Critical Review

Department of Morphology (Prof. K. KUROSUMI), Institute of Endocrinology,
Gunma University, Maebashi, Japan

Functional Classification of Cell Types of the Anterior Pituitary Gland Accomplished by Electron Microscopy

Kazumasa KUROSUMI

Received June 21, 1968

Light microscopy has revealed that the anterior lobe of the pituitary gland contains many kinds of glandular cells, which have been classified according to the staining behavior of each cell type after the use of various methods of differential staining. At the early stage in the development of pituitary morphology, the cell types were designated as acidophil, basophil or chromophobe suggesting their stainability, or they were called alpha cell, beta cell and so on, implying only the morphological difference between cell types (ROMEIS 1940). Physiology of the anterior pituitary which showed a great progress in recent years indicated six different hormones secreted from this gland. Then many authors attempted to correlate each of these six hormones with one of the various types of glandular cells, as cell types different in morphology from each other were believed to be different in function, too. Such a classification of pituitary cells in view of functional standpoint means the identification of the cellular source of the known anterior pituitary hormones. Each of the three cell types of the anterior lobe, viz. the acidophil, basophil and chromophobe, originally classified by pioneer microscopists was further divided into two or three subtypes as a result of the specific staining techniques of the light microscopic histochemistry, though some of these staining methods were not so widely used (HALMI 1950, DAWSON 1954).

Electron microscopy is far more advantageous than light microscopy even with the advanced techniques of histochemistry for morphophysiological studies of the anterior pituitary, because the electron microscope has extremely high resolving power which enables us to observe the structural characteristics of very small secretory granules of these cells. Furthermore, the electron microscope is very useful for the detection of minute changes in cell structure induced by various experiments which may help to determine the endocrine function of a given cell type in the anterior pituitary. Each cell type contains uniformly sized and characteristically shaped granules distinguishable from the granules of other cell types. This fact favors the differentiation of the cell types under the electron microscope. It is thus even possible to identify every cell in a given single section of the anterior pituitary. This state is more convenient for investigators than in the case of light microscopy which needs many different sections stained with different methods for the identification of cell types. Staining with dyes for light microscopy is sometimes unreliable, for the results of staining may change according to the method of the preceding fixation and also due to the small fluctuation in pH of the dye solution and other unknown factors. The characteristics in size and shape of the secretory granules observed with
the electron microscope are much more stable, though they also show slight changes according to the difference in fixation method and experimental conditions.

As the techniques in the research of cellular morphology and physiology advanced, the nomenclature of anterior pituitary cell types was altered: the names of cell types merely indicating their morphological characteristics were discarded, and a new terminology indicating the function of each cell type appeared. According to this modern classification of cell types, cells of the anterior pituitary gland are called such as thyrotroph, corticotroph and so on, indicating the name of the target organ of the hormone secreted by the cell concerned, or capping the abbreviated name of the hormone such as TSH-cell, ACTH-cell etc. (Purves and Griesbach 1951, Barnes 1963). Though the determination of the function of each cell type is not easy, it seems necessary to collect the results of many experimental studies such as those enumerated in the following chapter, before the final determination is established.

Methods for Determining the Function of Each Cell Type

Combinations of several methods in the following experiments are useful for the determination of the function of each cell type of the anterior pituitary. As the rat and mouse are most convenient for experimental manipulation, the pituitaries of these animals are frequently used and their function is best known. The rabbit, bat and some other mammals were also used for these studies.

1. Correlated light and electron microscopy

The functions of certain cell types were already known by light microscopy before the advent of the electron microscope. As to these cell types, the correlation between the light and electron microscopic findings is the easiest way to identify the function of a given cell type by electron microscopy. The so-called alpha cell (acidophil) which secretes the growth hormone and the beta cell (basophil) producing the thyrotrophic hormone (TSH) were first identified in electron micrographs by this way, because the morphological characteristics of these cells are quite remarkable and most of these characteristics can be recognized under both light and electron microscopes.

2. Ablation of target organs

The removal of certain endocrine glands, which are controlled by hormones of the anterior pituitary in the normal state, brings about a remarkable change in certain cells of the anterior pituitary. For example, castration causes in one type of the anterior pituitary cells enormous hypertrophy and a characteristic appearance like a signet ring. Such hypertrophic changes of certain cell types after the removal of peripheral endocrine organs imply a close mutual relationship between a hormone of the anterior pituitary and that of the peripheral endocrine organ which is the target of the former. The recent advance in endocrine physiology teaches us that most hormones of the peripheral target organs inhibit the secretion of the anterior pituitary hormones, which primarily promote the production of the hormones of the peripheral endocrine gland. This phenomenon is called the negative feed-back effect of the peripheral hormones, and this mechanism is essentially important for keeping the level of concentration of hormones constant in the circulating blood, i.e.,
for the homeostasis of hormones.

The site of action of the peripheral hormones as the negative feed-back is not limited to the anterior pituitary, but is predominantly concerned with a part of the brain, the hypothalamus. It is well known nowadays that the hypothalamus secretes neurohormones, most of which accelerate the production and release of the anterior pituitary hormones. Such neurohormones are the so-called releasing factors (RF) of the anterior pituitary hormones, produced in certain nerve cells in the hypothalamus, transported to the median eminence and released into the blood of the hypothysalportal system. As this peculiar vascular system leads the blood irrigated through the median eminence into every part of the anterior lobe of the hypophysis, neurohormones liberated at the median eminence exclusively come into contact with all types of anterior pituitary cells and stimulate their functions.

