A postnatal histogenetic study of the anterior pituitary of the mouse

By

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Introduction

During the half century that has elapsed since the reports of Fleisch (1884) and Schönemann (1892), studies dealing with the anterior pituitary have been directed toward a determination of distinct cell types based on morphological and physiological viewpoints, and it has come to be generally believed that the parenchymatous cells of the anterior pituitary are composed of three cell types (acidophile, basophile and chromophobe cells).

Romeis ('40) published the results of an extensive study of the anterior pituitary of several species. Employing the kresofuchsin-azan stain of Berblinger and Burgdorff ('35), he differentiated five cell types and, following a suggestion by Bailey and Davidoff ('25), designated them alpha through epsilon. Goldberg and Chai-koff ('52) supplemented the sixth cell type in the dog and named it "zeta-cell." Dawson and Friedgood ('38) and Dawson ('46) working on the anterior pituitaries of female rabbits and cats divided acidophiles into carminophile and orangeophile cells, but they failed in determination of these two types of acidophiles in the rat and mouse.

In recent years, two new staining techniques which have contributed greatly to developments in the field of pituitary histochemistry were reported successively: namely, the aldehyde-fuchsin and periodic acid-Schiff staining methods. The former devised by Gomori ('50) is an elastic stain in combination with a modified azan technique. After application of this method, Halmi ('50 and '52) differentiated clearly two types of basophiles (Gomori-positive beta- and negative
delta-cells) in the rat and mouse, and suggested that the beta- and delta-cells are correlated respectively with thyrotrophic and gonadotrophic functions ("51). The latter technique is staining with periodic acid followed by Schiff's reagent. This technique was established by McManus ("46) and elaborated by Hotchkiss ("48), and has been applied with conspicuous success by Catchpole ("49), Pearse ("49, '50, '52a, '52b, and '53) and Purves and Griesbach ('51a) in histochemical studies of the anterior pituitaries of various species. Purves and Griesbach ('51a, '51b and '51c) stated that in the rat pituitary there are two types of PAS positive cells designated as "gonadotrophs" and "thyrotrophs", and that the cells classified by Halmi as delta-cell include all gonadotrophic basophiles plus those thyrotrophic cells with low hormone content which are unstained by the Gomori stain. Later, the presence of these two types of basophiles in several animals was ascertained by many investigators (Yasuda, '53, Legait and Legait, '54; Wilson and Ezrin, '54; Ukei, '56; and others). In addition, it was advocated that in the rat anterior pituitary the gonadotrophs consist of central and peripheral ones, which are associated respectively with luteinising and follicle stimulating secretions (Purves and Griesbach, '54 and '55).

There are in addition, many reports on histogenetic studies of the anterior pituitary of various laboratory animals, but studies dealing with the mouse anterior pituitary have not been reported so frequently as other rodents, and only few investigations have been made (Yamada, '37a and '37b; Stein, Caldwell and Peters, '42; Baer, '42).

In the mouse, Yamada ('37a and '37b), one of the present authors, reported previously that the parenchymatous cells of the anterior pituitary consist of three cell types (the 1st, 2nd and 3rd types) corresponding respectively to chromophobe, basophile and acidophile cells. It was also pointed out that the chromophobes are further transformable into two types of chromophiles, although there is no mutual transition between the acidophile and basophile cells. Sano ("56), another of the present authors, reported results of a histological study of the anterior pituitary of the normal mature mice. According to this study, two types of basophiles comparable to the beta- and delta-cells reported by Romeis ("40) and Halmi ('50 and '52) were noted, but acidophiles could not be divided into the subtypes mentioned above. He further pointed out that in mature females there are sometimes observed chromophobe cells with an eccentrically situated nucleus and a cap-like structure consisting of many basophilic filaments.
at the cell periphery, and called them "peculiar chromophobe cells" for descriptive purposes.

In the present paper, there are presented results of a postnatal histogenetic study of the parenchymatous cells of the mouse anterior pituitary based on the cell classification described above.

**Materials and Methods**

One hundred and fifty-four mice of both sexes and of ages varying from immediately after birth to 150 days, were used. The young animals were allowed to remain with their mothers till about the 30th day after birth, after which male and female mice were bred separately. The pituitaries obtained were fixed with Zenker-formol-acetic acid and sublimate-formol-acetic acid solutions and embedded in paraffin. The materials were cut serially at four microns in a sagittal plane. Hematoxylin-eosin, Heidenhain's azan, Gomori's aldehyde-fuchsin, periodic acid-Schiff (modified by Coleman) (Coleman, '38) and Unna-Appenhein's methylgreen-pyronin staining methods were employed.

**Observation**

*At birth and 1 day:* At this stage, the majority of parenchymatous cells of the anterior pituitary are chromophobe cells (Fig. 1). Ill-defined, these cells have very little, colorless or faintly orangeophilic cytoplasm and a round or oval nucleus with abundant chromatin particles. The superior region of the anterior lobe adjacent to the residual cleft is almost entirely occupied by these chromophobe cells. There are seen in places groups consisting of several chromophobes which seem to have a naked nucleus (Fig. 1), and such groups have been designated as "Kernhaufen."

