

The Identification of Acidophilic Cells in the Human *Pars Distalis*¹

JAMES L. CONKLIN

Department of Anatomy, The University of Michigan,
Ann Arbor, Michigan

ABSTRACT Sections of human hypophyses were fixed in either formalin or Bouin's fluid and stained by a variety of acid stains. The stains were employed singly, in combination with each other and in conjunction with various mucoid staining procedures. After these procedures the chromophils of the *pars distalis* were classified as either acidophilic, mucoid, acidophilic-mucoid, modified or degranulated. The remaining cells which lacked specific chromophilic granules were classified as chromophobes.

The acidophils after the terminology of Ezrin were designated as cell types I, II and VIII which correspond to the *alpha*, *eta* and *epsilon* cells of Romeis.

Numerous, carminophilic type I cells were present in glands of all ages. Although usually pyramidal or oval in shape they also exhibited a variety of morphologic forms. The type I cells were further subdivided into small, light and dark cells on the basis of size and nuclear characteristics. The type II cell although present in all glands was most frequently observed in fetal and post-menopausal pituitaries. It was tinctorially identified by the prominent staining of its cytoplasmic granules with erythrosin.

The type VIII cell exhibited staining properties intermediate between cell types I and II. On the basis of tinctorial and morphologic properties it was tentatively identified as a modified type I or type II cell.

Prior to a study of pituitary cytogenesis the literature was searched for previous descriptions of pituitary cell types and the methods utilized for their demonstration. It was soon apparent from this literature survey that a great deal of confusion existed about the type and function of pituitary cells and the specificity of staining methods employed (see reviews: Purves, '61; Halmi, '63; Ezrin, '64; and Herlant, '64).

Factors which apparently contributed to this confusion were: (1) the utilization of selective but not specific staining methods to study glands from different species, (2) a lack of adequate morphological descriptions of specific cell types, (3) a lack of consistency in the use of nomenclature and (4) *overemphasis* on the correlation between cell types and concomitant endocrine disorders.

Presumably, less confusion would have occurred if certain guidelines common to all cytological studies had been observed. Some of the more significant of these are: (1) cells of different species which have the same tinctorial properties may not necessarily have the same function; (2) mor-

phologic parameters are variable and dependent on the physiological state of the cells; (3) cells that secrete specific hormones that are chemically consistent can be expected to exhibit consistent histochemical properties in spite of morphologic variability.

In order to clarify the cytology of the human *pars distalis*,² sections were examined after the application of a variety of histological and histochemical procedures. The specificity of the methods was evaluated and the chromophils of the normal human hypophysis were identified. Additionally, the histochemical cytology of the gland was compared with the published immunocytologic descriptions. The present report deals with a description of acidophils and chromophobes, while a subsequent report will describe the hypophyseal mucoid cells. For the sake of brevity and in observance of the above criticisms, only limited reference is made to studies of the hypophysis of other species.

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² The term *pars distalis* is employed as defined by Purves and Bassett ('63) and is homologous to the *pars anterior* and *pars intermedia* of most species.

MATERIALS AND METHODS

The material³ employed in the study consisted of 2,500 3–5 μ sections of human hypophyses obtained from 15 fetuses and stillborns; one female child, age — one year; three male adults of ages 27, 38, and 42 years; and three female adults of ages 37, 39 and 62 years. The postmortem interval preceding fixation was between three and five hours. Two additional adult pituitaries, fixed after ten and fourteen hours, were utilized to evaluate fixation but were not employed in the cytological studies.

When possible the glands were divided sagittally prior to fixation, and both frontal and sagittal sections were obtained from the same gland in order to have a basis for determining cellular distribution. The material was fixed in 10% neutral formalin (19 pituitaries) or Bouin's fixative (5 pituitaries). Formalin was utilized because an evaluation of staining procedures after this fixative would be most useful to other investigators who have access to existing, usable material which has been routinely fixed in formalin. Bouin's fixative was employed in order to have a means of evaluating cellular preservation by a fixative other than formalin.

The staining procedures which were evaluated were those previously reported by a number of investigators. These procedures were employed, where possible, as originally reported and were also modified as needed. In methods which utilized a sequence of stains, the stains were employed singly and in step-wise order, to be certain of the identity of the stained cells. As an illustration, in the use of the Masson trichrome method after aldehyde fuchsin different sections were stained in (1) aldehyde fuchsin (ald. f.); (2) Masson A (Mas. A); (3) Mas. A, B; (4) Mas. A, B, light green (lt. gr.); (5) lt. gr., ald. f., Mas. A; (6) ald. f., Mas. A, B; (7) ald. f., Mas. A, B, lt. gr. A similar procedure was utilized with all of the other methods employed.

The procedures for acidophilic cells which were evaluated are summarized below:

1. 0.1% azocarmine G (C. I. 50085) in 1% acetic acid at 60°C preceded by and

differentiated in aniline alcohol (0.1 cm³ aniline oil: 100 cm³ 95% alcohol) (Romeis, '40).

2. 2% orange G (C. I. 16230) in 1% aqueous phosphomolybdic acid followed by mordanting in 5% phosphotungstic acid (Romeis, '40).

3. 2% orange G in 5% aqueous phosphotungstic acid (Ezrin and Murray, '63).