If one surgically removes a certain peripheral endocrine gland or diminishes the activity of the gland by administration of anti-hormonic agents or radioactive isotopes which preferentially accumulate in that endocrine organ, the level of concentration of the hormone in the circulating blood considerably lowers or the hormone completely disappears from the blood. In this case, the effect of negative feed-back of the peripheral hormone is lost. As a result, the releasing factor, which is specific for the cell type of the anterior pituitary secreting the hormone stimulating the target organ that is removed in this experiment, may increase in the rate of secretion. Consequently, the target of this neurohormone, i.e., the corresponding cell type of the anterior pituitary is stimulated and becomes strongly hypertrophied. This change is highly specific and the other cell types of the pituitary show almost no reaction. Therefore, one can identify the cell type undergoing such a change with the producer of the hormone which would, in normal cases, stimulate the target organ removed in this experiment.

Such ablation of target organs was repeatedly performed by light microscopists, and the early experiments with the electron microscope in search of gonadotrophs and thyrotrophs was also carried out using this method of identification (Farquhar and Rinehart 1954 a, b). The producer cell of ACTH was also identified by means of electron microscopy of the altered structure in the anterior pituitary of the adrenalectomized rat (Siperstein and Allison 1965, Kurosumi and Kobayashi 1966).

3. Administration of hormones

Many kinds of hormone extracted from animal bodies were purified, and the chemical structures of some hormones were already determined. Furthermore, some natural hormones and similar substances with hormonal activity were artificially synthesized and became available commercially. The administration of selected hormones alters in both function and structure of a certain pituitary cell type, which is related functionally to the hormone administered. Minutia of the hormone-induced changes in the cell structure may be observed with the electron microscope and used for identification of cell types, if the physiological effect of the administered hormone is well known.

For this sort of experimentation, three kinds of hormones can be used: 1) the pituitary hormone, 2) the hormone of the target organ, 3) the hypothalamic releasing factor. Among these the hormones of the target organs were most frequently used,
because they are simple in chemical structure and easy to synthesize artificially. The effect of these peripheral hormones is inhibitory upon the pituitary cells, because they act as a former of the negative feed-back. On the other hand, the releasing factor has promoting action on the corresponding pituitary cells, and the result of RF administration is the same as the ablation of the target organ (unpublished data of the author's laboratory). The pituitary hormone itself may act as a depressor, as the short circuit feed-back mechanism by certain pituitary hormones was recently reported. However, no report was so far published on the electron microscope studies using pituitary hormones.

4. Various physiological conditions

Naturally occurring variations in the physiological conditions of intact animals without the administration of chemicals are good material for the studies on the anterior pituitary. Each stage of the estrous cycle, pregnancy, delivery and lactation, the hibernation and arousal from it in certain hibernating animals, and various stages in growth and maturation are accompanied with fine-structural alterations in the anterior pituitary gland. These are useful experiments performed by nature itself for the functional identification of certain cell types of the anterior pituitary; for example, Barnes (1962) identified FSH-, LH- and LTH-producing cells in the mouse pituitary after careful observations of each stage of the sexual cycle and pregnancy.

5. Various pathological conditions

Spontaneous or experimentally induced pituitary tumors are also good specimens for cytological and morphophysiological studies of the anterior pituitary gland. Some reports on the fine structure of the human pituitary tumors were published (Gusek 1962, Schelin 1962, Fukumitsu 1964, Kurosumi 1968), but the cell identification is quite difficult in such human specimens, unless clinical data on various endocrine activities in the patient body are thoroughly available. The estrogen-induced pituitary tumor of the rat consists chiefly of cells with characteristic structure of the mammotrophs (Hymer et al. 1961, Shimazaki et al. 1962, Schelin et al. 1964, Kurosumi et al. 1968), and this morphological finding coincides with the physiological data that this tumor-bearing animal shows a conspicuous development of the mammary glands.

Congenital malformations of the anterior pituitary in certain laboratory animals are sometimes reported. Hereditary dwarf mice have no somatotrophs (growth hormone-producing cells) in their pituitary glands as observed with both the light and electron microscopes.

6. Antotransplantation and tissue culture

It is known that an autograft of the anterior pituitary placed in abnormal places of the body such as the subcapsular region of the kidney continuously secretes prolactin; none of other hormones is produced and released from the graft. All the cell types other than the prolactin-producing cells (LTH-cells) are stimulated by the hypothalamic releasing factors (RF) specific for each cell type in the normal state. If the anterior pituitary is removed from the normal place which is associated with the hypothalamus via the hypophyseal portal system, most cells of the pituitary
The electron microscopy of such a prolactin-producing autograft indicated the ultrastructural characteristics of the mammotrophic cells (LTH-cells) (RENNELS 1962).

The tissue or organ culture of the adenohypophysis in a medium containing RF or IF (hypothalamic extract having these effects) was tried. Electron microscopy of such a cultured tissue may elucidate the cell types grown in the culture, but no report has been published, because none of RF or IF was purified in sufficient amount to be used for such a study.

7. Immunohistological studies

Fluorescent antibody method was applied to the anterior pituitary gland in search of ACTH-cell by light microscopy (MARSHALL 1951, LEZNOFF et al. 1962), but more detailed studies with the electron microscope using such immunohistological methods are required. Instead of a fluorescent substance, ferritin or peroxidase is conjugated to the antibody against a certain pituitary hormone for the use in electron microscopy. A precipitated ferritin-conjugated antibody upon the structure containing the antigen (hormone) is easily recognizable by the high density of ferritin, and likewise a peroxidase-conjugated antibody is also demonstrated under the electron microscope after incubation in the peroxidase-detecting media. These new immunohistological methods are expected to solve some unsettled debates on the cell identification of the pituitary as observed by electron microscopy. The results of these studies will be published by many laboratories soon.

