Electron Microscopic Studies on the Pars Tuberalis of the Rat Hypophysis*

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There have been a considerable number of studies on the fine structure of various parts of the adenohypophysis of several different species. The ultrastructure of the pars tuberalis in the mammalian hypophysis, however, was the subject of only a few previous studies, such as those on the cat (GREEN and VAN BREEMEN 1955), the rat (KOBAYASHI et al. 1963) and the rabbit (YOUNG et al. 1965).

In the present investigation, the normal structure of the rat pars tuberalis, as well as the fine structure of the organ under several experimental conditions, such as those after adrenalectomy, gonadectomy and thyroidectomy, are studied. Although the functions of the pars tuberalis are still unknown, the presence of the secretory activity in some cells of the pars tuberalis has been suggested morphologically. A short preliminary note on this work has already been reported in Japanese (OOTA 1965).

Materials and Methods

Adult male and female albino rats of Wistar strain were used in this study. In order to follow any changes in the pars tuberalis, several experiments were performed. Operation techniques are given under each heading. The tissues were obtained from the control and experimental animals according to the following procedures. After rapid decapitation without anesthesia, the skull was immediately cut open, the hypothalamic region was exposed, and the median eminence together with the pars tuberalis was removed. Most of the specimens were immersed in ice-cold 1% osmium tetroxide buffered at pH 7.4 with phosphate buffer (CAULFIELD 1957) for about 2 hours. Some of them were fixed in ice-cold 2% glutaraldehyde buffered at pH 7.4 with sodium cacodylate (SABATINI et al. 1963) for 2 hours followed by postfixation with 1% osmium tetroxide for 1 hour. The specimens were then dehydrated through an ascending series of ethanol and embedded in Epon 812 according to the method of LUFT (1961). Sections were cut with a Porter-Blum microtome and were stained with uranyl acetate followed by lead hydroxide (MILLONIG 1961) or lead citrate (REYNOLDS 1963). They were examined with a modified type of Hitachi HS-7 electron microscope under the accelerating potential of either 50 or 75 kV.

For comparison with thin sections examined in the electron microscope, the adjacent thick sections from the Epon-embedded specimens were stained with toluidine blue (YAMAMOTO 1963), and observed with an ordinary light microscope. Paraffin sections of Ciaccio-

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fixed specimens were also prepared and stained with azan, paraldehyde fuchsin, chrome alum hematoxylin phloxin, periodic acid-Schiff techniques.

Observations

A. Light microscopic findings

The pars tuberalis of the rat hypophysis consists of two or three layers of relatively small and irregular shaped epithelial cells lying close to the median eminence. As shown in Fig. 1, however, the structure of the pars tuberalis is rather complicated and the cellular characteristics may differ from region to region. The tuberalis cells are arranged in longitudinal cords, which may be seen as small globular areas when the median eminence or stalk are cut transversely. They spread along the ventral surface of the median eminence and extend to the posterior limit of the optic chiasma. Therefore, the whole surface area of the infundibular stalk is covered by the epithelial cells of the pars tuberalis.

The pars tuberalis is separated from the nervous tissue of the median eminence and infundibular stalk by a thin layer of connective tissue leading the capillary loops of the primary plexus of the hypophyseal portal system.

B. Electron microscopic findings

1. Fine structure of the pars tuberalis of the normal rat

The epithelial cells of the pars tuberalis can be classified into at least two types, that is, non-granulated and granulated cells according to the difference in size and appearance, as observed with the electron microscope.

a. The non-granulated cells

Fig. 2 represents a low magnification electron micrograph showing the contact region
between the median eminence and pars tuberalis. The pars tuberalis is composed of two or three layers of small flattened or polygonal epithelial cells. Most of the cells contain a relatively large electron dense nucleus with a single prominent nucleolus. The round or oval
nuclei are enveloped by double nuclear membranes and contain the dense karyoplasm which seems to be finely granular. The cytoplasm of the cell which belongs to this type is filled with a few mitochondria, rough and smooth endoplasmic reticula, lysosomes and multivesicular bodies (Fig. 3). Free ribosomes are also scattered throughout the cytoplasm, where they often form small clusters. The development of the Golgi apparatus is not so conspicuous. Since

Fig. 3. Electron micrograph of the non-granulated cell. A relatively large nucleus (N) containing a nucleolus (NC), a few mitochondria (M), rough-surfaced endoplasmic reticulum (ER) and free ribosomes (R) are observed in this type of epithelial cell. ×16,300
the formed elements of the cytoplasm are relatively few, the cytoplasmic matrix appears watery clear. Cells of this type form the major part of the pars tuberalis.