4. 0.3% acid fuchsin (C. I. 42685) in 1% acetic acid.

5. 0.7% ponceau R (C. I. 16150) in 1% acetic acid.

6. Masson A (a mixture of acid fuchsin and ponceau R, as above), with and without post-mordanting in Masson B (1% aqueous phosphomolybdic acid) (Masson, '29).

7. 2% aniline blue WS (C. I. 707) in 2% acetic acid (Masson C).

8. 2% light green SF yellowish (C. I. 42095) in 1% acetic acid.

9. 1% aqueous erythrosin B (C. I. 45430).

10. Mallory II (Humason, '62) (0.5% aniline blue and 2% orange G in 1% acetic acid).

11. 0.1% luxol fast blue MBSN (lot no. 136, E. I. Dupont Co.) in 95% alcohol (Kluver and Barera, '58).

12. 0.5% aqueous acid alizarin blue 2B (C. I. 1063) (Humason, '62).

These procedures were employed singly, in combination with each other (table 1), and with the following stains for mucoid cells: aldehyde fuchsin, resorcin fuchsin, alcian blue, periodic acid Schiff (PAS), aldehyde thionin and colloidal iron. The details of the mucoid procedures and the oxidation methods employed have been summarized elsewhere (Conklin, '67).

Several structural or tinctorial qualities were used to identify several cell types. These qualities were:

(1) the staining reaction of intracytoplasmic granules;

(2) the staining reaction of cytoplasm when granules were absent;

(3) the morphology of the cells, especially with regard to nuclei and cytoplasm.

³The material utilized in this study was obtained from the Department of Pathology and from the Embryology Research Collection of the Department of Anatomy.

TABLE 1
Summary of methods employed

Azocarmine	erythrosin, Mallory II
Orange G	erythrosin, Mallory II, acid alizarin blue (Helant, '60; Racadot, '62)
Aniline blue ¹	Mallory II (Humason, '62)
Light green ¹	azocarmine, orange G (Romeis, '40)
Acid fuchsin ¹	azocarmine, orange G, light green
Ponceau R ¹	azocarmine, orange G, aniline blue (Romeis, '40)
Masson A ¹	azocarmine, Masson B, C
Erythrosin B	orange G, light green
Luxol fast blue	Masson A, B, C (Masson, '29)
Acid alizarin blue	Masson A, B, light green
Masson A, B	
Masson B, C	

¹ These stains were employed with and without mordanting in Masson B.

mic granules with little reliance on size and shape of the cells;

(4) cellular distribution within the *pars distalis* and the nature of the distribution, i.e., whether the cells occurred singly or in clumps.

Adjacent sections were sometimes stained by different methods and cell counts were employed to aid in the identification of cell types. Cell counts were made by inserting a disc micrometer ruled into 49 squares into the ocular of the microscope and counting a minimum of 1,000 cells in a specific area of the gland. Comparison of the cell counts assisted in evaluating the specificity of certain staining reactions.

Various terms have been employed to indicate different classes of pituitary cells and also to identify individual cells within these classes. Romeis ('40) originally divided the cells of the *pars distalis* into three groups, the *acidophils*, *basophils* and *chromophobes*. Later, Pearse ('52a) employed the term *mucoïd* to identify the *basophils* of Romeis since the cells in this group exhibited some degree of staining after the PAS reaction. More recently, the term *mucoïd cells* has included cells which are stained by all methods for muco- and glycoproteins.

In the present report, the chromophilic cells of the *pars distalis* were classified as either acidophilic, mucoïd, degranulated or modified. The acidophils included those cells which exhibited an affinity for acid dyes and were not stained by mucoïd stains except after certain oxidative procedures (Conklin, '67).

Mucoïd cells were those whose granules were stained by mucoïd procedures such as PAS, alcian blue or aldehyde fuchsin. There are actually two groups of cells in the mucoïd category, i.e., cells whose granules stained only with mucoïd procedures (mucoïd cells) and cells whose granules were stained by mucoïd procedures and acidophilic dyes (acidophilic-mucoïd cells).

The degranulated cells were those that possessed morphological characteristics that permitted classifying them either as acidophilic or mucoïd, but which were lacking in specific cytoplasmic granules. It was considered that these and the modified cells were degranulated forms of either acidophilic or mucoïd cells.

The final chromophilic cell types were the modified acidophilic and mucoïd cells. These could be classified as either acidophilic or mucoïd because either a few cytoplasmic granules or vesicles were present that exhibited specific staining characteristics. Included in this group were the beta 3 cell of Ezrin and Murray ('63), the Crooke-Russell and Crooke hyaline cells, the gamma cell (Romeis, '40), and the vesiculate mucoïd cell (Pearse, '52b).

In the present study, chromophobic cells were identified by the absence of resolvable, cytoplasmic granules. Chromophobes were small and the nucleus was enveloped by a thin rim of cytoplasm (fig. 12). The nuclei were usually oval, contained a small nucleolus and exhibited a variable chromatin pattern. When the cells were of sufficient size to exhibit any specific staining response they were classified as a type of chromophil.