8. Correlated electron microscopy and bioassay of granule fraction

The secretory granules which are thought to contain the hormones are almost uniform in size in each cell type and are distinguishable from the granules of other cell types. Based on this fact, differential centrifugation of secretory granules of certain animal pituitaries by the sucrose density gradient method was performed. Each granule fraction obtained by this method consists of the secretory granules of one cell type of almost uniform size. Such isolated granule fractions were assayed by the hormonal activity on one hand, and on the other hand they were fixed, dehydrated, embedded and sectioned for electron microscopy. The characteristic size and shape of granules of a given fraction were compared with the morphology of the secretory granules in the tissue section. These correlated electron microscopic and physiological (biochemical) studies showed a good accordance with the results of the experimental morphological studies by the methods enumerated above (HYMER and McSHAN 1963).

Morphological Characteristics of Each Cell Type of the Rat Anterior Pituitary

Since the rat anterior pituitary has been most frequently observed and best known as to ultrastructure, the following descriptions are concentrated on the
Fig. 1. Survey electron micrograph of the normal rat anterior pituitary. Labelling coincides with the abbreviation of hormone produced by the corresponding cell. *FC* follicular cell, *BC* blood capillary. Fixation: glutaraldehyde osmium mixture. × 3,600
Fig. 2. Survey electron micrograph of the normal rat anterior pituitary. Labelling is the same as in the previous figure. Fixation: osmium tetroxide. $\times 3,600$
findings in the rat anterior pituitary gland. Structurally and functionally, the anterior lobe is most complicated in the pituitary gland, because it contains seven different cell types as observed with the electron microscope. Only one of them is thought to be non-secretory, for cells of this type contain no secretory granules, and are called the follicular cells, as they are so arranged as to form follicles (Fig. 1, 2). The other six cell types can be correlated with the six known hormones of this gland, and therefore it may be said that one pituitary cell type produces one hormone.

1. Somatotroph or STH-cell

Cells of this type, which are known to secrete the growth hormone (STH) and coincide with the typical acidophils of light microscopy, are most frequently observed in both the male and female anterior pituitaries. They are medium-sized and are shaped round, oval or polygonal. Round dense secretory granules about 350 mµ in diameter almost fill the entire cytoplasm (Fig. 3). The electron density of these granules is high and less variable after double staining with uranyl acetate and lead hydroxide, but they often appear to be less opaque, when the section was stained with lead alone. The internal texture of the granule is homogeneous.

Sometimes, one can observe intermingled small secretory granules less than 200 mµ with the usual large granules. It is not yet determined whether or not these cells are different in function from the usual somatotrophic cells.

The rough-surfaced endoplasmic reticulum usually consists of randomly oriented, flat or slightly dilated cisternae (Fig. 3), but sometimes it appears as well-developed parallel lamellae (Fig. 4). The Golgi apparatus is well formed in the latter case, and consists of many vesicles and vacuoles, but the lamellae are few. The elaboration of the secretory granules is seen in some of the cisternae of the Golgi apparatus. The budding of small vesicles from the cisternae of the rough endoplasmic reticulum towards the Golgi apparatus is frequently observed. The mitochondria are rather stout and shaped globular or like short rods. The mitochondrial cristae are moderate in number, and the intramitochondrial granules are either very few or absent at all. This cell sometimes contains rather many lysosomes (Fig. 4). They are round, oval or somewhat irregular in outline and contain a matrix of variable density with characteristic myelin figures.

The extrusion of secretory granules is performed by the way known as type IV (Kurosuni 1961) or reverse pinocytosis. After thyroidectomy, secretory granules of the somatotrophs are vigorously discharged, and this cell type sometimes becomes difficult to be distinguished from the non-granulated follicular cells. In the pituitary of young animals, the secretory granules of this cell are few in number and arranged along the cell surface. This may be brought about by the high degree of granule discharge in this stage of growth, which requires a great amount of growth hormone liberated.

On the other hand, Yamada et al. (1967) reported that the somatotrophs of a mouse of diabetic strain showed an abundance of secretory granules and the scarcity and irregular arrangement of the endoplasmic reticulum. This may be brought about by the inhibition of the discharge of the growth hormone due to the hyperglycemia in this animal.
Fig. 3. Two somatotrophs (STH) of the normal rat pituitary are illustrated. They contain elliptical nuclei, a number of large round secretory granules of high electron density and a few mitochondria. Fixation: osmium tetroxide. $\times 13,000$
Fig. 4. Part of a somatotroph of the normal rat. The rough ER and Golgi apparatus (G) are well developed. Moderate numbers of mitochondria (M) and lysosomes (L) are observed, but the secretory granules are relatively few. Fixation: osmium tetroxide. × 14,000
2. Mammotroph, luteotroph or LTH-cell

Cells of this type, which correspond to another kind of acidophils as observed by light microscopy, secrete prolactin or LTH. They are found abundantly in the female pituitary gland, but sparsely in the male organ. The size of the cell in the normal state is about the same as that of the somatotroph. The cell body is shaped round, oval or polygonal. This cell type is characterized by the secretory granules of irregular or elliptical shape and of large size. They are most frequently elliptical or dumb bell-shaped, but sometimes much more irregular in shape (Fig. 5). Spherical forms mingled with irregular ones are merely cross-cut profiles of elliptical or cylindrical granules, and only a few of them are thought to be actually spherical in three-dimensional shape. The size of these granules is the largest among secretory granules of various cell types of the anterior pituitary, and measures about 700 m\(\mu\) in maximum length. Generally speaking, the granules situated in the Golgi apparatus are smaller in size, but they are also irregular in shape. It is hardly considered that the irregularity in the granule shape of this cell type may be brought about by the fusion of primarily round granules, because the youngest granules in the Golgi cisternae are already irregular in shape.

The rough-surfaced endoplasmic reticulum and the Golgi apparatus of this cell are well developed in general, but show the tendency to atrophy after progesterone administration. The secretory granules are strongly accumulated in this state, because progesterone may suppress the discharge of granules (Fig. 5).

