Light and Electron Microscopical Studies on the Appearance of a Marginal Layer and Colloid in the Pituitary Cleft in Young Rats

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Summary. Studies on the marginal zone and colloid of the pituitary cleft in pituitaries of rats aged 2 to 88 days were performed by light and electron microscopy. From 10 days of age the intermediate lobe could be divided into two parts, i.e., the marginal layer and the intermediate lobe proper. The former part consisted of agranular cells facing the cleft; the latter part comprised granular cells stained with PAS-lead hematoxylin. In addition, the former part was separated from the latter by a basal lamina. In 2 and 6-day-old animals, however, there was neither a rising of the marginal layer nor an occurrence of the basal lamina, although the agranular cells and the granular cells could be distinguished in the intermediate lobe. Accordingly, it appears that the marginal layer of the intermediate lobe begins to occur after 6 days of age.

The PAS reactivity of the colloid in the pituitary cleft was generally weakly positive up to 20 days of age, and then from 26 days onward the reactivity became stronger, being strongly positive in 74 and 88-day-old rats. Consequently, it is suggested that the PAS positive substances begin to be stored in the cleft after birth and increase with the advance of age.

The pituitary cleft is derived from the cavity of the ectodermal Rathke's pouch; the cavity persists during postnatal life as this cleft in the rat and other mammals. In the rat pituitary, epithelial cells facing the cleft are known as marginal cells; the marginal cells of the intermediate lobe side in particular clearly differ from the cells of the intermediate lobe proper.

In some species the cavity of the cleft contains a large amount of colloid where exfoliated cells and cell debris are frequently seen. Changes in the amount of colloid have been described in mammals under several experimental conditions (ELLISON and WOLFE, 1934; ROMEIS, 1940; SELYE, 1943; FERRER, 1956; RAPP and BERGON, 1977; CIOLCA and GONZALEZ, 1978); these authors suggested that the construction of the cleft had some correlation with the mechanism of hormonal secretion in the pituitary. Furthermore, it was reported that intermedin and glycoproteins were present in the colloid (RAPP and BERGON, 1977; LEWIS et al., 1937; ANDERSSON and JEWELL, 1958). However, the chemical composition of the colloid remains largely unknown.

As regards the functional relationship between the contents of the cleft and the marginal cells, KUROSUMI et al. (1962) suggested a holocrine secretory mechanism as one of the means of production of the colloid for the reason that the exfoliated cells
and cell debris could be seen in the cleft. VANHA-PERTTULA and ARSTILA (1970) discussed the formation and absorption of the colloid by the marginal cells. There has been, however, few observations regarding the accumulation of the colloid, the appearance of the exfoliated cells and the development of the marginal layer with aging under normal conditions.

For this reason, the present authors deemed it valuable to study the appearance of the marginal layer and the colloid of the cleft in the young rat pituitary, in order to clarify the relationship between the pituitary cleft and the marginal cells of the intermediate lobe with the advance of age.

**MATERIALS AND METHODS**

Albino rats of the Wistar strain nourished under normal laboratory conditions (12L: 12D light control, 22°C temp.) were used in the present study.

According to the plan shown in Table 1, the animals were sacrificed by decapitation in a time period between moon and 2 p.m.

**Table 1. Number of rats used**

| Age (days) | 2   | 5   | 6   | 10  | 11  | 14  | 20  | 24  | 26  | 27  | 32  | 33  | 39  | 74  | 75  | 88  |
|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Light microscopy |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Male       | 4   | 6   | 4   | 6   | 2   | 10  | 4   | 7   | 8   | 5   |     |     |     |     |     |     |
| Female     | 4   | 3   | 4   | 5   | 1   | 11  | 5   | 7   | 12  | 5   |     |     |     |     |     |     |
| Electron microscopy |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Male       | 5   | 4   | 4   | 4   | 3   | 5   | 6   | 1   | 6   | 4   |     |     |     |     |     |     |
| Female     | 7   | 2   | 4   | 3   | 4   | 6   | 3   | 3   | 5   | 5   |     |     |     |     |     |     |