RESULTS

Examination of the pituitary gland following the application of a given acidophilic stain revealed that the granules of most cells were stained to some degree. Therefore, identification of specific cell types necessitated the use of several staining procedures since with few exceptions specificity was unattainable with a single technique. When the staining reactions of pituitary cells are described, reference to a specific cell is based on its identification after a variety of procedures.

Individual cells have been referred to by a variety of terms including alphabetical designations. Recently, it has been recommended that a functional terminology be employed for pituitary cells in the absence of an agreeable morphological terminology (van Oordt, '65). This can be done if a technique is employed which gives functional results. However, since it was necessary to employ some designation for the different cell types in the present report, the system of Ezrin ('63) which is analogous to the terminology of Romeis ('40) was employed. According to this system the chromophilic cells are indicated by Roman numerals I-VIII. Of these the definitive acidophils are types I, II, and VIII which correspond respectively to the alpha, eta and epsilon cells of Romeis. Cell types III-VII are mucoid cells which include the amphophils (Burt and Velardo, '54), beta, delta and gamma cells.

The staining characteristics of nine cell types are described (table 2). Cell type IX is a mucoid cell found in the posterior zone of the *pars distalis* which differs slightly from the anterior zone mucoid cells. A blank space in the table is an indication that a cell type either did not exhibit an affinity for a stain or was only weakly stained.

Since most cell types were stained by one or more of the acid stains, identification of acidophils was dependent upon determining which of the cell types were also mucoid cells. Application of mucoid staining procedures revealed that cell types III-VII and IX were stained by these methods (Conklin, '67). Of this group, cell types III, IV, VI, and IX were clearly acidophilic-mucoid cells. Therefore, of the

TABLE 2
Staining responses of pituitary cell types

Stain	I Acidophil	II Acidophil	III Mucoid	IV Mucoid	V Mucoid	VI Mucoid	VII Mucoid	VIII Acidophil	IX Mucoid posterior cell
Azocarmine	+	+	+	+		+	NR	+	+
Acid fuchsin	+	+	+	+		+	NR	+	+
Eosin	+	+	+	+		+	NR	+	+
Orange G	+	+	+	+		+	NR	+	+
Aniline blue	+	+	+	+	+	+	NR	+	+
Light green	+	+	+	+	+	+	NR	+	+
Ponceau R	+	+	+	+	+	+	NR	+	+
Acid alizarin blue 2R	+	+	+	+	+	+	NR	+	+
Erythrosin B	+	+	+	+	+	+	NR	+	+
Luxol fast blue	+	+	+	+	+	+	NR	+	+

+++ and + indicate either a strong or moderate affinity for the stain. A blank indicates either a weak or negative staining response. NR, no responses, are included for cell type VII which is a modified type of cell.
¹ When used independently orange G stains the cell quite intensely, however, if preceded by azocarmine, then the affinity for orange G is not exhibited.
² When employed after aniline blue, this stain shows less affinity for types III and IV than when used alone.

nine cell types only types I, II and VIII were distinct acidophils and are described in detail in this report.

Characteristics of the acidophils

Specific identification of the three types of acidophilic cells was possible when certain acid stains were employed in conjunction with procedures for mucoid cells (table 3).

Cell Type I

The type I cell (figs. 2-8) contained characteristically small, densely packed intracytoplasmic granules. The granules were specifically identifiable by being carminophilic following an azocarmine-orange G sequence of staining. The granules were unstained by mucoid stains although the cytoplasm of the cells was lightly PAS positive. The cells were present throughout the anterior zone and were rarely present in the posterior zone (fig. 1).

They were most numerous in the posterior region of the gland, antero-lateral to the remnants of the residual cleft. Large clusters of the cells also occurred in the central region of the gland around the connective tissue septum. In the anterior region of the gland they were more numerous inferiorly than superiorly. Very few other cell types were observed in the acidophilic zones and the cells occurred in large irregular clusters in close association with sinusoids. The typical, or most observed, type I cell ranged in size from small round cells (fig. 5) with scanty cytoplasm and a central nucleus to large oval cells 20-25 μ that contained abundant cytoplasm and an eccentric nucleus (figs. 2-4). The large cells were often binucleate and most of them exhibited a sparsely granulated, juxtannuclear zone (fig. 4) and often contained 1-2 intracytoplasmic vacuoles. The nuclei of the large type I cell exhibited some morphological

TABLE 3
The tinctorial properties of acidophil cells

Staining procedures employed	Cell type		
	I	II	VIII
PAS	pale pink	pale pink	pale pink
Performic acid,alcian blue, PAS	pale pink	pale pink	pale pink
Performic acid,alcian blue, PAS, orange G	dark yellow	pale yellow	dark yellow
Aldehyde thionin, PAS	pale pink	pale pink	pale pink
Aldehyde thionin, PAS, orange G	dark yellow	pale yellow	dark yellow
Colloidal iron, PAS, orange G	dark yellow	pale yellow	dark yellow
Herlant tetrachrome	pale orange	grey cytoplasm, red granules	light orange cytoplasm, pale red granules
Aldehyde fuchsin, without oxidation, Romeis counterstain	red	red-orange	red-orange
Aldehyde fuchsin, without oxidation, Masson trichrome	red	red-orange	red-orange
Aldehyde fuchsin, periodic acid oxidation, Masson trichrome	red	red-orange	red-orange
Performic acid, alcian blue, aldehyde fuchsin	light purple	light purple	light purple

variation. Although most nuclei were vesicular with a fine chromatin pattern and a prominent basophilic nucleolus, some cells contained a dark almost-pyknotic nucleus (fig. 6). The nucleolus in the latter type of cell was usually acidophilic. The cytoplasmic granules of the darkly nucleated cells were intensely carminophilic and the juxtannuclear zone was usually not evident. For purposes of reference three type I cells were distinguished. These were the small type I cell (with reference to size) (fig. 5) and the light (figs. 2-4) and dark type I cells (fig. 6) (with reference to nuclear staining).