Sometimes the plasma membranes between the adjacent cells are folded and separated from each other forming relatively wide intercellular spaces. As shown in Figs. 4 and 5, a dense colloid-like substance fills the intercellular space, into which a great number of micro-

**Fig. 4.** An electron micrograph showing the intercellular space among the non-granulated cell. An electron dense colloid-like substance (C) fills the space, into which projections of many cilia (CL) and microvilli can be observed. ×12,600
villi and cilia may project from the surface of surrounding non-granulated cells. The basal bodies of cilia are surrounded by a dense filamentous substance. On the cell borders frequently there is conspicuous thickening of plasma membranes of the adjacent non-granulated cells at the angle between the luminar and intercellular surfaces. These structures are quite similar in morphology to the junctional complexes described by FARQUHAR and PALADE (1963) in
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various epithelia. As shown in Fig. 5, the juxtaluminal "tight junction," that is the fusion of two plasma membranes, is relatively long and some electron dense material is accumulated in the cytoplasm near the junction. Adjacent to this structure, a large amount of similar electron dense material is also condensed along the intermediate junction. Sometimes desmosomes are revealed immediately below the intermediate junctions.

The mitotic figure of the non-granulated cell is rarely seen. Large masses of dense chro-

Fig. 6. An electron micrograph of the mitotic figure of a non-granulated cell. Large masses of dense chromosomes (CH) are shown. ×17,200
mosomes are scattered in the cytoplasm (Fig. 6).

b. The granulated cells

As mentioned above, most of the cells of the pars tuberalis are non-granulated, flattened or cuboidal in shape, and small in size. However, there are relatively large cells of another type intermingled with small non-granulated cells, though the frequency of occurrence of this

Fig. 7. A survey picture of a granulated cell. A conspicuous nucleolus (NC) is observed in a round nucleus (N). It also contains a number of mitochondria (M) and dark granules (SG), which may be secretory. ×11,200
cell type is considerably low (Fig. 2, 7). They contain large round or oval nuclei with a conspicuous nucleoli, a relatively large number of mitochondria, and well-developed rough and smooth endoplasmic reticula and Golgi apparatus (Fig. 7, 8, 9). Ribosomes are abundant and generally found in small clusters.

The most prominent characteristic of this cell type is the occurrence of many dark granules. The granules are usually scattered at random throughout the cytoplasm, but they are

![Fig. 8. An electron micrograph of the granulated cell. The cytoplasm of this cell contains well-developed rough-surfaced endoplasmic reticulum (ER) and many dark granules (SG), ×16,300](image-url)
frequently crowded in close proximity to the well-developed Golgi apparatus (Fig. 8). Each granule is enveloped by a single smooth membrane. The size and degree in density of these granules may vary considerably. The diameter of granules ranges from 110 to 230 μm, and the mean diameter is about 150 μm. The size range and some other morphological characteristics of the granules resembles those of the thyrotroph or corticotroph of the pars distalis of the rat (Kurosuni and Kobayashi 1966). Sometimes the electron dense granules encased in
smooth-membranous sacs are found at about the center of the Golgi area (Fig. 9). Therefore, the granules may conceivably be the secretory granules of a protein nature which are formed in the Golgi apparatus.

The cytoplasm of the granulated cells, unlike that of the non-granulated cells, may contain a well-developed rough endoplasmic reticulum and a great amount of glycogen particles.

Fig. 10. The pericapillary region of the pars tuberalis. A number of collagen fibrils (CF) and fibroblasts (FB) are contained within the relatively wide perivascular connective tissue space. Glycogen particles (GL) are abundant in the granulated cells of the pars tuberalis. ×11,700
as well as free ribosomes. Intermingled with small secretory granules, there are a few large dense bodies which may be thought to be lysosomes (DE DUVE 1963, NOVIKOFF 1963). They are generally spherical or oval in shape, and are enveloped by a smooth limiting membrane.

c. The pericapillary region

As previously stated, the pars tuberalis and the median eminence are separated from each other by a thin layer of the loose connective tissue, in which capillaries of the hypophyseal portal system are often inserted. A relatively wide perivascular connective tissue space occurs and it contains a number of collagen fibrils and fibroblasts (Fig. 10, 11). In this space are observed the inner basement membrane separating the endothelial cells from the perivascular space and the outer basement membrane intervening between the epithelial cells.