The pituitaries were immediately removed and immersed in fixatives. For light microscopy, the fixative used was 2% formaldehyde-2% glutaraldehyde with 0.01% picric acid in 0.1 M phosphate buffer (pH 7.4) solution. The tissues were kept in the fixative for 2-14 days at room temperature, briefly rinsed twice with 0.1 M phosphate buffer, and subsequently embedded in JB-4 resin (Polysciences, INC.). Horizontal sections, prepared in thicknesses of 3 μm, were stained with PAS-lead hematoxylin (SOROKIN and HOYT, 1978) or PAS-orange G.

Materials for electron microscopy were prefixed in 4% glutaraldehyde phosphate buffer (pH 7.4) solution, postfixed in 1% osmium tetroxide phosphate buffer (pH 7.4) at 4°C and embedded in Luveak 812 (Nakarai Chemicals, LTD.). Pale gold sections were cut with a Porter-Blum MT-1 ultramicrotome and stained with uranyl acetate and lead citrate before examination with an electron microscope (Hitachi H-300).

**OBSERVATIONS**

I. **Light microscopy**

Rats at seventy-four and eighty-eight days of age

The marginal layer consisted almost completely of a simple layer of cuboidal or flattened epithelial cells and was separated from the intermediate lobe proper by the basal lamina (Fig. 1). No marginal cells of the intermediate lobe were stained with
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Some marginal cells were ciliated cells; they were more abundant in the intermediate lobe side than in the anterior lobe side. The number of ciliated cells varied in different specimens.

The colloid in the cleft presented a positive PAS reaction, and exfoliated cells were often found in the cleft. They contained differently sized PAS positive vesicles, and seemed to undergo autolysis (Fig. 1b). In addition to these cells, variously sized and non-stained vacuoles were also seen in the colloid of the cleft (Fig. 1b).

Almost all cells of the intermediate lobe proper were stained with PAS-lead hematoxylin. They were mostly polygonal, though some were slender-spindle in shape (Fig. 1a). In some individuals, cells strongly stained with PAS-lead hematoxylin were present in the intermediate lobe proper, and the nuclei of these cells showed orangeophilic with Orange G stain.

The appearance of cysts showed individual variations and were found in several places in the pituitary—the anterior lobe, the intermediate lobe or the margin of the neural lobe. Many cysts contained PAS positive colloid and were variable in size. Some epithelial cells lining the cyst had PAS positive particles or cilia.

Young rats

1. Rat aged six to eleven days

In 11-day-old rats the PAS positive colloid, the exfoliated cells and red blood cells were all found in the cleft. In 6-day-old rats, however, no exfoliated cells were found although PAS weak positive colloid could be seen in certain ones.

In 6 and 11-day-old rats some cells of the intermediate lobe were weakly stained...
with lead hematoxylin, and lead hematoxylin positive cells, as a rule, showed a tendency to be on the neural lobe side of the intermediate lobe (Fig. 2). At these ages, however, the marginal layer was not clear because the boundary of the intermediate lobe proper was uncertain (Fig. 2). The existence of ciliated cells was also indistinct.

2. At fourteen to twenty days of age
The gap in the cleft was narrow, and the colloid was weakly positive in PAS reaction. Exfoliated cells and red blood cells were seen in some clefts.

In 14-day-old rats the marginal cells were mostly bilayer in nature though occasionally monolayer. Ciliated cells, although few, could occasionally be seen.

3. Twenty-four, twenty-six, thirty-two and thirty-nine days old
At these ages, some parts of the cleft by the sides of the horseshoe-shaped intermediate lobe (in horizontal sections) were frequently closed, while the remaining parts—rostral ends and the caudal bottom—were always open and contained the PAS positive colloid. At times the exfoliated cells and red blood cells could be seen in the colloid (Fig. 3).

In the intermediate lobe proper, developed cells contained many lead hematoxylin positive granules while small cells contained fewer of the granules (Fig. 3).