Type I cells were present in all the pituitaries examined from both fetuses and adults. Small and light type I cells were most numerous in younger subjects while after forty years of age there was an increase in the dark type I cells. Concomitant with the increase in dark cells, the membranes of the cells became less distinct and the cells occurred in large syncytial-like clusters. Occasionally, oddly shaped type I cells were observed in the pituitaries of older subjects (figs. 7-8). The significance of these cells was not determined.

Cell Type II

While the granules of the type II cells were colored by most acid stains only erythrosin stained them with an intensity which permitted their being distinguished on the basis of color (figs. 9-11, 13). However, the cells could additionally be identified by the sparseness and coarseness of the intracytoplasmic granules. The cells were scattered throughout the gland although they occurred most frequently near the periphery, along the superior margin. They were present most frequently as single cells intermingled with mucoid cells. Usually, the type II cell was smaller (15-20 μ) than the type I cell and ranged from round to pyramidal in form. Occasionally, a type II cell was larger than a type I cell but this was observed only under certain conditions (fig. 13). The cytoplasm of the type II cell was finely granular and lightly acidophilic and was colored a light grey after the Herlant procedure, the result of light staining with aniline blue and orange G. Few distin-

guishing characteristics were presented by the nuclei of the type II cells. The cells were rarely binucleated; the chromatin was coarse and the nucleolus was prominent. The nuclei were either centric or eccentric in location and when in the latter position a small nongranulated, juxtannuclear zone was evident in the cytoplasm. Type II cells, although variable in number, were observed in the pituitary gland of both sexes. They were most frequent in the fetal and post-menopausal pituitary.

Cell Type VIII

The type VIII cell (figs. 14-16) was round to oval in shape and ranged from slightly larger to slightly smaller than the type I cell. The nucleus was either centric or eccentric in position depending upon the shape of the cell. The larger, oval cells occasionally exhibited a small juxtannuclear zone. The nucleus was vesicular with moderately coarse chromatin and usually contained a small nucleolus. The cytoplasm of the type VIII cell was often poorly preserved (fig. 14) and sometimes fragmented. This occurred after fixation in either formalin or Bouin's fluid. A small juxtannuclear zone was sometimes observed in the larger, oval cells. The granules of the type VIII cell were morphologically distinct because of their large size and sparseness. They were stained by most acid dyes but exhibited the greatest affinity for orange G and ponceau R. The cells were observed in all pituitary glands. They were located individually in all areas of the anterior lobe including the acidophil zones and usually were in close association with the sinusoids. Type VIII cells were easily discriminated from the type I cells because of the marked difference in granule size and content.

It was difficult to distinguish between the type II and type VIII cells after routine procedures although it could be accomplished on the basis of difference in granule size. After the Herlant procedure the cells were more easily discriminated by the greater erythrosinophilia of the granules in the type II cell.

DISCUSSION

When a single stain is employed it is extremely difficult to distinguish between

the different types of acidophils. Little specificity is exhibited by acidophilic stains and the identification of individual cell types requires the employment of a sequence of stains. While Shanklin et al. ('59) described luxol fast blue as a specific stain for alpha cells it had an affinity for all acidophils. Erythrosin was differentially specific for the type II cell but it also stained the type VIII cell to some degree. Azocarmine, when employed with orange G, intensely stained the type I cells but again some measure of staining was exhibited by other acidophils and the acidophilic-mucoid cells.

The true acidophils included at least three types of cells. The carminophilia, oval shape and fine granulation of the type I cell (figs. 2-8) corresponded closely to the alpha cell described by Romeis ('40). Also, in agreement with Romeis, small, light and dark alpha cells were observed (figs. 4-6). Romeis noted little correlation between the kind of type I (alpha) cell and the physiological state of the subject. In the present study the small and light type I cells were predominant in the young while the dark type I cell was more frequent in older subjects.

Most investigators of hormone localization have not distinguished between the alpha cell and other acidophils. In not one of the several immunofluorescent studies (Leznoff et al., '60; Meneghelli and Scapinelli, '60) has a specific correlation been made between the alpha cell and the cells which are fluorescent. However, most investigators (see reviews) conclude that the type I (alpha) cell is the probable source of somatotropic hormone (STH).

Meneghelli and Scapinelli ('60) observed that while most acidophils were fluorescent with anti-STH a few acidophils were

not. The non-fluorescent acidophils would undoubtedly include the type II and type VIII cells observed in this study. The Herlant tetrachrome procedure clearly reveals two additional acidophilic cell types in the human pituitary and they have also been studied by electron microscopy (Pasteels, '63).