On the other hand, the administration of estrogen causes an opposite effect in the mammotroph, namely the rough endoplasmic reticulum is enormously hypertrophied and forms whorled or parallel-arranged stacks of rough-surfaced membranes and fills almost all the spaces in the cytoplasm, but the secretory granules are mostly discharged away and only a few of them remain within the cytoplasm (Fig. 6).

Such hypertrophic changes are much more remarkable when estrogen is administered continuously for a long time, and the anterior pituitary enlarges markedly and finally forms a kind of tumor. Most cells of such estrogen-induced adenoma of the anterior pituitary have a well-developed rough endoplasmic reticulum and irregularly shaped granules, both of which are the salient features of the mammotroph. The animal bearing such a pituitary tumor possesses more developed mammary glands as compared with controls, suggesting a hypersecretion of prolactin. As mentioned above, the identification of this prolactin-secreting type of cell as such was established by the observation on such artificially induced tumors (Hymer et al. 1961) and by that on the autograft transplanted in the renal capsule where only mammotrophs can survive (Renells 1962). Studies on the anterior pituitaries of female animals in different stages of the sexual cycle and in pregnancy reinforced the view that the cell containing irregular granules is the mammotroph (Barnes 1963). The so-called pregnancy cells which were thought to be the chromophobes by light microscopists are actually the degranulated and hypertrophied mammotrophs, that are faintly stained or not tinted at all due to the poverty of stainable secretory granules.

The production of secretory granules in the Golgi apparatus and their extrusion by the mechanism of reverse pinocytosis (type IV extrusion) were demonstrated in mouse mammotrophs (Sano 1962).
Fig. 5. A mammotroph of the rat treated with progesterone. The cell is moderate in size, and contains a large number of secretory granules, which may be suppressed in discharge. Golgi apparatus (G) and ER are moderately developed. Fixation: osmium tetroxide. ×14,000
Fig. 6. Mammotrophs of the rat 6 days after a single injection of estrogen. The cell body is hypertrophied and filled with abundance of rough ER. The secretory granules are few in number.

Fixation: osmium tetroxide. ×12,000
3. Thyrotroph or TSH-cell

The thyrotroph in light microscopy is known to be a small basophilic cell with an angular outline. Such a characteristic in cell contour is also detected by electron microscopy. The secretory granules are the smallest in the anterior pituitary and measure 100—150 m\(\mu\) in diameter. Most of them are spherical, but some are spindle-shaped. They are arranged along the cell surface and relatively few in number in the normal anterior pituitary. Most granules are electron dense, but a few less dense.
Fig. 8. Part of a thyrotroph of the rat 3 weeks after thyroidectomy. The cisternae of rough ER are expanded and contain colloid-like substance along with dark spherical granules. Lysosomes (L) are seen at the lower right corner. Fixation: osmium tetroxide. × 23,000

ones are intermingled. The cell organelles such as the rough endoplasmic reticulum, Golgi apparatus and mitochondria are poorly developed in the normal state, and the cytoplasm itself is also small in amount (Fig. 7).

The identification of the cells with ultrastructural characteristics mentioned above with the thyrotrophin (TSH)-secreting cells was performed through the experiments of thyroid ablation. The results of surgical ablation (Farquhar and Rinehart 1954 b,
Saki 1962, Cardell 1964, Kurosumi and Oota 1966), radiothyroidectomy (Lundin and Schelin 1964) and administration of propylthiouracil (Barnes 1963) are essentially identical. Within a few days after thyroidectomy, the thyrotrophs undergo a marked hypertrophy with the well developed Golgi apparatus and rough endoplasmic reticulum, whose constituent cisternae are enormously dilated and contain homogeneous colloid-like substance. The secretory granules are decreased in number. Dense round granules appear in the dilated cisternae of the rough endoplasmic reticulum at about the 10th day after surgical thyroidectomy (Fig. 8). Such intracisternal granules occur only in the thyrotrophs after thyroidectomy, and are not observed in any other cells of the pituitary of the thyroidectomized rat. Neither in the gonadotrophs of castrate pituitary, nor in the corticotrophs after adrenalectomy, are observed such intracisternal granules. Lysosomes are increased in the thyrotrophs of the thyroidectomized animals. It is considered that the accumulation of lysosome substance in the Golgi cisternae may hinder the transport and condensation of secretory substance in the Golgi apparatus, and consequently newly synthesized secretory protein may remain in the cisternae of the rough endoplasmic reticulum. In such a case, the secretory substance may be condensed in the dilated rough cisternae to form dense intracisternal granules.

A slight enlargement and decrease in electron density of the secretory granules occur in the thyrotrophs of the thyroidectomized rat, suggesting incomplete condensation of the secretory substance in the Golgi sacs, as known by observation on the pituitary of the rat thyroidectomized and thereafter administered with thyroid hormone.

During artificial hibernation, the thyrotrophs of the bat contain a great number of secretory granules (Kobayashi and Herman 1966). This does not mean the hyperfunction, but the activity of the thyrotroph is lowered and the discharge of secretory granules may be suppressed. Such a morphological finding coincides well with the data of a physiological examination which showed a decreased I\textsuperscript{131} uptake in the hibernating state.

4. FSH-gonadotroph or FSH-cell

Gonadotrophic hormones secreted from the anterior pituitary are divided into two kinds, i.e., the follicle stimulating hormone (FSH) and the luteinizing hormone (LH). The latter in the male is also called interstitial cell stimulating hormone (ICSH). The third hormone which has an effect on the gonad is prolactin (luteotrophic hormone, LTH). This hormone is sometimes classified in the gonadotrophins; but often excluded from them, because it has a strong extragonadal activity, i.e., stimulation of the mammary gland. Furthermore, the former two hormones have common characteristics, namely they are glucoprotein but the last is a simple protein; the probable producers of the formers are basophilic but that of the last is acidophilic in light microscopic preparations.