II. Electron microscopy
Rat at seventy-five days of age
The marginal cells of the intermediate lobe had neither secretory granules nor electron
lucent vesicles. They did have, however, a considerable number of mitochondria and frequently large dense bodies like lipid droplets (Fig. 4). Short microvilli could be seen on the free surface of the marginal cells. Ciliated cells were often present in the marginal layer. A wide space with amorphous contents was occasionally seen between the basal lamina of the marginal layer and that of the intermediate lobe proper; little intercellular space was found in the lateral areas of marginal cells.

Since in the marginal region of the anterior lobe no basal lamina separating agranular cells from granular cells existed, neither was a marginal layer similar to that of the intermediate lobe found. The agranular cells usually contained a considerable number of mitochondria with an occasional dense body. Both agranular and granular cells near the cleft formed wide intercellular spaces containing colloid.

In the cells of intermediate lobe proper, numerous secretory granules, 200–350 nm in diameter, were dispersed throughout the cytoplasm, and a number of mitochondria could be seen. There was not much rough endoplasmic reticulum, however. Electron lucent vesicles were abundant, some of these cells being dark. In these dark cells, electron lucent vesicles were more abundant than secretory granules. Many dark cells could be seen in some pituitaries. An expanded intercellular space was rarely observed.
Fig. 5. Intermediate lobe of the pituitary in a 6-day-old male rat. The marginal layer has not yet formed. However, it can be discerned between the granular and the agranular cells; these two types of cells being intermingled at this time. Intercellular spaces are found throughout the intermediate lobe and are variable in size. $\times 2,800$. Inset. Some granular cells face the cleft lumen directly although the agranular cells predominantly occupy a portion near the cleft. A 5-day-old. CL cleft lumen. $\times 10,000$
Young rats

1. At two, five and six days of age

Granular cells and agranular cells could be distinguished in the intermediate lobe. However, the marginal layer had not yet been formed, neither was a basal lamina separating the agranular cells from the granular cells present (Fig. 5). The granular and the agranular cells of the intermediate lobe were intermingled at this time. Although the agranular cells of this lobe predominantly occupied a portion near the cleft, some granular cells facing the cleft could be seen (Fig. 5). Both cells had a small volume of cytoplasm. The granular cells contained a few secretory granules. These granules, however, were not always arranged in a single row along the periphery of the cell (Fig. 6). Furthermore, the granular cells contained a sparse number of electron lucent vesicles (Fig. 6). The agranular cells sometimes contained a few dense bodies. No ciliated cells were observed, but dark cells of the intermediate lobe proper were observed. Intercellular spaces variable in size were found throughout the intermediate lobe (Fig. 5).

In the junction of the intermediate and the neural lobes on 2-day-olds, a narrow gap remained in part between the intermediate lobe and the neural lobe, where fibroblasts and collagen fibers were observed. This suggests that the nerve supply had not yet been established there (Fig. 7).

2. At ten and fourteen days of age

In some places of the intermediate lobe the marginal layer was observed; in other places the basal lamina marking the boundary of intermediate lobe proper had not yet formed. Both a monolayer or multilayers of the agranular cells existed. Ciliated cells
T. Ismi and T. Ishibashi were encountered at times (Fig. 8).

In the marginal region of the anterior lobe, the rough endoplasmic reticulum was relatively well developed in the granular cells, but there was far less development of the organelles in the agranular cells. There were few ciliated cells, and the intercellular space was expanded.

In the cells of the intermediate lobe proper in 10-day-olds, the secretory granules were unevenly distributed or dispersed throughout the cytoplasm. In addition, they were occasionally arranged in a single row along the periphery of the cell in those cases when there were only a few present. Mitochondria were numerous. There were few electron lucent vesicles, however, in the cells except for the dark cells, which were frequently encountered at this time.

3. Twenty days of age

Here, the characteristics of the marginal layer of the intermediate lobe did not differ from that of the adult. In the space between the marginal layer and the intermediate lobe proper, neural fibers and terminals containing light core vesicles were frequently seen. Occasionally some parts of the space with amorphous contents were widened and the undulating basal lamina of the marginal layer was visible (Fig. 9).

