells in various animals MS cells were quite rare in normal adult rats, but MS cells were usually encountered in lactating and pregnant females [126, 127]. Adenomatous rat pituitaries contained MS cells [128]. MS cells were also observed in mice [32, 129], musk shrews [127], bats [130], cows [131], sheep [132, 133], rhesus monkeys [134], and humans (fetal, [135–137]; normal adult, [138]; adenomatous adult, [139–141]). MS cells in rat pituitary tumor lines are well known [142–144]. However, Shirasawa *et al.* [145] could not detect any MS cells in the fetal and male adult bovine pituitary glands using three different immunohistochemical methods. The difference between the report of Fumagalli and Zanini [131] (nursing cows and virgin cows) and that of Shirasawa *et al.* [145] (fetal and adult bulls) is partly due to the difference in the age and sex of animals used. In mice, MS cells were further divided into two subtypes, the small, round, solid secretory granular type and the vesicular secretory granular type [129].

Ishibashi and Shiino [127] found two types of colocalization of GH and PRL. One type was that GH and PRL are colocalized in the same secretory granules within a single cell, as described by Nikitovitch-Winer *et al.* [126]. The other type is that GH-secretory granules and PRL-secretory granules were intermixed within closely aggregated and interdigitated cell-clusters which consist of GH and PRL cells in pregnant rats and female musk shrews. This type is similar to the multinucleated mammosomatotrophs in cows reported by Fumagalli and Zanini [131]. This finding suggests a possibility that the enhanced stimulation of hormone secretion induce the fusion of the secretory cells.

2. Development of MS cells

Using the reverse hemolytic plaque assay, MS cells were detected in neonatal and adult male and female rat pituitaries [146], in bovine pituitaries [147] and also in human pituitaries [135, 138]. Hoeffler *et al.* [33] reported that MS cells were 35.8% of all GH and/or PRL secreting cells of 5-day-old male rats. In adult male rats about one third of all GH and/or PRL secreting cells are MS cells [146]. Leong *et al.* [148] reported that about 5% of all pituitary cells were MS cells in adult male



FIG. 22. A mammosomatotroph in a 5-day-old male rat, which was identified by the double immunocytochemical method using antisera to GH and PRL. GH was labelled with small gold particles and PRL was labelled with large gold particles. Bar=500 nm.

rats. The data of the relative proportion of MS cells shown above could not be directly compared, because the mode of data description was different between the two reports.

Chatelain *et al.* [12] immunocytochemically observed MS cells in rats at 21 days of fetal age. In neonatal rats, MS cells were found and these cells resembled the type III cells of GH or PRL cells (Fig. 22). The frequency of occurrence of MS cells during the neonatal period was not so high as reported by Hoeffler *et al.* [33]. In fetal mice at 15.5 days of gestation, a few pituitary cells colocalized GH- and PRL-mRNA, but the majority of cells containing PRL-mRNA did not express GH-mRNA [16].

3. Developmental and physiological significance of MS cells

Several possibilities were presented to explain the significance of MS cells. (i) One is that MS

cells are a transitional cell type for the conversion of GH cells to PRL cells, or PRL cells to GH cells. (ii) Another possibility is that MS cells are progenitor cells for GH and PRL cells [135]. (iii) The other possibility is that the MS cell is an independent type of cell, and may be terminally differentiated. Analysis of the data which had been reported so far, and possible future data may clarify the genesis and physiological roles of MS cells.