It is repeatedly discussed whether FSH and LH are produced from cells of different types, or they are produced in one and the same cell type and secreted alternately. In the rat two different types of gonadotroph were advocated by light microscopists, and it was described that both are different in localization in the anterior pituitary and that the peripheral gonadotrophs produce FSH, while the
Fig. 9. An FSH-gonadotroph of the normal rat containing an irregular nucleus (N), expanded cisternae of ER, slender mitochondria (M) and many secretory granules, some of which are large and less opaque. BC blood capillary. Fixation: osmium tetroxide. × 19,000

As early as 1954, an electron microscopic study was carried out in order to identify the gonadotrophic cells of the anterior pituitary, and two types of gonadotrophs were indicated in the pituitary of castrate rats (Farquhar and Rinehart 1954 a). In that report, however, these authors did not show the fine-structural characteristics of the two types of gonadotroph in the normal pituitary. Later Farquhar demonstrated certain electron micrographs and supplied brief descriptions concerning the cell identification of the rat pituitary in a review of Purves (1961). In this series of illustrations, she divided gonadotrophs into two types: one was rich in expanded cisternae of the rough endoplasmic reticulum and contained evenly distributed secretory granules, while the other was characterized by flocculent clear areas scattered among dark secretory granules. She thought that the former might be FSH-secreting cell and the latter LH-secreting. Rennels (1963) reappraised this problem and demonstrated two types of gonadotrophs in the rat pituitary. These were essentially the same as those which appeared in the review of Purves (1961) and were explained as two functionally different types of gonadotrophs by Farquhar. Rennels (1963), however, did not draw any conclusions as to the functional significance of these two types of gonadotrophs.

On the other hand, Barnes (1962) showed two functionally and morphologically distinct gonadotrophic cell types each indicating production of FSH and LH in the mouse as a result of observations on the anterior pituitaries in various stages of estrous cycle and in pregnancy. As recent studies on the pituitaries of persistent estrous and diestrous rats (Kurosumi and Oota 1968) showed a good accordance with the concept of Barnes (1962, 1963), the author wants to adopt the cell identification of gonadotrophs proposed by Barnes.

The cell identified as the FSH-producer by the criteria according to Barnes (1962) is the so-called gonadotroph or delta cell of the light microscopic designation, and is a large round cell occurring along the blood capillary (Fig. 1, 2). The FSH-cell contains many secretory granules widely variable both in size and electron density. The granules are often classified into two classes, one is larger in size and tends to be lower in density and the other smaller and denser (Fig. 9). Granules of the first class are about 0.7—1 \( \mu \) in diameter and are often seen as round flocculent areas without membranes when fixed with osmium alone, but the fixation with glutaraldehyde accompanied either simultaneously or successively with osmium demonstrates relatively denser content and well preserved membranes (Fig. 10). Secretory granules of the smaller type are about 200 m\( \mu \) in diameter and regularly spherical. Their interior is fine granular in either fixation and this texture is more remarkable in granules with less dense content. The classification of granules into these two types is for convenience' sake and is superficial, because one can observe the transition between the larger and smaller granule types and it is difficult to separate them sharply. It is evident that the secretory granules are produced in the Golgi apparatus irrespective of their size.

The cisternae of the rough endoplasmic reticulum are moderately dilated, and their content appears transparent after osmium fixation but it is dark after fixation with mixed glutaraldehyde and osmium tetroxide (Fig. 1, 2, 9, 10). The mitochondria are slender having the cross diameter of about a half of that of the mitochondria in
the somatotroph. They are provided with many cristae arranged in a transverse direction and a considerable number of intramitochondrial granules (Fig. 9).

After castration, the endoplasmic reticulum undergoes a strong dilatation and an accumulation of internal substance which is amorphous and homogeneoucys. The faint filamentous texture seen in the intracisternal substance either of normal or castrate pituitary may be the precipitates of the proteinaceous substance brought about by fixation. No intracisternal granules appear in every stage of the castrate. Expanded cisternae may fuse with one another and a large space occurs at about the center of such a changed FSH-cell. The nucleus and cytoplasmic matrix containing organelles and secretory granules are pushed away towards the periphery of the cell, forming an odd shape like a signet ring (Fig. 11) (Farquhar and Rinehart 1954a, Ichikawa 1959, Yoshimura and Harumiya 1965, Girod and Dubois 1965).

Large clear granules seen in the normal FSH-cell almost disappear from the FSH-cell in the castrate, but the size of smaller granules is slightly increased and the content of these granules becomes a little less dense, implying the incomplete condensation of secretory granules in the Golgi apparatus in such a pathological condition.

None of the features of granule discharge suggesting the mechanism of reverse pinocytosis (type IV extrusion of Kurosumi 1961) was observed in either normal or castrate FSH-gonadotrophs, and consequently a possibility of diacrine secretion or

Fig. 10. Part of an FSH-gonadotroph of the normal male rat. The limiting membranes of secretory granules are relatively well preserved. Note the variation of size and electron density of the granules. The content of the rough ER appears dark. Fixation: glutaraldehyde osmium mixture. \( \times 46,000 \)
Fig. 11. A signet-ring cell (FSH-gonadotroph) of the female rat 12 weeks after ovariectomy. The nucleus (N) is pressed away by a large cavity which is made by fusion of expanded cisternae. Fixation: osmium tetroxide. × 6,000
transmembranous permeation (type V extrusion) might be suggested.