Fig. 11. The pericapillary region of the pars tuberalis. The cytoplasmic layer of the endothelial cells lining the portal capillaries is extremely thin except for the area containing the large nucleus. Note fenestrations of the endothelial cells (arrows). Capillary lumen (CA), perivascular connective tissue space (PS), fibroblast (FB). × 19,000
of the pars tuberalis and the space. The cytoplasmic layer of the endothelial lining of the portal capillaries is extremely thin except for the area containing the large nucleus. Fenestrations are often encountered through the cytoplasmic sheet of the endothelial cells. The nucleus of the endothelial cell is relatively large and often has numerous infoldings. These cells contain only a small number of mitochondria, vesicular and tubular elements of the endoplasmic reticulum, with either free or attached ribosomes, multivesicular bodies and the Golgi appa-

![Image](image_url)

Fig. 12. A survey picture of the pars tuberalis of the adrenalectomized rat. Conspicuous granulated cells with many dark secretory granules (SG) are intermingled. ×8,800
ratus. Occasionally rounded pinocytotic vesicles are demonstrated (Fig. 11).

2. Fine structure of the pars tuberalis of rats under various experimental conditions

Twelve virgin female rats were adrenalectomized through the dorsal approach. After the operation, the animals were kept alive on a 1% sodium chloride solution as drinking water.

Fig. 13. An electron micrograph of the region of the intercellular space of the pars tuberalis of a thyroidectomized rat. An accumulation of electron dense materials (C) in the intercellular space is prominent. Junctional complexes (JC) between non-granulated cells are observed, ×26,000
The pars tuberalis were fixed for electron microscopy on the third, fifth, and seventh day of the postoperative stage. As shown in Fig. 12, the pars tuberalis of adrenalectomized rats appears to be similar in structure to those of the control animals. The two types of epithelial cells, non-granulated and granulated, are also revealed in these operated animals. There is neither a tendency for the secretory granules to increase in number, nor for the amount of the cytoplasm to change.

Fig. 14. A high power electron micrograph of a non-granulated tuberalis cells of a thyroidectomized rat. Numerous longitudinal sections of the cilia are observed. ×40,800
Effect of ovariectomy on the fine structure of the pars tuberalis was also studied. Specimens of pars tuberalis from animals at 6 and 12 postoperative weeks were taken and examined under the electron microscope. Four animals for each stage were used. No significant morphological changes in the fine structure of the pars tuberalis were demonstrated in the ovariectomized rats.

Eight virgin female rats were thyroidectomized. Half of the animals were sacrificed at

Fig. 15. A part of the granulated tuberalis cells of a thyroidectomized rat. They contain various organelles such as the rough endoplasmic reticula (ER), mitochondria (M), Golgi apparatus (GO), multivesicular body (MB) and lysosomes (L). × 25,700
the end of the second week after the operation and the remainder were sacrificed at the end of the third week. The fine structure of the pars tuberalis was studied with the electron microscope. However, no further changes could be demonstrated following these experimental procedures. As in the normal, an accumulation of electron dense material is seen in the intercellular space (Fig. 13). The cell borders between the non-granulated cells also show conspicuous junctional complexes and frequently, desmosomes which are characterized by the accumulation of the electron dense material around them, are demonstrated immediately below the intermediate junctions. Occasionally numerous cilia are projected into the space (Fig. 14). The cytoplasm of the cells is filled with rough and smooth endoplasmic reticula, mitochondria, Golgi apparatus, multivesicular bodies and lysosomes (Fig. 15). Electron dense secretory granules, 110—230 m\(\mu\) in diameter, are also demonstrated in the granulated cells. All these histological features are quite the same as those in control animals.

Discussion

In 1955 Green and Van Breemen demonstrated the presence of relatively large amounts of vacuoles and reticular structures in the cells of the cat pars tuberalis. They also observed a few granule-containing cells among many non-granulated cells by using both the phase contrast and electron microscopes. In the rat pars tuberalis, however, Kobayashi et al. (1963) reported that a few mitochondria were revealed in the cytoplasm, but neither secretory granules nor vacuoles were encountered. On the basis of these observations Kobayashi et al. (1963) denied any characteristic functional differentiation of epithelial cells in the rat pars tuberalis into the glandular (endocrine) tissue. Recently Young et al. (1965) investigated the ultrastructure of the rabbit adenohypophysis and described the cells of the pars tuberalis as containing numerous small, dense, smooth membrane-bound vesicles and numerous ribosomes, but no secretory granules.