The type II cell exhibited a somewhat specific affinity for erythrosin and thus was tinctorially distinguished from the other cells. The type VIII cell stained with less specificity but was morphologically distinguishable. The type II and type VIII cells correspond respectively to the eta and epsilon cells of Romeis ('40) (table 4). Although Romeis did associate the epsilon cell with a specific hormone these cells were always present in all pituitaries. Eta (type II) cells occurred only in the last trimester of pregnancy and were suggested to be the source of a fetal growth hormone. In the present study, both type II and type VIII cells were observed in all pituitaries. Type II cells occurred most frequently in pituitaries of a term fetus (fig. 13) and a post-menopausal female. Pasteels ('63) has also reported the presence of the eta cell in a fetal pituitary. The failure of Romeis to observe "pregnancy" or eta (type II) cells in non-pregnant subjects may be the result of the close similarity in tinctorial properties of the eta and epsilon (type VIII) cells.

The function of the eta and epsilon cells is uncertain. As the result of transplantation studies Herlant ('60, '63) and Pasteels ('61) have concluded that erythrosinophilic cells are the source of prolactin in rodents. Later, Pasteels ('63) demonstrated the ultrastructural similarity of eta cells in the rodent and human hypophysis. Although Emmart et al. ('63) reported

TABLE 4
Classification of acidophils

Author	System		Terminology	
Romeis ('40)	Greek	alpha	eta	epsilon
Ezrin ('63)	Roman	type I	type II	type VIII
van Oordt ('65)	functional (tentative)	somatotropic cell	lactotropic cell	
This report	Roman, descriptive	type I	type II	modified cell

the immunofluorescent localization of prolactin in the rodent they did not correlate this localization with any specific cell type. The higher incidence of the type II cell in the last trimester of pregnancy in both mother and fetus is suggestive of a lactotropic function.

The function of the epsilon (type VIII) cell remains obscure. Purves ('61) and Halmi ('63) have suggested that it is an inactive eta cell, while Herlant ('64) proposed that this cell elaborated the hormone, ACTH. Tinctorially and morphologically the type VIII cell is intermediate in properties between the type I and type II cells. Until further evidence is available the type VIII cell is best classified as a modified form of one of the other specific cell types.

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LITERATURE CITED

- Burt, A. S., and J. T. Velardo 1954 Cytology of the human adenohypophysis as related to bioassays for tropic hormones. *J. Clin. Endocr.*, 14: 979-996.
- Conklin, J. L. 1967 A survey of histochemical procedures for the demonstration of mucoid cells in the human *pars distalis*. (In manuscript.)
- Emmart, E. W., S. S. Spicer and R. W. Bates 1963 Localization of prolactin within the pituitary by specific fluorescent antiprolactin globulin. *J. Histochem.*, 11: 365-373.
- Ezrin, C. 1963 In: Discussion Generale, *Cytologie de l'adenohypophyse*. Ed. by J. Beniot and C. Da Lage. Paris. Editions of C.N.R.S., p. 346.
- 1964 The pituitary gland. *Ciba Clinical Symposia*, 16: 71-100.
- Ezrin, C., and S. Murray 1963 The cells of the human adenohypophysis in pregnancy, thyroid disease and adrenal cortical disorders. In: *Cytologie de l'adenohypophyse*. Ed. by J. Beniot and C. Da Lage. Paris. Editions of C.N.R.S., pp. 183-200.
- Halmi, N. S. 1963 Some unsolved problems of anterior pituitary histophysiology. In: *Cytologie de l'adenohypophyse*. Ed. by J. Beniot and C. Da Lage. Paris. Editions of C.N.R.S., pp. 19-32.
- Herlant, M. 1960 Etude critique de deux techniques nouvelles destinées a mettre en evidence les differentes categories cellulaires presentes dans le glande pituitaire. *Bull. Micr. appl.*, 10: 37-44.
- 1963 Apport de la microscopie electronique a l'etude du lobe anterieur de l'hypophyse. In: *Cytologie de l'adenohypophyse*. Ed. by J. Beniot and C. Da Lage. Paris. Editions of C.N.R.S., pp. 73-90.
- 1964 The cells of the adenohypophysis and their functional significance. In: *Internat'l. Rev. Cytol.* Ed. by G. H. Bourne and J. F. Danielli. Academic Press, New York, 17: 299-382.
- Humason, G. H. 1962 *Animal Tissue Techniques*. W. H. Freeman and Company, San Francisco.
- Kluver, H., and E. Barrera 1958 A method for the combined staining of cells and fibers in the nervous system. *J. Neuropathol. Exp. Neurol.*, 12: 400-403.
- Leznoff, A., J. Fishman, L. Goodfriend, E. McGarry, J. Beck and B. Rose 1960 Localization of fluorescent antibodies to human growth hormone in human anterior pituitary glands. *Proc. Soc. Exp. Biol. Med.*, 104: 232-235.
- Masson, P. 1929 Some histological methods: Trichrome stainings and their preliminary technique. *J. Techn. Methods*, 12: 75-90.
- Meneghelli, V., and R. Scapinelli 1961 L'ormone somatotropo umano e prodotto dalle cellule acidofile preipofisarie. *Atti Della Societa Medico-Chirurgica de Padova*, 36: 21-28.
- Pasteels, J. L. 1961 Secretion de prolactine par l'hypophyse en culture de tissus. *C. R. Acad. Sci. (Paris)*, 253: 2140-2142.
- 1963 Etude experimentale des cellules responsables de la secretion de prolactine chez les mammiferes. In: *Cytologie de l'adenohypophyse*. Ed. by J. Beniot and C. Da Lage. Paris. Editions of C.N.R.S., pp. 137-148.
- Pearse, A. G. E. 1952a Observations on the localization, nature and chemical constitution of some components of the anterior hypophysis. *J. Path. and Bact.*, 64: 791-810.
- 1952b The cytochemistry and cytology of the normal anterior hypophysis investigated by the trichrome-periodic acid Schiff method. *J. Path. and Bact.*, 64: 811-826.
- Purves, H. D. 1961 Morphology of the hypophysis related to its function. In: *Sex and Internal Secretion*. Ed. by W. C. Young, The Williams and Wilkins Company, Baltimore. 1: 161-239.
- Purves, H. D., and E. G. Bassett 1963 The staining reactions of the *pars intermedia* cells and their differentiation from *pars anterior* cells. In: *Cytologie de l'adenohypophyse*. Ed. by J. Beniot and C. Da Lage. Paris. Editions of C.N.R.S., pp. 231-242.
- Racadot, J. 1962 Sur La Mise En Evidence Des Types Cellulaires Adenohypophysaires Par La Methode De Herlant Au Bleu D'Alizarine Acide *Bull. Micr. appl.*, 12: 16-20.