Acidophile cells are small in size and seen only in small numbers (Fig. 1). These cells have a somewhat dark nucleus surrounded by few cytoplasmic granules, and there is noted no occurrence of such fully granulated acidophiles seen in adults.

Although, at this stage, it is almost impossible with few exceptional cases, to identify basophile cells in hematoxylin-eosin or azan sections, the presence of cells with glycoprotein containing granules in the cytoplasm is faintly recognizable after application of the periodic acid-Schiff technique. These cells vary in size and form but are smaller
than those in adults, and faintly pink staining fine granules are seen distributed sparsely in the cytoplasm. There are occasionally observed cells with strongly PAS positive droplets in the cytoplasm. In sections stained by Gomori’s aldehyde-fuchsin stain, almost all cells remain unstained.

At 2 to 4 days: There occurs an increase in number and size of acidophiles. It can with much difficulty be pointed out that several basophiles exist in the azan preparations. These cells are oval, polygonal or irregular in shape. They have oval nuclei with abundant dust-like chromatin particles and a conspicuous nucleolus, possessing fine, grey staining granules in the cytoplasm.

At 5 and 6 days: The increase in number and size of acidophiles proceeds further, but their characteristic features are similar to those described before.

Basophiles indicate an increase in number and size as in acidophiles, and are distributed in the anterior and central portions of the anterior lobe. In aldehyde-fuchsin preparations, however, only one or two cells with fine purple granules around the nucleus are found in some sections.

Chromophobe cells positively exceed in number the other cell types, and many “Kernhaufen” are seen here and there.

In methylgreen-pyronin sections, there are found many cells with delicated filamentous or granular pyroninophile materials in the cytoplasm (Fig. 2). These materials, however, are diffusely distributed throughout the cytoplasm in general and do not show the characteristic localizations seen in adults.

At 7 to 9 days: Cytoplasmic granules of basophile cells increase in number, size and stainability for several dyes. Thus, the cells become easily discernible in azan or PAS sections.

At 10 and 12 days: Many acidophiles at this stage possess a round or oval nucleus containing relatively abundant chromatin particles and one or two conspicuous nucleoli, and their nuclei are surrounded by narrow bands of fully granulated cytoplasm (Fig. 3).

Basophiles are found more numerosely than during the first 9 days. They are oval or polygonal in shape and smaller than those in adults. Thie cytoplasm are moderately filled with fine granules, staining with aniline blue or PAS reagent (Figs. 4 and 5). Rarely there are seen some vacuoles in the cytoplasm. Cells with strongly PAS positive droplets in the cytoplasm are occasionally observed. Nuclei of basophiles contain abundant dust-like chromatin particles and reveal some-
what dark casts in general. By utilization of the PAS staining technique, it can be recognized that basophiles are especially concentrated in the so-called “sex-zone” and situated dispersedly in the other portions, as it is able to disclose basophilic granules more markedly by this method than by other staining procedures. In many sections, the presence of several cells with aldehyde-fuchsin positive granules in the cytoplasm is clearly noted at this stage. These cells are situated mainly in the central portion of the anterior lobe. They contain a dark nucleus with abundant, dust-like to granular chromatin particles, and their small cytoplasm are polygonal in form.

Although the so-called “Kernhaufen” is seen at places and a number of chromophobes are provided with a colorless cytoplasm showing an indistinct cell boundary, there are also observed chromophobe cells with a small amount of cytoplasm which contains granular or flocculent materials around the nucleus. The majority of these chromophobe cells have small, round or oval nucleus with abundant chromatin particles and a conspicuous nucleolus.

At 15 and 18 days: All cell types increase in number and size, with their characteristic features similar to those described for 10- and 12-day-old mice.

At 20 days: The increase in number and size of acidophiles proceeds. Many acidophiles are densely packed with intensely acidophilic granules in the cytoplasm (Fig. 6). At this stage, acidophiles with a negative image of Golgi’s apparatus or a perinuclear halo are noted for the first time. Although acidophiles generally have somewhat dark nucleus with abundant dust-like chromatin granules, some possess a light vesicular nucleus with few coarse chromatin particles.

The number of basophiles increases considerably, when compared with other stages described before. Nevertheless the cells appear far less in number than in adults. At this stage, two types of basophile cells described previously (Sano, ’56) can be distinguished at first in each section. Delta-cells are mainly found in clusters in the anterior portion of the anterior lobe (“sex-zone”). They are oval in form, and possess fine PAS positive granules in the cytoplasm and a somewhat dark nucleus showing a delicate chromatin network. Beta-cells which are far less in number than delta-cells are dispersedly located in the central area of the anterior lobe. They indicate an irregular form are their coarse cytoplasmic granules react positively with aldehyde-fuchsin as well as with PAS reagent. Their nuclei which contain densely arranged, abundant chromatin particles show a darker cast than the
delta-cells.