Female rats which received daily injections of estrogen for 30 days beginning immediately after birth become persistent diestrous after they have grown up. Survey light microscopy indicates that the ovaries of such persistent diestrous rats are very small and contain neither Graafian follicles nor corpora lutea. The FSH-gonadotrophs are very few and atrophic in the anterior pituitary of such animals. LH-cells of this animal are almost normal in structure (Kurosuni and Oota 1968). Good agreement of the electron microscopic findings in the anterior pituitary with those of light microscopy of the ovaries clearly indicates that the decrease in FSH secretion is the only change of the pituitary in these persistent diestrous rats and that the identification of gonadotrophs by Barnes (1962) holds correct.

5. LH-gonadotroph or LH-cell

The LH-cell identified first in the mouse by Barnes (1962) is also found in the rat pituitary, having the same morphological characteristics in both species. The LH-cell is medium-sized and often situated along the blood capillary. The cell body is usually round or polygonal, but sometimes appears to be irregular. Secretory granules about 250 mμ in diameter are regularly spherical and mostly electron dense, but some less dense granules may be mingled. The interior of the granules is always homogeneous, and no granular texture as seen in FSH-cell granules can be observed (Fig. 12). The accumulation of granules at one pole of the cell facing the blood capillary was reported (Kurosuni and Oota 1968). Most of the mitochondria are rod-like, but a few are spherical. They are in the same range in size as the mitochondria of STH-cells. The development of the rough-surfaced endoplasmic reticulum and the Golgi apparatus is moderate or rather weak. The rough cisternae are randomly scattered among the secretory granules.

The secretory granules and other structural components of LH-cells resemble those of STH-cells, but the granule size of the LH-cell is always smaller than that of the STH-cell. Moreover, the STH-cells undergo marked degranulation after thyroidec- tomy, whereas the LH-cells are indifferent to this experimental condition. After an injection of progesteron, the secretory granules are strongly accumulated in the cytoplasm (Fig. 12). The same tendency was observed in the late stage of pregnancy. On the other hand, injection of estrogen causes a depletion of granules (Fig. 13). In this state, the cell organelles are relatively well preserved, but the secretory granules are decreased in number and margination of granules (preferential localization of granules along the cell surface) is remarkable. These morphological features well coincide with the physiological fact that estrogen may promote the release of LH from the pituitary (Kobayashi et al. 1968).

Changes in LH-cells after castration are less remarkable than those in FSH-cells, but castrate LH-cells are hypertrophic in every respect; namely the cell body is enlarged, somewhat irregular in shape, and contains relatively well developed endoplasmic reticulum, though the cisternae are not dilated. The secretory granules are relatively small in number, and not marginated but distributed rather evenly through the cytoplasm. It is remarkable that, in this state, many features of granule discharge by the mechanism of reverse pinocytosis can be observed. This finding may be reflected to the high activity of this cell after the removal of the
As mentioned above, LH-cells were almost normal in the pituitary of the persistent diestrous rat, when FSH-cells were strongly atrophic; but in the persistent estrus of the rat which was produced by administration of a relatively small amount of estrogen for 5 days after birth, LH-cells showed an extraordinary accumulation of secretory granules suggesting a high degree of inhibition in the discharge of granules, while FSH-cells remained almost unchanged (Kurosuni and Oota 1968). Absence or shortage of LH in the blood may cause a failure in the initiation of ovulation, though the follicles can grow by the stimulation of FSH. This condition may bring the state of persistent estrus in these neonatally treated rats.

The possibility that the two kinds of gonadotrophic hormone, FSH and LH
may be derived from different cell types is very likely as described in this chapter, but another possibility that, e.g., the FSH-cell may produce a small amount of LH along with the main product of this cell, i.e., FSH, and a similar condition in the LH-cells cannot be completely excluded.

6. Corticotroph or ACTH-cell

For many years, a great number of attempts were made to relate ACTH secretion to some one of the cell types demonstrated by stainings for light microscopy. Among many researchers, some claimed that ACTH might be secreted by acidophil cells (FINERTY and BRISENO-CASTREJON 1949), whereas others reported that the origin
of ACTH seemed to be basophils (Smelser 1944). Marshall (1951) demonstrated the localization of ACTH in basophils of the hog pituitary by the fluorescent antibody method, and Leznoff et al. (1962) indicated the presence of ACTH in some of the basophils of the human pituitary, using the same technique with more highly purified antigen.

Then Siperstein (1963) used autoradiographic technique in light microscopy and identified a unique cell type in the anterior pituitary of adrenalectomized rats. This adrenalectomy cell had the fastest rate of protein turnover following adrenalectomy and was assumed to be the site of ACTH production. She suggested that the adrenalectomy cell might be derived from chromophobes.

These controversies concerning the cellular source of ACTH, which could not be solved by light microscopy, were handed over to electron microscopists. In 1957, Farquhar described cells she presumed to be corticotrophs as containing no secretory granules and few formed elements and encircling follicles or ductules filled with colloid-like substance. This cell type, now called follicular cell, is known not to be changed after adrenalectomy and seems to have other functions than ACTH production. Herlant and Klastersky (1963) demonstrated the so-called corticotroph in the chronically stressed rat, but the cell they showed cannot be distinguished morphologically from the thyrotroph.

Rennels (1964) studied electron microscopic alterations in the rat anterior pituitary after scalding, which has been known to cause a discharge of a great amount of ACTH stored in the pituitary. In spite of careful observations, he failed to find a marked loss of secretory granules from any type of granulated pituitary cells. Then, Siperstein and Allison (1965) observed the rat anterior pituitary following adrenalectomy with the electron microscope, and identified the so-called adrenalectomy cell which was previously demonstrated by the senior author with a technique of light microscopic autoradiography (Siperstein 1963). They reported, however, that neither adrenalectomy cells nor corresponding cells were found in the pituitary of the normal rat.