The following reports are favorable to the transitional cell hypothesis. GH cells appeared earlier than PRL cells in rats and humans [14, 149]. In fetal mice, GH synthesis preceded PRL synthesis [17]. Stratmann and Ezrin [150] previously showed the possibility of the transition of GH cells to PRL cells by estrogen treatment using both electron microscopy and autoradiographical detection of ^3H -thymidine uptake. They stated that some of the previously existing GH cells proliferated and were converted into PRL cells. Frawley's group had reported a large amount of evidence for the transition from GH cell to PRL cell, or PRL cell to GH cell using a hemolytic plaque assay [33, 38, 151, 152]. Using transgenic mice, Borrelli *et al.* [153] clearly showed that some stem-PRL cells were derived from part of the stem-GH cells, stating that PRL cells originated from the GH cell lineage. One of the transcription factors for GH and PRL genes was the same, GHF-1 or Pit-1 [6, 154-156]. GH and PRL molecules were considered to be derived from a common ancestor molecule [157, 158]. Lira *et al.* [156] suggested that thyroid stimulating hormone-secreting cells (thyrotroph) as well as GH and PRL cells are derived from a common lineage of pituitary cells.

The analysis of factors of the transition of one type to the other type is required. Borrelli *et al.* [153] stated that estrogen is essential for the genesis of stem-PRL cells from stem-GH cells in mice. Insulin inhibited GH synthesis and secretion [159], and also reduced the number of fetal GH cells *in vitro* [160]. On the contrary, insulin stimulated PRL synthesis through the activation of a PRL gene promoter [161]. Inoue *et al.* [162] recently induced the transition of GH secreting cells to PRL secreting cells by insulin or insulin-like growth factor (IGF-1) in their newly established pituitary clonal cell line [163]. Thus, insulin, and/

or IGF-1, is closely associated with the development of GH and PRL cells, and probably MS cells. If stimulation of PRL synthesis and secretion can induce the transition from GH cell to PRL cell, which may occur through the transitional cell of an MS cell, excessive stimulation of PRL secretion may enhance the occurrence of MS cells. Our preliminary study showed that estrogen treatment (50 μg for 3 days) increased the number of MS cells (Fig. 22) about twice in the neonatal male rats. Similarly, estradiol increased the proportion of MS cells in a monolayer culture of male pituitary cells [164].

Chronic stimulation of GHRH using transgenic mice caused a hyperplasia of MS cells [165], although some of the MS cells in those transgenic mice were morphologically similar to those in adult mice, but others were morphologically different, and relatively close to those in adenomatous human pituitaries. Provided that the MS cell is the common progenitor cell, and this progenitor cell of MS cells exists even in adult pituitaries, MS cells in adenomatous tissues may be derived from unregulated proliferation of preexisting MS cells.

The volume of data accumulated so far seems to strongly support the theory that the MS cell is a transitional cell from a GH cell to a PRL cell. However, it is probable that PRL cells transform to GH cells through MS cells as shown in Porter *et al.* [151, 152]. If such bidirectional conversion between GH cells and PRL cells occurs in rat pituitaries, MS cells may be the common progenitor cells. Currently, further study is still needed to determine which possibility stated above holds for the rat pituitary.

IV. CONCLUSIONS

A large amount of evidence indicated that the GH cell and PRL cell populations were morphologically and functionally heterogeneous. A correlation between morphologically and functionally different subtypes remains to be studied. Analysis at a single cell level is needed for further clarification. Multiple molecular variants of GH and PRL have been reported. It is probable that such molecular variants of the two hormones may play different physiological roles.

Heterogeneity of pituitary GH and PRL cells at different levels (morphological, functional and molecular) may be the integrated outcome of various differences in the molecular variants of hormones, the intracellular age of hormones, the difference in receptors on the pituitary cells for hypothalamic regulatory hormones, the difference in mechanisms of intracellular signal transduction, and the location of pituitary cells in the gland. The maturing process of GH and PRL cells may be associated with these heterogeneities. Heterogeneity of GH and PRL cells in itself alters with age: the relative proportion of each subtype of GH and PRL cells changed with age. Age-related changes in GH and PRL secretion were studied, and the decrease in GH and PRL syntheses were clearly explained.

MS cells may be the transitional cell between GH cells and PRL cells, or a common progenitor cell of GH and PRL cells. Further study is needed to clarify the significance of MS cells. From the

view of the developmental and maturing process of pituitary cells, the hypothetical schema for explaining the morphological heterogeneity of GH and PRL cells, and the relationship among GH, PRL and MS cells is described (Fig. 23).