There occurs an increase in number of chromophobes with a slender cytoplasmic band around the nucleus. Rarely there is observed the "peculiar chromophobe cell" reported previously (Sano, '56).

At 30 and 35 days: Acidophiles show considerable increase in number and size, and their cytoplasm are in general densely packed with brilliant acidophilic granules (Fig. 7). The majority of nuclei of these cells appear to be light vesicular as in adults, and cells with a cartwheel-like nucleus are observed in small numbers. There are found at this stage many cells with a negative image of Golgi's apparatus or a perinuclear halo but less in number than in mature mice. Acidophiles with basophilic filamentous materials around the nucleus or at the cell periphery are found in considerable frequency, it not so abundant as in adults (Fig. 8).

Two types of basophiles become clearly distinguishable, as their cytoplasmic granules increase more and more in number, size and stainability for several dyes (Figs. 9 and 10).

At 40 days: At this stage, conspicuous cytological changes take place in the anterior pituitaries of female mice; viz. marked occurrence of delta-cells with hyaline vacuoles in the cytoplasm and of the "peculiar chromophobe cell."

In female pituitaries some delta-cells reveal one, two or more hyaline vacuoles in the cytoplasm (Fig. 11), and their PAS positive cytoplasmic granules are finer when compared with those at 30 and 35 days of age, whereas in males, differing from those in females, delta-cells still show development in number, size and stainability. On the other hand, beta-cells show no difference between the two sexes as regards their cytological appearances, and aldehyde-fuchsin positive cytoplasmic granules increase in number and size when compared with the foregoing stages.

Although many chromophobes reveal an appearance of a naked nucleus and the so-called "Kernhaufen" still remains at places in considerable numbers, chromophobe cells with scanty cytoplasm around the nucleus are seen more frequently than at the beforementioned stages. In the majority of female mice examined at this stage, if not so frequent as in adult females, peculiar chromophobe cells are more or less abundantly recognized here and there (Fig. 12). There is, however, considerable variation in the number of these cells in individual animals. On the other hand, in methylgreen-pyronin preparations, chromophobes with filamentous pyroninophile materials around the
nucleus are found frequently in both sexes.

Many acidophiles contain a light vesicular nucleus, and some have a cartwheel-like nucleus. Cells with a negative image of Golgi’s apparatus or a perinuclear halo are also found frequently. Occasionally there are seen cells with cytoplasmic vacuoles. The occurrence of cells with basophilic filamentous materials showing characteristic localizations in the cytoplasm as mentioned above is as great as in adults.

At 50 days: Acidophiles are somewhat less in number than in adults. Although the majority of them are in general medium in size, there are occasionally found some large acidophile cells which contain a very light vesicular nucleus and a sparsely granulated cytoplasm. Cells with a cartwheel-like nucleus are observed numerously when compared with those at the abovementioned stages.

In females, delta-cells possess in general few fine cytoplasmic granules. Delta-cells with clearly PAS positive granules in the cytoplasm are fewer in number than those described for 40-day-old mice, whereas cells with one, two or more hyaline vacuoles in the cytoplasm are encountered frequently. On the other hand, in male pituitaries there is seen no occurrence of vacuolization or degranulation in the cytoplasm in delta-cells. In males the cells show an increase in number and size. Their cytoplasmic granules become coarser than at the aforementioned stages and are densely packed in the cytoplasm. Thus, at this stage, delta-cells which contain clearly PAS positive cytoplasmic granules in males exceed markedly those in females both in number and size.

Beta-cells do not show any difference between both sexes, and their cytoplasmic granules are stained strongly with Gomori’s aldehyde-fuchsin.

In female pituitaries, peculiar chromophobe cells are found numerously as in adults, and are seen in almost all sections.

At 60 and 65 days: All types of chromophiles of the mouse anterior pituitary, from this stage onward, indicate a fully developed appearance in both sexes (Figs. 19, 20, 21 and 22).

As regards the number of acidophiles, there is recognized no difference between both sexes. Although at this stage many acidophiles contain heavily granulated cytoplasm, there are also observed sparsely granulated cells here and there. Acidophile cells vary in size, and the majority of them consist of medium sized ones. The medium sized cell is fully granulated, in general. The cell is round or oval in shape, but frequently elongated or polygonal. Their nuclei are round
or oval in form and light vesicular, and there are frequently seen cartwheel-like nuclei. Moreover, considerable numbers of these medium sized cells possess a negative image of Golgi’s apparatus or a perinuclear halo.

In small numbers, there are observed large acidophile cells which contain a round or oval, lighter vesicular nucleus than in medium sized cells and a sparsely granulated cytoplasm. These cells indicate generally a prominent negative image of Golgi’s apparatus or a perinuclear halo.

Small acidophile cells are found somewhat numerously. Their round or oval nuclei have relatively abundant coarse chromatin particles, and are surrounded by a densely granulated, narrow cytoplasmic band.

In methylgreen-pyronin preparations, there are clearly observed a considerable number of acidophiles with filamentous or granular, pyroninophile materials in the cytoplasm. These materials are mainly situated around the nucleus or at the cell periphery in the form of calottes.