In the present study, however, it is demonstrated that the cells of the rat pars tuberalis can be divided clearly into two groups, i.e. non-granulated and granulated cells. The majority of the cells which constitute the pars tuberalis are non-granulated cells. These cells contain a rather small amount of cytoplasm and a few cytoplasmic organelles. The junctional complexes between the adjacent cell membranes are often conspicuous. Furthermore, characteristic features including projection of many microvilli and cilia into the intercellular space are demonstrated in the masses of non-granulated cells. These morphological characteristics may correspond to the chromophobe cells described in the pars distalis of the rat (Kurosumi 1965). On the other hand, the granulated cells intermingled with smaller non-granulated cells are relatively large in size. They are characterized by the presence of many secretory granules of varying density scattered through a considerable amount of the cytoplasm containing moderate numbers of cell organelles. The morphological characteristics of these granulated cells resemble those of the cells regarded as a type of basophils (Farquhar and Binehart 1954a, b, Ichikawa 1959, Kurosumi and Kobayashi 1966). Embryologically the pars tuberalis is closely related to the pars distalis of the hypophysis. Therefore, it may be conceived that the cells of the pars tuberalis are similar in structure to those of the pars distalis. In the recent investigation by Kurosumi and Kobayashi (1966), a new type of granulated cells, the so-called corticotroph, of the rat pars distalis was known to undergo remarkable morphological changes after adrenalectomy, including a change in the number of secretory granules. Furthermore, the thyrotrophs and gonadotrophs of the rat also show a characteristic change with the enlargement of the rough surfaced endoplasmic reticulum either after thyroi-
decotomy (Farquhar and Rinehart 1954 b) or gonadectomy (Farquhar and Rinehart 1954 a). However, the fine structure of the granulated cells found in the rat pars tuberalis showed no marked changes after adrenalectomy, thyroidectomy and gonadectomy in this study. Therefore the granulated cells of the pars tuberalis may not correspond exactly to the thyrotroph, corticotroph or gonadotroph of the pars distalis, in spite of the morphological similarity to each of them.

In an earlier histochemical study of the rat pars tuberalis, Halmi (1950) demonstrated a few cells faintly stained with the periodic acid-Schiff technique. Similar cells which showed positive PAS-reaction were also found in the basophil cells of the pars distalis (Halmi 1950). Lestroh (1963) reported that a considerable increase of PAS-positive material occurred in cells of the pars tuberalis of the rat 6 months after hypophysectomy. Using the electron microscope, Stutinsky et al. (1964) investigated the changes in epithelial cells of the pars tuberalis of the rat following hypophysectomy. They found the granulated cells in the pars tuberalis of these experimental animals and considered these cells to be the synthetic site of follicle stimulating hormone and adrenocorticotropic hormone. Recently Fand (1965) reported the successful demonstration of α-glycerophosphate dehydrogenase which might be a histochemical criterion of the production site of the thyroid stimulating hormone in epithelial cells of the pars tuberalis of the human hypophysis. He reported that the positive reaction to α-glycerophosphate dehydrogenase was limited to the PAS-positive cells of the pars distalis, although the number of these cells was restricted. In spite of many speculations which assumed the production of some of the anterior pituitary hormones from the granulated cells of the pars tuberalis, the function of the cells filled with many secretory granules found in the rat pars tuberalis in this electron microscopic study cannot be accurately interpreted at present. In other words, the electron dense granules in some cells of the pars tuberalis clearly indicate the possibility of secretion of a certain proteinous substance, but the secretory substance cannot be identified yet with any of the known trophic hormones of the anterior pituitary, and the nature of it remains uncertain.

As shown in the present observation, some of the cells of the pars tuberalis contain considerable amounts of scattered glycogen particles. Therefore there arises the question of whether the positive PAS-reaction of certain cells of the pars tuberalis described by several investigators may be caused by the presence of massive glycogen particles or due to the secretory granules. However, the present investigation could not solve this problem.

Summary

The pars tuberalis of the rat hypophysis was studied with the electron microscope. The pars tuberalis consists of several layers of relatively small epithelial cells lying along the ventral surface of the median eminence. The epithelial cells may be classified into two types. One is the non-granulated cell constituting the major part of the pars tuberalis. These cells show a morphological structure similar to the chromophobes of the pars distalis. Most of them contain relatively large nuclei, a few mitochondria, rough and smooth endoplasmic reticula, and Golgi apparatus. In some places, desmosomes and junctional complexes are observed between the adjacent non-granulated cells. Sometimes, a colloid-like substance fills the intercellular space, into which a great number of microvilli and cilia may project. The other cell type is granulated cells which are large in size and contain a large number of mitochondria, well-developed rough and smooth endoplasmic reticula and Golgi apparatus. The most prominent characteristic of this cell type is the presence of many dark granules, about 150 nm in diameter.
in diameter. These cells resemble the basophils of the pars distalis. The presence of these granulated cells suggests a possible secretory function of the pars tuberalis. In order to clarify the function of the granulated cells, animals were also investigated under several experimental conditions such as adrenalectomy, thyroidectomy and gonadectomy. However, there was no marked morphological change in the fine structure of the pars tuberalis following these experimental conditions.

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