The author and his co-worker (Kurosumi and Kobayashi 1966) found a new type of granulated cell in the pituitaries of both normal and adrenalectomized rats. This cell type is characterized by small secretory granules about 150—200 m\(\mu\) in diameter which are often seen as vesicles with a dark central core. This cell is relatively large in size and extends several cytoplasmic processes (Fig. 14). As this cell type undergoes marked changes after adrenalectomy, it was suggested for this cell to be the producer of ACTH and called corticotroph. The most striking characteristic of this cell type is a wide variety in form and electron density of its secretory granules: namely, some of them are solid dense granules like those of the thyrotroph, but some others are quite less dense and look like empty vesicles or those with a central core (Fig. 15). Such characteristic morphology of secretory granules depends upon the cell function and preparation technique. If the ACTH-cell is stimulated by a strong stress, solid dark granules increase, while vesicular granules predominate in the cell several days after adrenalectomy (Fig. 16) (Kurosumi and Kobayashi 1966l). On the other hand, the secretory granules of ACTH-cells fixed with osmium alone are mostly vesicular with or without cores, whereas fixation with glutaraldehyde and osmium makes many granules solid (Fig. 17).
Fig. 14. A corticotroph (ACTH) of the normal rat anterior pituitary. The cell body is large in size and sends out some processes (arrows), one of which abuts on the blood capillary (top of the figure). Fixation: osmium tetroxide. × 12,000
Fig. 15. Part of a corticotroph illustrated in the previous figure. The nucleus (N) is seen at the lower right corner. Most of the secretory granules appear to be cored vesicles. Fixation: osmium tetroxide. × 31,000

Fig. 16. Part of a corticotroph of the rat 5 days after adrenalectomy. Most secretory granules are vesicular. The cisternae of rough ER are dilated and contain dark homogeneous substance. N nucleus. Fixation: osmium tetroxide. × 21,000
The most characteristic change of this cell after adrenalectomy is the accumulation of a colloid-like substance in the cisternae of the rough endoplasmic reticulum (Fig. 16). This corresponds to the changes in the thyrotroph after thyroidectomy or in the gonadotroph after castration. It is well known that the hormones of the anterior pituitary are protein, and are produced by ribosomes attached to the outer surface of the membranes of the rough endoplasmic reticulum. Newly synthesized protein may accumulate in the cavity of the endoplasmic reticulum. Therefore, the strong accumulation of a substance within the rough cisternae may indicate preceding accelerated production of secretory protein. High protein synthesis in the corresponding cell after ablation of the target organ is known through the biochemical examination, and well coincides with the finding by electron microscopy.

On the contrary, a continuous administration of adrenocortical hormones to the experimental animal for several days may induce changes of pituitary corticotrophs as considered to be hypofunctional and atrophic: cisternae of the rough endoplasmic reticulum are collapsed and secretory granules are strongly accumulated as a result of the suppression of discharge (Fig. 17).

Corticotrophs in the anterior pituitaries of gonadectomized or thyroidectomized rats were observed with electron microscopy (Kurosumi and Oota 1966). They showed slight changes after castration or thyroidectomy: the chief changes were the increase in number of secretory granules especially those of solid ones, but the endoplasmic reticulum was almost unchanged as compared with the cells after ablation.
of their own target organs. As the changes in the corticotrophs are always less marked than those in the gonadotrophs or thyrotrophs after gonadectomy or thyroidectomy, respectively, it is evident that the corticotroph is an independent cell type which is different from both the gonadotroph and thyrotroph.

Hymer and McShan (1963) obtained good evidence of the hormonal activities of separated granule fractions which were related to the respective cell types of the rat pituitary by electron microscopy. They found the ACTH activity in association with a particulate fraction which contained microsomes and small granules less than 140 \( \mu \)m. This size is comparable with the cores of secretory vesicles in the corticotroph identified by Kurosumi and Kobayashi (1966). In a subsequent similar study, Perdue and McShan (1966) found that ACTH was associated with a pellet of granules having maximum diameters of 140 and 200 \( \mu \)m, which were related with TSH and gonadotrophins, respectively. Though the precise diameter of ACTH granules was not shown in this study, this result clearly coincides with the morphological study of the rat corticotrophs which have the secretory granules 150—200 \( \mu \)m in diameter (Kurosumi and Kobayashi 1966).

On the other hand, Yamada and Yamashita (1967) studied the mouse pituitary and discovered a new cell type which had not been described previously in this animal by Barnes (1962, 1963) or any other researcher. Vesiculated secretory granules are one of the most remarkable characteristics of this cell type and are consistent with the same characteristics of the rat corticotrophs. They observed the salient response of this cell to the adrenalectomy and hydrocortisone administration; the change in the former experiment was reverse to the latter and this cell was identified as the ACTH producer. In the same animal, Wu (1967) also demonstrated a specific response of ACTH-cells to a severe cold stress.

7. Follicular cell or non-granulated cell

Only one type of the anterior pituitary cells contains no secretory granules and this type is thought not to produce hormones but to be an undifferentiated or immature type of glandular cells.

They are arranged to encircle a relatively narrow intercellular space which is either empty or filled with colloid-like substance. Microvilli are extended into this space from the surface of surrounding cells, and terminal bars (junctional complexes) adjoin these non-granulated cells with each other (Fig. 18). As such an arrangement of cells is reminiscent of follicles found in certain endocrine glands such as the thyroid, these cells are called follicular cells. However, the contour of such a follicle is not smooth and is not covered with the basement membrane. The periphery of such a follicle is thus complexly intermingled with other types of cells, and such a cell mass may be more reasonably called a pseudofollicle.

The follicular cells are rather small in size and contain a small amount of cytoplasm with few cisternae of the rough endoplasmic reticulum but an abundance of free ribosomes. The Golgi apparatus is localized in the region between the nucleus and the cell surface facing the follicular lumen. Cilia can be observed occasionally on the luminal surface.