Regarding the delta-cell, at this stage, the differences between both sexes are more conspicuous than at the aforementioned stages. In female pituitaries, a number of these cells contain some hyaline vacuoles and minute PAS positive granules in small numbers in the cytoplasm (Figs. 13, 15 and 18), while in males delta-cells are densely packed with cytoplasmic granules showing a clear PAS positive reaction (Figs. 14 and 16). These cells in males surpass those in females in size and number (Figs. 13, 14, 15 and 16). Nuclei of the delta-cells in both sexes, are large, oval or round in form and show a delicate chromatin network.

On the other hand, beta-cells few in number, are seen scattered in the central area of the anterior lobe. These angular or irregularly shaped cells contain coarse granules which are stained with Gomori’s aldehyde-fuchsin and PAS reagent. Their nuclei are oval, sometimes irregularly shaped and are usually smaller and darker than in delta-cells.

In female pituitaries, peculiar chromophobe cells are more numerous seen than at the abovementioned stages. They are mainly situated in the upper region of the anterior lobe adjacent to the residual cleft and scattered in other portions (Fig. 17). But they are only rarely found in males. Their nuclei are round or oval in shape and contain relatively abundant, dust-like to coarse chromatin particles. These nuclei are eccentrically situated in the cytoplasm. In the peri-
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pheral zone of these cells, there is recognized a cap-like structure consisting of many basophilic filaments or granules stained with hematoxylin or pyronin. In places, the "Kernhaufen" still remain in the mature animals. Chromophobes with scarce cytoplasm containing granular or flocculent materials are frequently observed throughout the anterior lobe. After application of the Unna-Pappenhein stain, moreover, the presence of chromophobes with filamentous pyroninophile materials around the nucleus is recognized both in males and in females.

At 70 to 90 days: The glands of the 70- to 90-day-old mice resemble closely those found at 60 and 65 days of age. In some cases, however, there are observed basophile cells with an irregularly shaped, shrunken nucleus, especially in beta-cells.

At 100 to 120 days: Although all cell types, at this stage, show characteristic features which are generally similar to those described for 60 and 65 days of age, in female pituitaries delta-cells with hyaline vacuoles in the cytoplasm are somewhat fewer in number when compared with those described for 60- and 65-day-olds. The number of such cells, however, exceeds markedly that of males.

At 150 days: In chromophiles, cells with an irregularly shaped nucleus appear more frequently than at the aforementioned stages. Acidophiles show a slight decrease in number when compared with those of young mature mice.

Mitotic activity: There is found no difference in the frequency of mitosis in both sexes. At birth and one day of age, although mitotic figures can be observed in large numbers, their cytoplasm are generally colorless. From this stage mitotic activity indicates an increase and attains a maximum at about five days of age. From this stage onward, mitoses decrease rapidly in number with advancing age. In young mature mice, dividing cells are found only rarely. Mitotic figures observed in the present experiment belong almost to chromophobe and acidophile cells, and the present authors did not encounter mitoses of cells which can be identified clearly with basophiles.

Discussion

Acidophile cell

Small and coarsely granulated acidophiles observed in only small numbers at birth, increased in number and size with advancing age. At about 10 days of age, acidophiles with a narrow band of fully granu-
lated cytoplasm around the nucleus were found here and there (Fig. 3). There appeared cells with a negative image of Golgi’s apparatus at about 20 days of age. At about 30 days, cells with light vesicular and cartwheel-like nuclei seen in adults were recognized, and cells with a negative image of Golgi’s apparatus came to be found frequently (Fig. 7). After application of the Unna-Pappenheim stain, although acidophiles with pyroninophile materials in the cytoplasm were observed numerously, these materials were seen scattered diffusely in the cytoplasm in general until about the 20th day after birth (Fig. 2). From the 30th day on, they were situated in characteristic localities in the cytoplasm as seen in adults (Fig. 8). Thus, at about 30 days of age, many acidophiles showed an appearance similar in cytological details as those in adults but less in number and size.

It is believed that the presence of abundant ribonucleic acid in cells is closely connected with protein-synthesis (Caspersson, ’41) and that in embryonal stages, many cells contain abundant RNA (Brauchet, ’41). It can thus be assumed from the present results that many acidophiles begin to work actively on the secretion of hormone(s) associated with cells at about 30 days of age. It is of interest that this stage coincides exactly with the weaning period in mice.