- Romeis, B. 1940 Inner sekretorische Drüsen II. Hypophyse. In: Handbuch der Mikroskopischen Anatomie des Menschen 6. Ed. by W. von Mollendorf, published by Julius Springer, Berlin.
- Shanklin, W. M., T. K. Nassar and M. Issidores 1959 Luxol fast blue as a selective stain for alpha cells in the human pituitary. *Stain Technol.*, 34: 55-58.
- van Oordt, P. G. W. J. 1965 Nomenclature of the hormone producing cells in the adenohypophysis. A report of the International Committee for Nomenclature of the Adenohypophysis. *Gen. Comp. Endocr.*, 5: 131-134.

PLATE 1

EXPLANATION OF FIGURES

- 1 The *pars distalis* (PD) and *pars nervosa* (PN) of the human pituitary gland. (AZ) anterior zone and (PZ) posterior zone of the *pars distalis*. The dark areas in the center are colloid filled remnants of the residual cleft. Aldehyde fuchsin and Masson. $\times 200$.
- 2 A portion of an acinus containing oval and pyramidal type I cells. Note the relation to the sinusoid(s). Aldehyde fuchsin and Romeis' azan. $\times 2000$.
- 3 A variant morphological form of the type I cell. Note the numerous vesicles (light areas) in the cytoplasm. Aldehyde fuchsin and Masson. Wratten no. 22 filter. $\times 2600$.
- 4 An oval type I cell which contains a prominent justanuclear area. Note the density and size of the cytoplasmic granules in this and the other figures. The nucleus is typical of the light type I cell. Aldehyde fuchsin and Romeis' azan. Wratten no. 22 filter. $\times 2600$.
- 5 A group of small type I cells. Herlant tetrachrome. $\times 2300$.
- 6 A group of dark type I cells. Note the pronounced nuclear density. Herlant tetrachrome. $\times 2300$.

