A Histochemical Study of Mucoid Cells in the Pars Distalis of the Human Hypophysis [1]

JAMES L. CONKLIN

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

ABSTRACT   Sections of the human hypophysis were fixed in either formalin or Bouin's fluid and stained by a variety of mucoid and acid stains in order to demonstrate the staining characteristics of the mucoid cells of the *pars distalis*.

Five types of mucoid cells were revealed and designated as cell types III, IV, V, VI and IX according to the classification system of Ezrin. Cell types III, IV, V and VI were present throughout the *pars distalis* while cell type IX was observed only in the posterior zone.

The cells were tinctorially distinguished by the PAS-orange G affinity of the type III cell, the alcian blue-PAS reactivity of the type IV cell, the alcian blue staining of the type V cell and the affinity of the type VI cell for alcian blue-PAS-orange G. Cell type IX was recognized by the affinity of its cytoplasmic granules for most of the stains employed. Modified and degranulated forms of these cells were observed and are also described.

In a previous paper (Conklin, '66) some of the factors contributing to the confusion surrounding hypophyseal cytology were reviewed. This discussion of interpretation of pituitary cytology is applicable not only to the acidophilic and chromophobic cells (Conklin, '66) but also the mucoid cells.

In the present investigation, a variety of staining procedures was employed to demonstrate the staining characterisics of the mucoid cells of the human hypophyseal *pars distalis*. In this way it was hoped that the identity and characteristics of various mucoid cell types could be definitively established.

MATERIALS AND METHODS

The material studied and the composition of some of the stains employed have been described (Conklin, '66). The remainder are summarized below and in table 1.

Aldehyde fuchsin was prepared with basic fuchsin (C.I. 42500) according to the method of Gomori ('50). Aldehyde thionin was prepared as reported by Ezrin and Murray ('63) with thionin obtained from the Coleman and Bell Co. (Lot no. 61105) and Chroma (C.I. 52000).

The reagents for the colloidal iron and PAS procedures were prepared as described by Mowry ('58).

Alcian blue 8GX (C.I. 74240) was employed as an 0.1% solution in 95% alcohol or 3% acetic acid. Acidified resorcin fuchsin was employed as described by Krutsay ('60). Oxidizing procedures were utilized as called for in various techniques. In addition, sections were stained without prior oxidation.

The oxidizing procedures employed were:

1. Lugol's solution: Weigert's variation (Humason, '62), for ten minutes followed by a ten minute bleach in 5% sodium thiosulfate; 2. periodic acid: 0.5% periodic acid in 70% alcohol, for ten minutes; 3. performic acid: generated by combining 90 cm³ 98% formic acid, 10 cm³ 30% hydrogen peroxide and 1 cm³ concentrated sulfuric acid two hours prior to use, for five minutes; 4. acidified potassium permanganate: 0.5% sulfuric acid and 0.5% potassium permanganate (1:1), for two minutes followed by a one minute bleach in 2% potassium metabisulfite.

In contrasting the results obtained with the various procedures the criteria described previously (Conklin, '66) were again employed.

OBSERVATIONS

An attempt was made to identify all the cells in the *pars distalis* and to describe the tinctorial and morphological characteristics of each type of cell. The findings were compared with those in earlier investiga-

TABLE 1

Methods

Periodic acid Schiff (PAS)	Mowry's modification ('58)
Alcian blue, aldehyde fuchsin	Adams and Swettenham ('58)
Alcian blue, PAS, orange G	Gomori ('50)
Aldehyde fuchsin, Masson trichrome	Elftman ('59)
Aldehyde fuchsin, PAS, orange G	Romeis ('40)
Aldehyde fuchsin, Romeis counterstain	Paget and Eccleston ('60),
Aldehyde thionin, PAS, orange G	Ezrin and Murray ('63)
Colloidal iron, PAS, orange G	Ezrin et al. ('58)
Herlant's tetrachrome	Herlant ('60)
Resorcin fuchsin	Krutsay ('60)

tions, and an effort was made to ascertain reasons for discrepancies.

The system of Ezrin ('63) was used for designating the several cells. In this system the chromophilic cells are indicated by Roman numerals I-VIII, definitive acidophils being types I, II, and VIII and mucoid cells being types III–VII. The staining reactions of nine cells are summarized in table 2, type IX being a mucoid cell found only in the posterior zone of the *pars distalis* (Conklin, '66).