At 60 days of age acidophiles attained a mature state in cytological detail (Fig. 19). They varied in size, and there were observed three varieties; medium sized cells accounting for the majority, large cells in small numbers and small cells lying between the two. The medium sized cell usually possessed a light vesicular nucleus and densely or moderately granulated cytoplasm. However, there were found numerously among these medium sized cells, cells with a negative image of Golgi’s apparatus, with a cartwheel-like nucleus and with basophilic filamentous materials in the cytoplasm. The majority of the large cells showed a negative image of Golgi’s apparatus and basophilic filamentous materials in the sparsely granulated cytoplasm. The small cells had a somewhat dark nucleus surrounded by a narrow band of densely granulated cytoplasm. Siperstein et al. (’54) emphasized for the rat pituitaries that the presence of degranulated acidophiles with an enlarged Golgi’s image, which is strictly confined to the female, is observed from maturation onward, and that this phenomenon is the result of follicle function and oestrogen release. On the other hand, in the present observations, although we could not find such acidophiles with the enlarged Golgi image as pointed out by them, there were frequently observed slightly degranulated
acidophiles with the negative image of Golgi's apparatus without any sex difference in adult mice.

The number of acidophiles at about 150 days of age showed a slight decrease when compared with that described for young mature mice.

As far as acidophiles are concerned, there was found no significant difference between both sexes throughout this experiment.

In immature mice pituitaries, especially until about 30 days of age, the number of acidophiles showed a marked increase with advancing age. On the other hand, there were frequently observed mitotic figures of acidophile and chromophobe cells during this period, and there were also found cells which can be acknowledged as a transition form between acidophile and chromophobe cells at all stages of this experiment. Such cells showed indistinct cell boundaries and possessed a somewhat dark or vesicular nucleus similar to that of an acidophile cell, containing granular or flocculent materials in the cytoplasm. From these facts, it can be admitted that an increase in number of acidophiles, in addition to mitotic division of cells, is due to the transition from chromophobes to acidophiles.

Dawson and Friedgood ('38), Dawson ('46) and others distinguished two types of acidophiles (carminophile and orangeophile cells) in various animals, based on their morphology and function. Although there was also recognized the presence of these two types of acidophiles in the present study, the authors cannot agree, at least in the mouse, with the opinion that these cells represent respectively independent cell types, as the carminophile and orangeophile acidophiles of the mouse anterior pituitary did not reveal definite appearances even for the same staining method, as in the case of Ukai's results ('56) on a study dealing with the hamster hypophysis.

**Basophile cell**

The present authors differentiated two types of basophiles. The first type cell was represented by an oval or polygonal cell which contains fine cytoplasmic granules stained by the PAS technique and a somewhat dark, oval nucleus. These cells were located in clusters in the anterior portion and scattered in the other portions of the anterior lobe. The second type cell was polygonal or irregular in shape, with coarse cytoplasmic granules stained with aldehyde-fuchsin as well as with PAS reagent and a dark nucleus. These cells were situated in
small numbers in the central portion of the anterior lobe. As reported previously (Sano, '56), it can be acknowledged that the first and second type cells correspond respectively to the delta- and beta-cells proposed by Romeis ('40) or Halmi ('50 and '52). Purves and Griesbach ('51a) stated for the rat pituitary that two types of PAS positive cells designated as "gonadotrophs" and "thyrotrophs" are distinguishable, and that the cells classified by Halmi as delta-cells include all gonadotrophic basophiles plus those thyrotrophic cells with low hormone content which are unstained by the Gomori stain. Although they showed a schematic figure revealing the distribution of these two type cells, the distribution of the first and second types of basophiles in our experiment dealing with the mouse is closely similar to that of their gonadotrophs and thyrotrophs.

In PAS sections, glycoprotein containing cells were faintly discerned at immediately after birth, but aldehyde-fuschin positive cells were almost not found until about 10 days of age. These cells of two types increased more and more in number, size and stainability with advancing age, and at about 20 to 30 days of age they became clearly distinguishable (Fig. 9). Meanwhile, PAS positive cytoplasmic granules of these two type cells were generally very fine, but there were sometimes found small cells with strongly PAS positive droplets in small numbers in the cytoplasm, especially at the so-called "sex-zone," and such cells were only rarely encountered in adults. However, the significance of the presence of such cells is at present obscure.

At about 40 days of age, marked sexual difference occurred in the delta-cell. In females there began to appear delta-cells with hyaline vacuoles in the cytoplasm (Fig. 11), and their cytoplasmic granules decreased in number and size when compared with those of the immediately previous stage. Furthermore, the majority of delta-cells were occupied by cells with hyaline vacuoles in the cytoplasm at 50 days of age, after which in slightly decreasing number delta-cells attained a mature state at about the 60th day. Superstein et al. ('54) reported that between days 35 and 42, degranulation of gonadotrophs in the female rat take place, and that this fact almost certainly is due to the release of gonadotrophic hormones, coinciding with the first oestrous. In the present study dealing with the mouse anterior pituitary, there were obtained results for delta-cells which can be believed to be liquefaction of cytoplasmic granules rather than simple degranulation. This fact suggests that the delta-cells may enter promptly a secretory phase at this stage.
Purves and Griesbach ('54 and '55) stated for the rat that the gonadotrophs consist of central and peripheral ones, which are associated respectively with luteinizing and follicle stimulating secretion. Superstein et al. ('54) stated for rat female pituitaries that the central and peripheral gonadotrophs are distinguishable at 35 days of age, and assumed that the degranulation in these two types of gonadotrophs is correlated with FSH- and LH-secretion. Their data, however, are not sufficient for ascertaining this assumption at the stage of first ovulation. On the other hand, our results showed that there were observed no morphological differences between the delta-cells distributed in the sex-zone and centrally situated ones, and that in the same specimens, the occurrence of cells with hyaline vacuoles in the cytoplasm was simultaneous in both areas mentioned above.