The overview of heterogeneities of GH cells and PRL cells gives the impression that such a wide spectra of heterogeneities, at various levels from the molecular to the pituitary level, could probably be highly helpful for endocrine functions. Such heterogeneity can give the pituitary gland enough flexibility to respond to any demands for hormone secretion.

ACKNOWLEDGMENTS

The author would like to express cordial thanks to Dr. K. Wakabayashi, Gunma University (Maebashi, Japan) for kindly supplying of the antisera against GH and PRL, and to Dr. J. A. Martial, University of Liege (Belgium) for the rat GH cDNA and rat PRL cDNA. The author is also grateful to Dr. S. Raiti, the National Hormone and

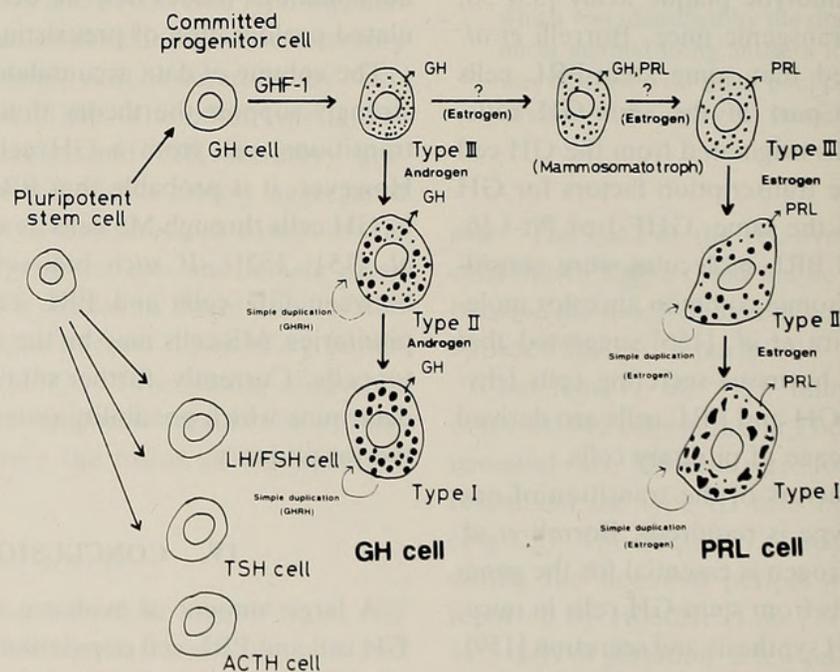


FIG. 23. Possible relationship among GH, PRL and MS cells. Pituitary glands consist of GH cells, PRL cells, gonadotrophs (LH/FSH cells), thyrotrophs (TSH cells), corticotrophs (ACTH cells) and folliculo-stellate cells (not shown in the figure). Pituitary specific transcription factor GHF-1 is known to be involved in the development of GH and PRL cells, and probably TSH cell. The conversion of GH cells is stimulated by androgen, and that of PRL cells by estrogen. This conversion may be bidirectional. Pituitary cells proliferate by the mode of self-duplication. GHRH stimulates the mitosis of GH cells, and estrogen that of PRL cell. Mammomatotrophs (MS cells) may be the transitional cells between GH cells and PRL cells. From several reports, part of PRL cells may be derived from part of GH cells. This transition from stem-GH cells into stem-PRL cells may probably be stimulated by estrogen.

Pituitary Program (University of Maryland School of Medicine, Baltimore, MD, USA) and the NIDDK, NIH (Bethesda, MD, USA) for the RIA kit. This study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan, and by the Itoh Science Foundation.

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Takahashi, Sumio. 1992. "Heterogeneity and Development of Somatotrophs and Mammatrophs in the Rat." *Zoological science* 9, 901–924.

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