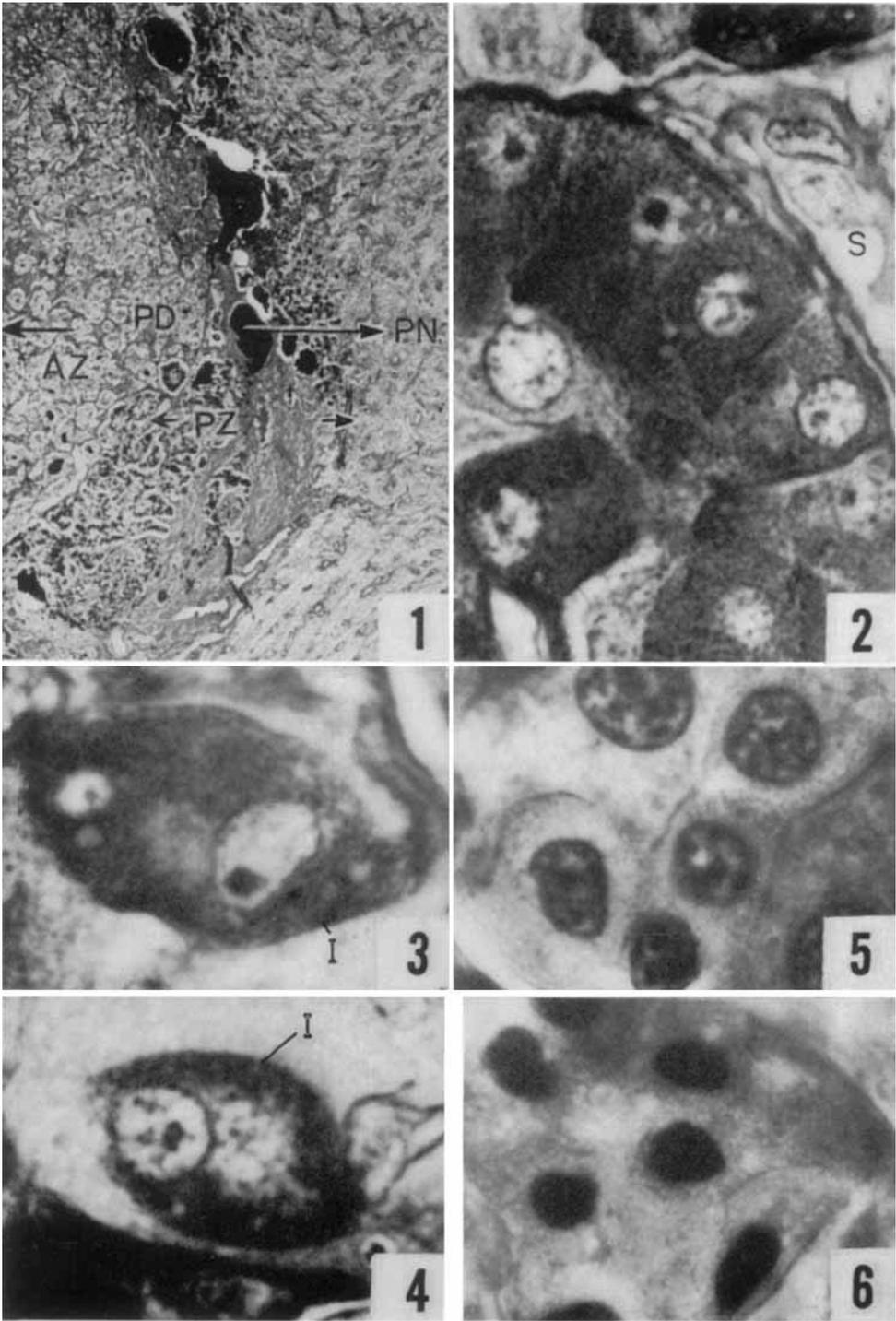
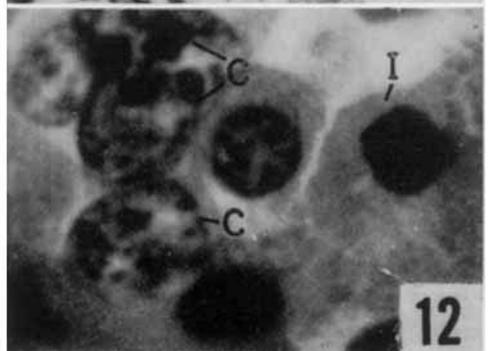
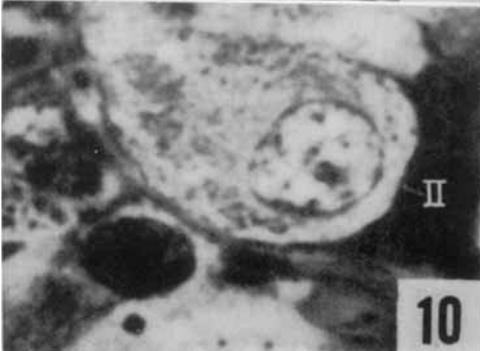
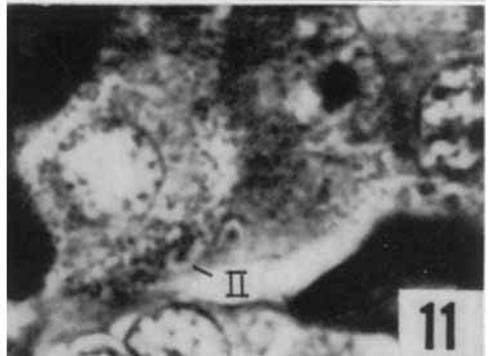
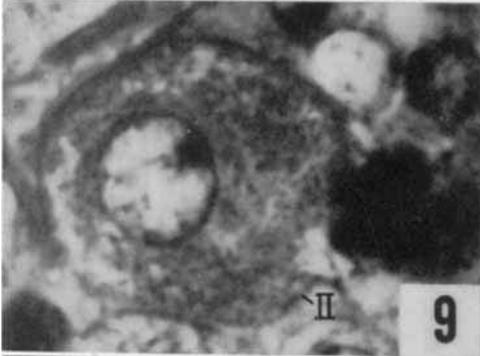
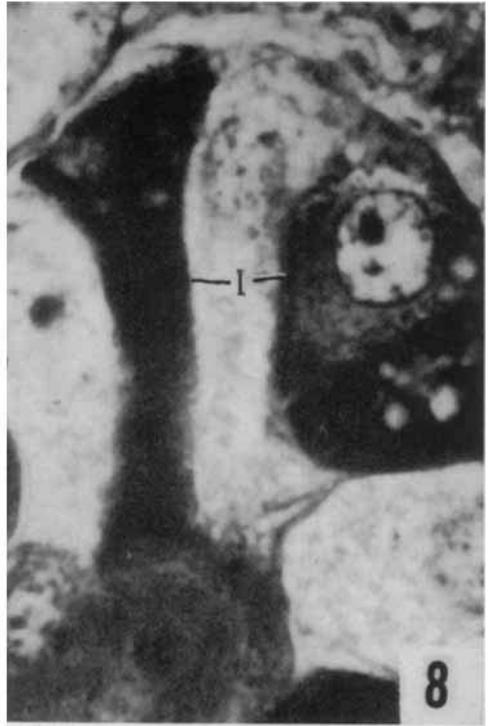
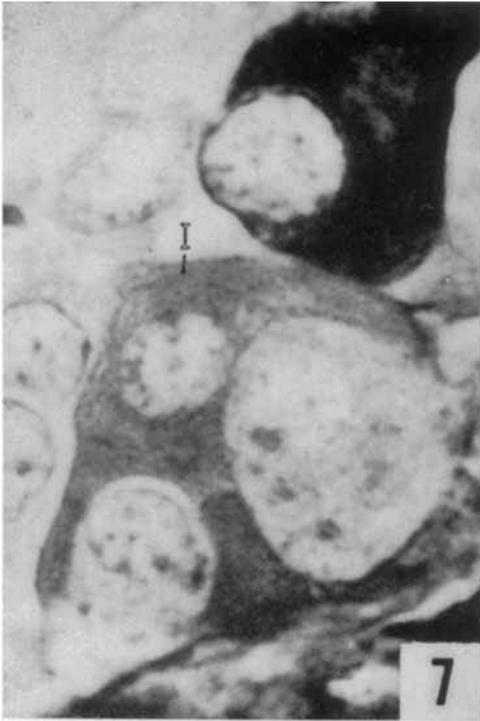
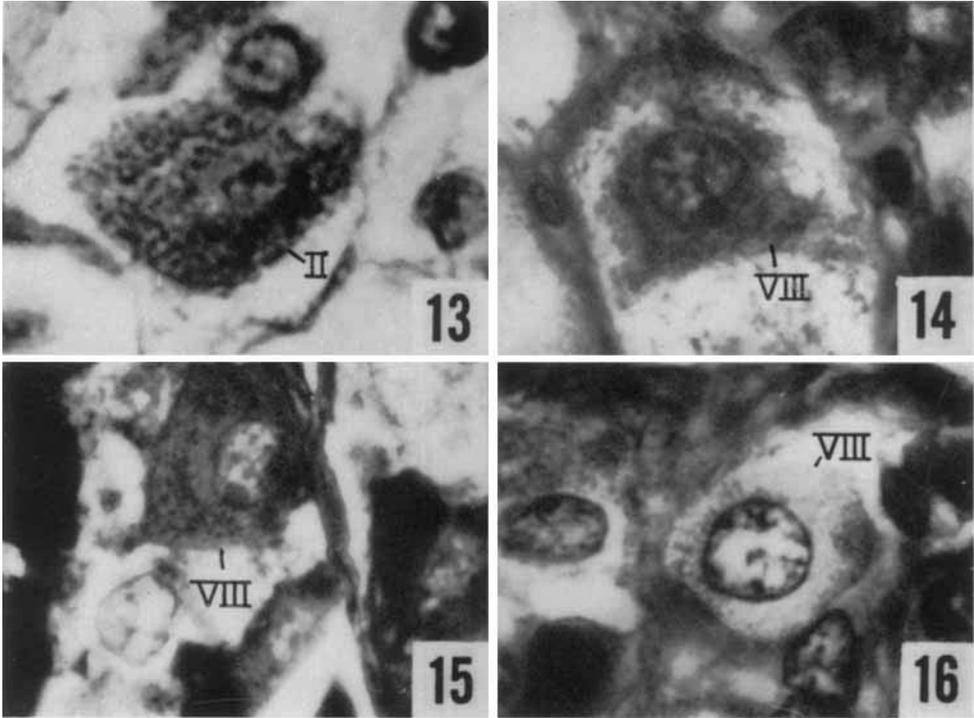


PLATE 2

EXPLANATION OF FIGURES

- 7-8 Pleimorphic forms of the type I cell. Aldehyde fuchsin and Masson. $\times 2300$.
- 9 A type II cell. Note the coarseness of the cytoplasmic granules as contrasted with the granules of the type I cell. Herlant tetrachrome. Interference green filter. $\times 2600$.
- 10-11 Type II cells after staining with Masson trichrome. $\times 2300$.
- 12 Chromophobes (c) and other cell types. Note the paucity of cytoplasm and lack of granulation in the chromophobic cells. Herlant tetrachrome. $\times 2000$.





EXPLANATION OF FIGURES

- 13 A type II cell from the hypophysis of a term fetus. The cell contains numerous granules and is larger than the adult type II cell. Herlant tetrachrome. Interference green filter. $\times 2000$.
- 14 A type VIII cell. The poor preservation of the cytoplasm is characteristic of this cell. Herlant tetrachrome. Interference green filter. $\times 2000$.
- 15-16 Type VIII cells exhibiting differences in granulation. Observe the prominent nucleolus in the cell in figure 15. Note the similarity in appearance between the cell in figure 16 and the type II cells. Aldehyde fuchsin and Masson. $\times 2000$.