During the same period, such a phenomenon concerning the delta-cell in male pituitaries did not occur and the increase in number and size of cells continued. Thus, from 60 days on, delta-cells in males predominated those in females number and size (Figs. 13, 14, 15 and 16). On the contrary, in males cells with cytoplasmic hyaline vacuoles were far fewer in number than in females.

Regarding the beta-cell, there was found no difference between both sexes. These cells did not appear at immediately after birth in the sections, and these cells were recognized at first with much difficulty at 5 or 6 days of age. At 10 to 20 days of age, the presence of beta-cells was clearly discerned, and the fully granulated cells seen in adults were encountered for the first time at about 30 days of age (Fig. 10). These cells increased in number and size more and more, until they attained a mature state at about 60 days of age. Sugiyama ('41, in the rat and mouse) and Gorbman and Evans ('43, in the rat) reported that ability to store the thyroid-colloid begins at the final stage of gestation. In the present study, the process of development of the beta-cell was too late for assuming that the cells are necessary to stimulate thyroid function. This fact may suggest that the thyroid gland of the mouse in the suckling period is supported partly by maternal stimulants acquired through lactation or that endogenous demand of thyrotrophic hormone is so great that the hormone does not yet accumulate in the stored form in the pituitary of the mouse, or that the beta-cells have no correlation to thyroid function.

The present authors did not encounter mitoses of cells which can be acknowledged clearly to be basophiles, throughout the experiment,
though basophiles increased in number with advancing age. Moreover, chromophobe cells containing granular or filamentous materials in the cytoplasm with more or less indistinct cell boundaries were always found throughout the anterior lobe. Some of their nuclei had abundant dust-like chromatin particles and showed a delicate chromatin network, and were similar closely to those of basophiles. Thus, the authors arrived at the conclusion that such chromophobes are transformable to basophile cells.

**Chromophobe cell**

At immediately after birth chromophobes account for the greater portion of the parenchymatous cells of the anterior lobe (Fig. 1). Although the number of cells increased gradually, they declined in percentages, since chromophiles augmented markedly in number. In immature mice, as in adults, there were found chromophobe cells with granular or flocculent materials in the cytoplasm and indistinct cell boundaries. The authors think, as mentioned above, that some of these chromophobes are transition forms between chromophobes and two types of chromophiles.

It should be noted that in females at about 40 to 50 days of age a marked occurrence of peculiar chromophobe cells described previously was recognized (Fig. 12). These cells had an eccentrically situated, somewhat dark nucleus and a cap-like structure consisting of many basophilic filaments in the cell periphery. These cells were mainly situated in the upper region of the anterior lobe adjacent to the residual cleft and scattered in the other portions (Fig. 17). It is clear that the distribution of the peculiar chromophobe cells is similar to that of common chromophobes. In our laboratory, it was confirmed in the mouse, in accordance with the results of Desciin ('36 and '40), that the peculiar chromophobe cell is found with extreme abundance during pregnancy (Kato, '56) and lactation (Yamada, Sano, Kato and Mizutani, '56), and that the cap-like structure contains abundant ribonucleic acid (Kato, '56). The present data showed, moreover, that the peculiar chromophobe cells were encountered only rarely in male pituitaries throughout the experiment. In addition, the occurrence of these cells in females was simultaneous with the appearance of hyaline vacuoles in the cytoplasm of delta-cells at about 40 days of age. These facts suggest that the peculiar chromophobe cell is connected intimately with the sexual function of female mice.
Although peculiar chromophobe cells were found numerously only in females, after application of the Una-Pappenheim stain, the presence of chromophobes with filamentous pyroninophile materials around the nucleus was always discerned both in males and in females throughout this experiment. These cells can be regarded as cells comparable to the early secretory stage in the hypothesis advocated by Abolins ('52), and it is clear that these chromophobes are in an undifferentiated state, from the cytological details.

**Summary**

Hypophyses of mice ranging from birth to 150 days of age of both sexes were examined in this experiment.

1. Acidophile cells which were found in only small numbers at immediately after birth showed a gradual increase in number and size with advancing age, and at about 30 days of age many acidophiles showed, though small in size, a similar appearance as seen in adults. At about 60 days of age the cells attained a fully developed state in cytological detail. The number of acidophiles indicated a slight decrease at the age of 150 days. On the other hand, as far as the acidophiles were concerned, there was found no significant difference between both sexes throughout this experiment.

2. Two types of basophile cells could be distinguished. The delta-cell, which contains PAS positive, fine cytoplasmic granules, was situated mainly in the anterior portion of the anterior lobe and partly in the central area of the lobe, and the beta-cell which reacts positively with aldehyde-fuchsin as well as with PAS reagent was located in the central region of the lobe. The presence of the former was faintly recognized at a time immediately after birth, while the latter was almost not found till about 10 days of age. These two types became clearly distinguishable at about 20 to 30 days after birth.