In the marginal region of the anterior lobe, the morphological features were almost the same as those of 10 and 14-day-old rats.

4. Twenty-seven, thirty-three and thirty-nine days of age

The marginal cells of the intermediate lobe were divided into light cells and dark cells. Both types had an abundance of mitochondria and Golgi complexes but little rough

Fig. 7. Intermediate lobe (IL) and neural lobe (NL) of the pituitary in a 2-day-old male rat. A narrow gap partly remains between the intermediate lobe and the neural lobe, and a fibroblast (F) and collagen fibers (asterisk) are found there. ×2,000
Fig. 8. Intermediate lobe (IL) and anterior lobe (AL) of the pituitary in a 10-day-old male rat. 

a. The marginal layer of the intermediate lobe appears in some places on the right, in other places on the left; however, the basal lamina forming the boundary of the intermediate lobe proper does not yet appear at this point in time. The intercellular spaces (IS) are wide in the marginal region of anterior lobe. Arrows: basal lamina, CL cleft lumen, D a dark cell of the intermediate lobe. ×3,000. 

b. A ciliated cell (C) is seen in the marginal layer. ×3,000
endoplasmic reticulum and no secretory granules like that of the adults. Certain dense bodies, such as lipid droplets, were occasionally present although the dense bodies were smaller than those of the adults. Although many of the ciliated cells were light, some dark ciliated cells were still encountered.

In the marginal region of the anterior lobe, the morphological appearances of the agranular and granular cells were almost the same as those of the adults. Ciliated cells could be seen at times. The granular cells did not contact the cleft lumen or the marginal cells of the intermediate lobe because they were overlaid with agranular light cells and ciliated cells directly facing the cleft lumen (Fig. 10).

Exfoliated cells and red blood cells were frequently observed in the cleft. The exfoliated cells without secretory granules had many vacuoles of various sizes and densities and had scanty numbers of microvilli on the cell surface (Fig. 11).

At this stage there was little space between the anterior lobe and the intermediate lobe in certain places (Fig. 10), where the cells of the two lobes directly contacted each other, and a number of microvilli formed on the apical plasma membrane. No junctional complexes, however, occurred between the cells of the two lobes (Fig. 10b). Thus it is suggested that a latent space of the cleft may be widened at this place. The agranular cells of the anterior lobe were light and frequently localized in small aggregations near the portion where the marginal cells of the intermediate lobe were mostly dark (Fig. 10).
Fig. 10. Intermediate lobe (IL) and anterior lobe (AL) of the pituitary in a 27-day-old male rat. a. There is little space between the lobes. At this place the cells of the two lobes directly contact each other, and a number of microvilli occur on the apical plasma membrane. Note that, being overlaid with the agranular light cells, the granular cells (asterisks) of anterior lobe do not contact the marginal cells (M) of the intermediate lobe. Almost all the marginal cells are dark at this place. ×3,000. b. The space between the lobes enlarged is similar to the state shown in Figure 10a. There are no junctional complexes between the marginal cells (M) of the intermediate lobe and the agranular cells (A) of the anterior lobe, although the junctional complexes (arrows) can be seen between the same type of cells. Microvilli (arrowheads) are present in the contact zone of the anterior lobe (upper side) and the intermediate lobe (lower side). ×20,000
DISCUSSION

A gap containing colloid is found between the anterior and the intermediate lobes in the rat, porcine, ovine and bovine pituitary among others. It shows the derivation of Rathke's pouch and apparently takes a fissure-like conformation, or cleft. Reports claim that the cleft varied in volume with the colloid and that the volume was altered by several experimental conditions. Furthermore, an increase of the amount of colloid with the advance of age was noted (Höser, 1942; Wolfe, 1943). Bassett (1951) described how no colloid was seen in the calf or fetal materials. It is unknown, however, exactly when the colloid in the cleft occurs or starts to increase under normal conditions.