Anterior zone mucoid cells

Upon evaluation of the staining reactions (table 2) it was evident why many investigators failed to make a distinction between the four cell types. No mucoid stain was exclusively specific for a single cell type. In addition, the staining reactions were influenced by pretreatment of the tissue sections. This was illustrated by staining with aldehyde fuchsin. No oxidation or oxidation in Lugol's solution or periodic acid before aldehyde fuchsin gave similar results. The only difference was the increased intensity of the staining which occurred when oxidation was employed. After performic acid or permanganate oxidation, staining with aldehyde fuchsin was even less precise and various acidophils were also stained. Similar nonspecificity was also exhibited by alcoholic alcian blue. Aqueous alcian blue was more specific after performic acid treatment of the tissues as was aldehyde thionin following permanganate oxidation. Resorcin fuchsin yielded results which were comparable to those obtained with aldehyde fuchsin. Three of the four mucoid cell types were stained by the PAS reaction.

The aldehyde thionin-PAS-orange G techque gave variable results. Staining with aldehyde thionin was inconsistent. Thionin obtained from Chroma gave the most intense staining when a suitable stain was obtained. The intensity of staining with either aldehyde thionin or colloidal iron was never as great as that exhibited by other procedures. Also, the results after aldehyde thionin-PAS-orange G were found to be dependent on the concentration of sulfuric acid employed in the oxidizing solution (table 3).

The most specific staining of mucoid cells, which also resulted in distinct color difference, was achieved by the use of the combined performic acid-alcian blue-PAS-organge G procedure. The basis for the specificity was (a) the PAS-orange G affinity of the type III cell, (b) the alcian blue-PAS reactivity of the type IV cell, (c) the unique staining of the type V cell by alcian blue and (d) the affinity of the type VI cell for alcian blue, PAS and orange G (figs. 22–25).

Description of cell types

Cell type III. The type III cell presented the characteristics of an acidophilic-mucoid cell (figs. 2–4). The cells occasionally occurred in clusters in the central area of the gland but were most frequently present as scattered, individual cells in the posterior part of the anterior zone. Typically, the nuclei were eccentric and contained a prominent nucleolus. Chromatin varied from sparse to dense and was usually lightly stained. The cells were round or oval and the diameter ranged from 20–25 μ. The intracytoplasmic granules were numerous and varied in size. Cytoplasmic vacuoles were rarely observed and 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
PAS			+++	+		+++	NR		+++
Aldehyde thionin, permanganate oxidation				+	+++		NR		
Aldehyde fuchsin, no oxidation			+	+	+++	+	NR		+
Aldehyde fuchsin, periodic acid oxidation			+	+++	+++	+	NR		+
Aldehyde fuchsin, performic acid oxidation			+++	+++	+++	+++	NR	+	+
Aldehyde fuchsin, permanganate oxidation	+		+++	+++	+++	+++	NR	+	+++
Alcian blue, aq., performic acid oxidation				+++	+++	+	NR		
Alcian blue, alcoholic, performic acid oxidation	+	+	+	+++	+++	+	NR	+	+
Resorcin fuchsin,[1] permanganate oxidation		+	+++	+++	+++	+++	NR	+	+++
Colloidal iron				+	+++		NR		

[1] The staining response of resorcin fuchsin is identical to aldehyde fuchsin after all oxidation procedures with the exception of the staining of cell types I and II.

+, ++ and + indicate either a strong or moderate affinity for the stain.

A blank indicates either a weak or negative staining response.

NR, no response, is indicated for this cell because it is a modified form and will be considered separately.

TABLE 3

Tinctorial properties of pituitary mucoid cells

Method	III	IV	V	VI	IX
PAS	red	pink	pale pink	red	red
Performic acid, alcian blue, PAS	red	blue-purple	turquoise blue	red-purple	red
Performic acid, alcian blue, PAS, orange G	umber	blue-grey	turquoise blue	brown	umber
Aldehyde thionin, PAS [1]	red-purple	light blue to unstained	purple	red-purple	red-purple
Aldehyde thionin, PAS, orange G [1]	bright red	light grey blue to colorless	purple	red-purple	bright red
Aldehyde thionin, PAS, orange G [2]	bright red	light purple	purple	red	bright red
Colloidal iron, PAS, orange G	bright red	red-purple	blue-purple	red	bright red
Herlant tetrachrome	blue	blue	pale blue	blue-black	blue
Aldehyde fuchsin, without oxidation, Romeis' counterstain	dark blue	red-purple	purple	red-purple	red-purple
Aldehyde fuchsin, without oxidation, Masson trichrome	light red-purple	dark red-purple	purple	dark red-purple	dark red-purple
Aldehyde fuchsin, periodic acid oxidation, Masson trichrome	red-purple	red-purple	purple	red-purple	red-purple
Performic acid, alcian blue, aldehyde fuchsin	purple	purple	blue-black	purple	purple