3. At 40 to 50 days of age, marked sexual differences occurred in the delta-cell. In females at this stage delta-cells with hyaline vacuoles in the cytoplasm were encountered frequently, and their cytoplasmic granules decreased in number and size when compared with those at just before this stage. On the contrary, in males, such a phenomenon concerning delta-cells did not occur. Thus, in adults, delta-cells in males predominated those in females in number and size, although in males the cells with cytoplasmic hyaline vacuoles were far less in number than in females.
4. As far as the beta-cell was concerned, there was found no difference between both sexes throughout this experiment.

5. In females, a marked occurrence of the "peculiar chromophobe cell" was observed from the 40th day on, although these cells were only rarely encountered in males throughout this experiment. The cells possessed an eccentrically situated, somewhat dark or vesicular nucleus and a cap-like structure consisting of many basophilic filaments at the cell periphery.

6. The increase in number of the acidophile cell depended mainly on mitotic division of the cell itself and partly on a transition from chromophobe cells. On the other hand, the number of basophile cells increased almost entirely by means of a transition from chromophobes.

Literature cited

A ll o l i n s, L. 1952 The visualization of pyronin, of the RNA-system, indicating cytoplasmic protein synthesis in the anterior pituitary of the guinea pig. Exp. Cell Research, 3, 1-9.


B a i l e y, P., and L. M. D a v i d o f f 1925 Concerning the microscopic structure of the hypophysis cerebri in acromegaly. Am. J. Pathol., 1, 185-207.

B e r b l i n g e r, W., und B u r g d o r f 1935 Neue Färbemethode zur Darstellung der Gewebestandteile der Hypophyse des Menschen. Endokrinol 15, 381-388.

B r a c h e t, J. 1941 La détection histochemique et le microdosage des acides pento-senucléiques (tissue animaux-développement embryonnaire des amphibiens). Enzymologia, 10, 87-96.


C a t c h p o l e , H. R. 1941 Distribution of glycoprotein hormones in the anterior pituitary gland of the rat. J. Endocrinol., 6, 218-225.


D a w s o n , A. B., and H. B. Friedgood 1938 Differentiation of two classes of acidophiles in the anterior pituitary of the female rabbit and cat. Stain Technol., 13, 17-21.


A postnatal histogenetic study of the anterior pituitary of the mouse

Pathol., 20, 665-666.


H a l m i, N.S. 1950 Two types of basophils in the anterior pituitary of the rat and their respective cytophysiological significance. Endocrinol., 47, 289-299.


——— 1952 Differentiation of two types of basophils in the adenohypophysis of the rat and mouse. Stain Technol., 27, 61-64.


L e g a i t, H., et E. L e g a i t 1954 Modifications de structure du lobe distal de l'hypophyse au cours de la couvaison chez la Poule Rhode-Island. Essai d'interprétation de la valeur des deux types principaux de cellules cyanophiles. C. R. Assoc. Anat., 41, 188-199.


——— 1952a Observations on the localization, nature and chemical constitution of some components of the anterior hypophysis. J. Path. and Bact. 64, 791-809.

——— 1952b The cytochemistry and cytology of the normal anterior hypophysis investigated by the trichrome-periodic acid Schiff method. J. Path. and Bact., 64, 811-827.


P u r v e s, H. D., and W. E. G r i e s b a c h 1951a The site of thyrotrophin and gonadotrophin production in the rat pituitary studied by McManus-Hotchkiss staining for glycoprotein. Endocrinol., 49, 244-264.

——— 1951b Specific staining of the thyrotrophic cells of the rat pituitary by the Gomori stain. Endocrinol., 49, 427-428.


R o m e i s, B. 1940 Hypophyse. Handbuch der mikroskopischen Anatomie des Menschen, herausgegeben von W. v Möllendorff, Bd. 6, 2/III. Springer Verlag, Berlin.

S a n o, M. 1956 Cellular changes in the anterior pituitary of the mouse following Pituitrin-injection. Folia anat. jap. 29, 247-277.


S i p e r s t e i n, E., C. W. N i c h o l s, Jr., W. E. G r i e s b a c h and I. L. C h a i k o f f 1954


Ukei, T. 1956 On the postnatal development of the parenchymatous cells of the anterior pituitary in the hamster. Arch. hist. jap., 10, 433-454. (in japanese)


Yasuda, M. 1953 The two types of “basophiles” in the fowl pituitary. Arch. hist. jap., 5, 327-335.