Barnes (1961) first described the fine structure of cilia which occurred in the anterior pituitary. She noted that the pituitary cilia possess a 9 + 0 fibril pattern,
Fig. 18. Follicular cells of the normal rat anterior pituitary. Microvilli and a cilium (arrow) extend into the narrow follicular cavity ($F$). Fixation: osmium tetroxide. $\times 8,000$
and assumed their function to be sensory. Cilia may occur in any type of the pituitary cells, but most frequently they are associated with the follicular cells. The complete motile cilia with the typical 19+2 fibril pattern are observed in connection with follicular cells in the rat pituitary, though some 9+0 cilia are also found especially in other cell types. The sensory theory of the pituitary cilia is weakly founded. They are probably rudiments of the Rathke's pouch which is lined with ciliary pharyngeal epithelia and inefficient in the adult stage.

In the follicular cells a few slender mitochondria are contained. Irregularly formed dense bodies probably one of the lysosomes are observed. No secretory granules are contained in the follicular cells, but in very rare cases a few granules are observed. This may indicate the possibility of transition from the follicular cell into other granulated cells.

In the dog pituitary gland, Kagayama (1965) showed the follicular cells extend-

![Fig. 19. A diagrammatic representation showing the ultrastructural characteristics of all the cell types in the normal rat anterior pituitary.](image-url)
ing long cytoplasmic processes among parenchymal cells and sometimes containing a small number of granules 150—200 μ in diameter. The follicular cells were assumed to be the producer of ACTH by FARQUHAR (1957), and RENNELS (1964) indicated that these cells were the only cells which showed recognizable changes after scalding. The size of granules occasionally found in the follicular cells is in the same range as those of the corticotroph. Therefore, it may be presumed that the follicular cells are capable of changing themselves into the ACTH-producing cells in such a case of emergency as exposure to strong stressful stimuli.

As the follicular cells contain no secretory granules as a rule, they appear in light microscopic preparations as unstainable cells and reasonably called chromophobes. In the dog pituitary, however, KAGAYAMA (1965) demonstrated another cell type called chromophobe cell as it contains no granules and quite different from the follicular cells. Till now no cells corresponding to the dog chromophobes could be found in the rat anterior pituitary.

The so-called chromophobes in light microscopy are considerably high in frequency of occurrence and were called chief cells by some histologists, but the follicular cells or non-granulated cells are very few in electron microscopic fields. This discrepancy may be based on the fact that cells with a few granules are judged “unstained” in light microscopic preparations, whereas they are classified into one of the granulated cells as the secretory granules, though sparse, can be detected by electron microscopy. The “chromophobes” in the light microscopy may thus include, besides follicular cells, many types of granulated cells in degranulated or immature stages.

In Figure 19, morphological characteristics of each cell type of the rat anterior pituitary as seen with the electron microscope are diagrammatically illustrated.

Summary

Recent progress in endocrine physiology showed six different hormones secreted from the anterior pituitary gland, but their cellular sources were not completely elucidated, when the morphological technique was confined to the light microscopy. Application of the electron microscope is very advantageous to the field of functional morphology of the pituitary gland. Within a few years, the identification of cellular sources of the six known hormones of the anterior pituitary has been established in the rat and mouse.

Methods for determination of endocrine function of each cell type distinguished by electron microscopy are discussed in detail. Some of them have been fully utilized by electron microscopists who are engaged in functional morphology of the anterior pituitary gland, but a few remaining ones are still in the trial stage.

The nomenclature of the anterior pituitary cells indicating the morphological characteristics is now replaced by a new terms implying the function, namely indicating the hormone which is secreted by a given cell type.

As the rats have been most frequently observed and best known as to function, the description of this paper is concentrated to the rat anterior pituitary. The electron microscopical features of each of the seven cell types in either normal, pathological or experimental condition are described, and some representative electron micrographs are illustrated.
電子顕微鏡による下垂体前葉の機能的細胞分類（内容自抄）

近年、内分泌生理学の進歩によって、下垂体前葉から6種の異なったホルモンが分泌されていることが示されたが、形態学的研究の技術が光学顕微鏡に限定されていた間は、これらの前葉ホルモンの源流となる細胞は、まだ完全には明らかにされていなかった。電子顕微鏡の応用は下垂体の機能的形態学の領域にとって非常に有用である。この数年間にラットおよびマウスにおいて、下垂体前葉の6種の既知のホルモンについて、それぞれの産生細胞の同定が確立された。

電子顕微鏡によって区別される各細胞型の内分泌機能を決定する方法が、詳細にわたって考察された。その方法の中の若干は、下垂体前葉の機能的形態学の電子顕微鏡的研究に充分に利用されているが、次三の方法はまだ試行の段階に過ぎない。従来から用いられていた形態学的特徴を指示する下垂体前葉細胞の命名法は、今や機能を示唆する新しい名称、すなわちそれぞれの細胞によって分泌されるホルモンを示す名称によっておきかえられつつある。

ラットはこの種の研究のために最もしばしば観察されており、その機能について最もよく知られているので、本論文の記載はラットの下垂体前葉の所見を中心とした。正常、病的、あるいは実験的条件下における下垂体前葉の7種の細胞型の各々の電子顕微鏡像が記載され、若干の代表的電子顕微鏡写真が図示されている。

References


Gusek, W.: Vergleichende licht- und elektronen-mikroskopische Untersuchungen menschlicher
Cell Types of the Anterior Pituitary 361


Rennels, E. G.: An electron microscope study of pituitary autograft cells in the rat. Endocrinology


Wu, W.-D.: Light and electron microscopic observations on cytological changes in the anterior hypophysis of the mouse following exposure to cold. Fol. anat. jap. 43 : 263–289 (1967).