[1] The acidified permanganate was prepared with 1% H_2SO_4.
[2] The acidified permanganate was prepared with 0.5% H_2SO_4. (Ezrin and Murray, '63).

dense granulation of the cell prevented observation of a juxtanuclear zone. The cells were present in pituitaries from subjects of all ages. In summary, the type III cell contained cytoplasmic granules which were strongly PAS positive while the cytoplasm exhibited a strong affinity for orange G and light green.

Cell type IV. The type IV cells contained granules which were stained by both mucoid and acid stains (figs. 8–10). They occurred most frequently in the inferior and anterior areas of the gland and were rarely present in the acidophil zone. The cells, about 18–22 μ in diameter and usually oval in shape, occurred in groups and were often mixed with type III and type VI cells. The nuclei were eccentric, contained a prominent nucleolus and exhibited a coarse chromatin pattern. Large vacuoles were often persent in the cytoplasm (fig. 10). In summary, the type IV cell was characterized by numerous granules which were stained by both acidophilic and mucoid stains. Specific identification of the cell was possible because of the weak staining of its granules by the PAS reaction.

Cell type V. The type V cells (figs. 11–14) occurred throughout the gland but were least frequent in the inferior and anterior margins of the anterior zone.

While many of the cells were small, 12–15 μ in diameter, others were 20–25 μ in diameter. They varied in shape from round to pyramidal. They occurred frequently as single, scattered cells interspersed between other cell types except in the junctional area of the anterior and posterior zones. In this region, the cells were quite numerous and were clumped together in irregulare groups. After the colloidal iron and aldehyde thionin procedures, scattered, coarse purple granules and vesicles were prominent in the cytoplsam (fig. 13). This was in contrast to the staining after alcian blue when fine granules were colored a brilliant, turquoise blue (fig. 14). Most of the large, alcian blue positive cells were unstained by aldehyde thionin and colloidal iron. The variable appearance of the stained material suggested that different substances were being stained.

The type V cell was identified by its strong affinity for most mucoid stains, except PAS, and lack of affinity for acid dyes. Of all the mucoid cells this was the only cell type which was not also acidophilic and was therefore a "pure" mucoid type.

Cell type VI. The type VI cell was almost identical in appearance to the type IV cell (figs. 15–17). Both cell types were of the same size and shape. They exhibited similar nuclear and cytoplasmic characteristics with regard to chromatin, granules and vacuoles. The cells differed only in distribution within the gland and the tinctorial properties of the granules after certain stains. The type VI cells were most frequent in the superior and central areas of the gland. They were less frequent in the inferior and anterior areas and rarely occurred in the acidophil zone. The type VI cell could be identified because its granules were moderately stained by the PAS reaction, alcian blue and orange G. This cell type was classified as an acidophilic-mucoid cell.

Modified and degranulated cells

In the modified type III cell, the cytoplasm was devoid of granules except in a juxtanuclear position (fig. 6). The granules were stained by aldehyde fuchsin, aldehyde thionin, alcian blue or PAS. After an alcian blue-PAS sequence they were alcian blue positive.

The degranulated type III cell was identified by the light green or pale blue staining of the cytoplasm (fig. 7). In all other properties it was similar to the modified and typical type III cells.

The modified type IV cell contained a few scattered granules and numerous vesicles (fig. 10). Both the granules and the vesicles exhibited the same staining characteristics as the typical type IV cell. Some degranulated type IV cells could be identified by morphological features. However, it is doubtful that *all* degranulated cells of this type were identified.

Degranulated type V cells, if present, were not identified. Partially degranulated cells of this type were observed in one gland and were identified by the tinctorial properties of the granules.

The type VI cell exhibited two altered forms. In the first, sparse granules and vacuoles were present in the cytoplasm

(figs. 17–19). These exhibited staining properties similar to the granules of the unmodified cell. Many degranulated and partially granulated cells were also observed. The degranulated cells were slightly larger than the granulated type VI cell but other characteristics were similar.