In the present study the PAS reactivity of the colloid in the cleft appeared weakly positive by 20 days of age although only a certain part of the cleft contained the PAS positive colloid. From 26 days of age onward, the PAS reactivity strengthened and appeared strongly positive in 74 and 88-day-old rats. Hence, it seems that the PAS positive substances begin to be stored in the cleft after birth and increase with age, and it can be inferred that the liquid in the fetal Rathke’s pouch may either not contain PAS positive component or only as a very thin solution.

It has long been known that exfoliated cells and red blood cells can be observed in the cleft (Selye, 1943). In our study they were observed in the cleft from 10 days of age, and not in younger rats. The exfoliated cells contained different sized PAS positive vesicles. Their vesicles may fuse with each other into larger ones and fill up within the cytoplasm in time, and subsequently may dissolve into the colloid of the cleft.

Fig. 11. Intermediate lobe (IL) and anterior lobe (AL) in a 39-day-old female rat. Exfoliated cells (E) are observed in the cleft. The exfoliated cells without secretory granules have many vacuoles of various sizes and densities, but there are few microvilli on the cell surface. Arrows show cilia. ×4,000
as a result of autolysis. At the light microscopic level it appears that these cells arise from the marginal cells of the anterior lobe side. It could not be firmly established, however, as to which side of the cleft the exfoliated cells arise, because no satisfying pre-conformation of the exfoliated cells could be found by electron microscopy. In any case, the present authors believe that these cells are one of source of the colloid, in common with the theory by Kurosumi et al. (1962).

Red blood cells were often seen in the colloid of the cleft. In this regard, there is the possibility of an artificial leakage of blood in the course of specimen preparation. One must ascertain in the future whether the appearance of red blood cells in the cleft is an artefact or not.

In our observations the marginal layer of the intermediate lobe and the ciliated cells were found in 10-day-old rats, but were not observed before that age. Accordingly, it appears that the marginal layer of the intermediate lobe is formed and borders the intermediate lobe proper by the basal lamina sometime after 5 or 6 days of age in the rat; lobulation of the intermediate lobe by the connective tissue will also occur simultaneously. In 2, 5 and 6-day-old rats the marginal layer of the intermediate lobe has not yet been differentiated and the basal lamina separating it from the intermediate lobe proper is also indistinct. Nevertheless, the intermediate lobe cells can be differentiated into the lead hematoxylin weakly positive and the negative groups, namely, the granular and the agranular cells.

The marginal cells cannot be identified before the granulation of the primordial cells of the intermediate lobe begins. In the mouse, granular cells first appear in the central part of the intermediate lobe at the embryonic stage; the marginal cells are also identifiable at the same stage (Eurenius and Jarskår, 1975). On the contrary, in the rat, the granular cells of the intermediate lobe were found on the side of the neural lobe rather than that of the cleft. Similarly, Chatterjee (1974, 1975) mentioned that cytodifferentiation of the intermediate lobe cell began at the side nearest the neural lobe before the establishment of either a nerve or vascular supply, on the basis of observations of fetal and perinatal rabbits. Svalander (1974) described how direct cell to cell contact was not found between the intermediate lobe and the neural lobe, and axons and synaptic contacts were not observed in the intermediate lobe during the embryonic development of the rat, though secretory granules were already found in the cells of the intermediate lobe at the same embryonic stage. Furthermore, in our observations, direct contact between the intermediate lobe and the neural lobe was not completely established even in 2-day-old rats. Here it appears that the granular cells of this lobe first arise in the juxta-neural lobe area, and it is supposed that the initiation of granule formation is under some control other than direct neural effect, although some effects from the neural lobe side induce the granulation of the primordial cells of the intermediate lobe. Consequently, after the beginning of granulation the remaining agranular cells facing the cleft lumen will still be ungranulated and become the marginal cells. However, the possibility cannot be completely overlooked that mesenchymal cells which have penetrated from the neural lobe side affect either the differentiation of the primordial cells or the formation of the marginal layer in the intermediate lobe.

Finally, it is suggested that there is simultaneity in the appearance of PAS positive colloid, exfoliated cells, ciliated cells and the marginal layer.
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