**Explanation of Figures**

**Plate 1**

Fig. 1. Pituitary of 1-day-old male. Many chromophobes seen. Ill-defined these cells have very little, colorless or faintly orangeophilic cytoplasm and a round or oval nucleus with abundant chromatin particles. Zenker-formol-acetic acid fixation. Hematoxylin-eosin stain. ×600

Fig. 2. Pituitary of 5-day-old female. There are observed many cells with delicate filamentous or granular pyroninophilic materials in the cytoplasm. These materials, however, are diffusely distributed throughout the cytoplasm and do not show any characteristic localization such as seen in adults. Zenker fixation, Methylgreen-pyronin stain. ×1400

Fig. 3. Pituitary of 10-day-old male. Acidophiles possess a round or oval nucleus containing relatively abundant chromatin particles and one or two conspicuous nucleoli, and their nuclei are surrounded by a narrow band of fully granulated cytoplasm. Sublimate-formol-acetic acid fixation. Azan stain. ×1600

Fig. 4. Pituitary of 10-day-old male. At the center, a basophile cell with fine granules is seen. Sublimate-formol-acetic acid fixation. Azan stain. ×2000

Fig. 5. Pituitary of 10-day-old male. Basophiles are oval or polygonal in shape. Their cytoplasm are moderately filled with fine granules staining with PAS reagent. Sublimate-formol-acetic acid fixation. PAS stain. ×1700

Fig. 6. Pituitary of 20-day-old male. Many acidophiles are densely packed with intensely acidophilic granules in the cytoplasm. At the left lower corner, a delta cell is seen. Sublimate-formol-acetic acid fixation. Azan stain. ×700.

**Plate 2**

Fig. 7. Pituitary of 30-day-old female. Many acidophiles are seen. Their cytoplasm are densely packed with brilliant acidophilic granules. majority of their nuclei appear
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To be light vesicular as in adults. There are found cells with a negative image of Golgi's apparatus or a perinuclear halo. Sublimate-formol-acetic acid fixation. Azan stain. ×600.

Fig. 8. Pituitary of 30-day-old female. There are observed acidophiles with pyroninophile materials around the nucleus or at the cell periphery as seen in adults (indicated by arrows). Zenker fixation. Methylgreen-pyronin stain. ×1400.

Fig. 9. Pituitary of 30-day-old female. Two types of basophiles are distinguishable (B₁ and B₂ represent respectively delta and beta-cells). Sublimate-formol-acetic acid fixation. Azan stain. ×1500.

Fig. 10. Pituitary of 30-day-old male. Two beta-cells are seen (indicated by arrows). Sublimate-formol-acetic acid fixation. Aldehyde-fuchsin stain. ×700.

Fig. 11. Pituitary of 40-day-old female. There are seen delta-cells with hyaline vacuoles in the cytoplasm (indicated by arrows). Sublimate-formol-acetic acid fixation. Azan stain. ×1400.

Fig. 12. Pituitary of 40-day-old female. Two “peculiar chromophobe cells” are present (indicated by arrows). Sublimate-formol-acetic acid fixation. Azan stain. ×1600.

Plate 3

Figs. 13. and 15. Pituitary of 60-day-old female. The micrographs show PAS-positive cells in the anterior portion of the anterior lobe. Some of these cells contain hyaline vacuoles in the cytoplasm. Sublimate-formol-acetic acid fixation. PAS stain. ×600 (Fig. 13), and ×150 (Fig. 15).

Figs. 14. and 16. Pituitary of 60-day-old male. In males delta-cells are densely packed with cytoplasmic granules showing PAS positive reaction. These cells in males predominate those in females in size and number. Sublimate-formol-acetic acid fixation. PAS stain. ×600 (Fig. 14), and ×150 (Fig. 16).

Fig. 17. The distribution of the “peculiar chromophobe cell” in the anterior pituitary of the mature female mouse. A, B, C and D represent respectively sagittal planes marked by a, b, c and d by a bird’s-eye view of the mouse pituitary.

Plate 4

Fig. 18. Pituitary of 60-day-old female. There are seen some delta-cells with hyaline vacuoles in the cytoplasm. Sublimate-formol-acetic acid fixation. Azan stain. ×1700.

Fig. 19. Pituitary of 60-day-old female. Small acidophile cells with round cytoplasm and a centrally situated round nucleus are observed. Many medium sized acidophile cells reveal oval or elongated cytoplasm and an eccentrically situated light vesicular nucleus. Zenker-formol-acetic acid fixation. Azan stain. ×1300.

Fig. 20. Pituitary of 130-day-old female. Beta-cells (indicated by arrows) are angular in form and contain dense dark blue, coarse granules. Their nuclei show a very dark cast. Sublimate-formol-acetic acid fixation. Azan stain. ×1500.

Fig. 21. Pituitary of 130-day-old male. Delta-cells (indicated by arrows) are round or polygonal in form. Their nuclei show a light cast. Their granules staining pale blue with aniline blue are very fine, and are diffusely distributed throughout the cytoplasm. Sublimate-formol-acetic acid fixation. Azan stain. ×1500.

Fig. 22. Pituitary of 110-day-old female mouse. There are seen two peculiar chromophobe cells (indicated by arrows). Sublimate-formol-acetic acid fixation. Azan stain. ×1500.
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Plate II

K. Yamada, M. Sano and T. Ito