Posterior zone mucoid cells

In adult pituitaries, mucoid cell types III, IV, V and VI were present in the posterior zone (figs. 26–29) as wall as a type designated as cell type IX. In the pituitaries of a child and a fetus, most of the cells in the posterior zone were of the type IX variety.

Cell type IX. Morphologically, cell type IX resembled the type IV and type VI cells (figs. 26–28). It was usually oval in shape with an eccentric nucleus. It was an acidophilic-mucoid cell (tables 2, 3). It was similar in some staining properties to the type III cell and in others to cell types IV and VI. It was this duality of properties which revealed the type IX cell since it demonstrated no unique histochemical properties. Type IX cells were not observed in the anterior zone although the lack of unique staining properties may have prevented their recognition.

DISCUSSION

In the present study, five types of mucoid cells have been found in the *pars distalis.* Four of these cells were in the anterior zone and were also variably present in the posterior zone. The fifth mucoid cell (type IX) was observed only in the posterior zone of the *pars distalis.* The correlation between the cells observed in this study and those previously reported is presented in table 4. Not included in table 4 is the cell designated as type VII by Ezrin ('63). The cell is a modified type III cell and probably corresponds to the Crooke-Russell cell (Crooke, '35).

In comparing the staining properties of the five cell types it was apparent that specific staining of the four anterior zone mucoid cells was achieved only after two techniques: colloidal iron-PAS-orange G (Ezrin et al., '58) and alcian blue-PAS-orange G (Adams and Swettenham, '58; Pearse and Van Noorden, '63). Techniques utilizing aldehyde fuchsin (Elftman, '59) or aldehyde thionin (Ezrin and Murray, '63) did not selectively color all of the cell types. The same limitation followed the use of the Herlant ('60) tetrachrome procedure. The aldehyde thionin technique did produce selective staining when the oxidizing agent was modified (see table 3) as suggested by Elftman ('59). Of the methods that were selective, the alcian blue-PAS-orange G technique produced the most intense and most consistent staining.

Fand ('55) reported that the posterior zone cells differed in enzymatic activity from cells of the anterior zone. Other investigators (Pearse, '56; Purves and Bassett, '63) have questioned this view. In this study, it was observed that the cells of the posterior zone were variable in type. Typical anterior zone cells were often observed in this area in adult pituitaries (figs. 26–29). A fifth cell type was also observed in this region in all pituitaries. This cell exhibited histochemical properties that were similar in one or more respects to those of cells in the anterior zone. It is possible that the type IX cell was observed by Fand and not by Pearse or Purves and Bassett. The technique employed by the latter investigators would not distinguish the type IX cell from other mucoid cell types.

In the present study mucoid cells were observed in the pituitaries of subjects of all ages. This is in contrast to the report of Swanson and Ezrin ('60) who observed no delta (type V) cells in pituitaries of subjects under ten years of age. Midgley ('63) found anti-LH fluorescent cells in pituitaries of children and newborn infants as well as subjects of other ages. The LH cells corresponded to the turquoise-colored, alcian blue positive cells (type V) (figs. 11–14). The failure of Swanson and Ezrin to demonstrate these cells in young subjects may be because of the technique employed. Not all of the type V cells are demonstrated by the colloidal iron or aldehyde thionin procedures. The reasons for this are not clear. The fact that Midgley demonstrated fluorescence in degranulated type V cells suggests that the granules seen after histochemical procedures are either a particular form of the hormone or a "carrier" substance.

TABLE 4

Comparison of pituitary cell types

This study	Type III	Type III altered	Type IV	Type IV altered	Type V	Type V altered	Type VI	Type VI altered
Ezrin ('63)	III		IV		V		VI	
Ezrin ('63)	beta 1	beta 3	beta 2	gamma	delta 1		delta 2	gamma
Pearse ('52)	maximal mucoid, punctate mucoid	Crooke-Russell	disperse mucoid	vesiculate mucoid			maximal mucoid, intermediate mucoid	vesiculate mucoid
Adams and Swettenham ('58)	R cell		S cell	amphophils	S cell		R cell	
Pearse and Van Noorden ('63)	R cell	R cell	S2 cell	S2 cell	S1 cell		S2 cell	S2 cell
Jerasik ('61)	Bg2		Bt1		Bt2		Bg1	
Burt and Velardo ('54)	normal basophils	hyaline basophils	amphophils	amphophils			normal basophils, amphophils	
Herlant ('64)			delta		gamma		beta	
Romeis ('40)	delta		beta	gamma			delta	gamma
Crooke ('35)		Crooke-Russell cell, hyaline cell						

All immunofluorescent studies have localized ACTH in a mucoid cell type. Pearse and Van Noorden ('63) designated this cell as the R 2 cell because of its tinctorial properties after the alcian blue-PAS-orange G procedure. The modified forms of the R 2 cell are the Crooke-Russell cell and the Crooke hyaline cell. These cells correspond to the type III and modified type III cells. As Pearse noted, the properties of the granules undergo changes when the cells are modified; they no longer stain with PAS and are alcian blue positive.

The source of the gonadotropic hormone, LH(ICSH), has been identified as the S 1 (type V) cell by Midgley ('63). Herlant ('64) and Pearse and Van Noorden ('63) have attributed LH secretion to a PAS positive cell. In vitro studies of the PAS reactivity of LH are in agreement with this conclusion (Fand and Thorell, '62). The weak PAS reactivity of the LH-cell suggests that the hormone may not be demonstrated in situ by histochemical methods.

The sources of FSH and TSH have been tentatively identified. Cruikshank and Currie ('58) localized TSH in one of the mucoid cells. Koffler and Fogel ('64) localized FSH in one of the aldehyde fuchsin positive cells. The S 2 cell described by Pearse and Van Noorden contains granules which are both PAS and alcian blue positive. In the present study, it was noted that after the alcian blue-PAS or alcian blue-PAS-orange G procedures the S 2 cells could be divided into two distinct subtypes (table 3, figs. 22–25). Rather than transitional types, these cells are the probable sources of FSH and TSH. Since the type IV (S 2) cell is more numerous in fetal pituitaries (unpublished observation) it is the probable source of TSH. The type VI (S 2) cell is more prevalent in adults, especially postmenopausal women, and may be the source of FSH.

For the present, the function of the type IX cell is unknown. Its presence in the region comparable to the pars intermedia of other species is suggestive of a comparable function.

ACKNOWLEDGMENTS

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1 The junctional zone between the *pars distalis* (PD) and *pars nervosa* (PN). Anterior zone (AZ) and posterior zone (PZ) of the *pars distalis*. The dark areas (upper center) are colloid-filled remnants of the residual cleft. Aldehyde fuchsin and Masson. × 200.

2 An oval, type III cell. Note the prominent nucleolus and coarse cytoplasmic granules. Interference green filter. Alcian blue-PAS-orange G. × 2000.

3 A type III cell. Observe the abundant cytoplasmic granules. Colloidal iron-PAS-orange G. × 23000.

4 A round, type III cell. Note the coarseness of the cytoplasmic granules. Aldehyde thionin-PAS-orange G. × 2300.

5 A partially degranulated type III cell. Aldehyde fuchsin and Masson. × 2000.

6 A modified type III cell. Cytoplasmic granules are present only in the juxtanuclear area. Aldehyde fuchsin and Masson. × 2000.

7 A degranulated type III cell. Herlant tetrachrome. × 2000.

PLATE 1

PITUITARY MUCOID CELLS
James L. Conklin
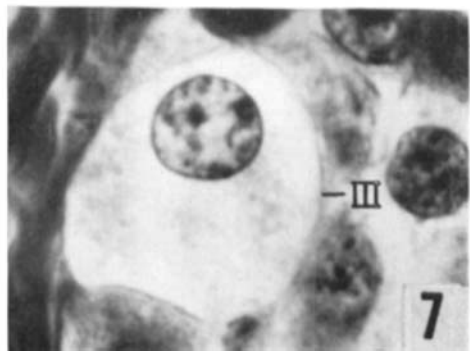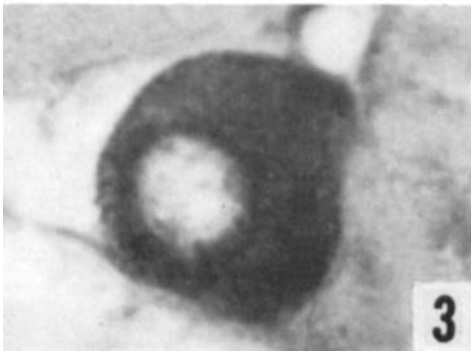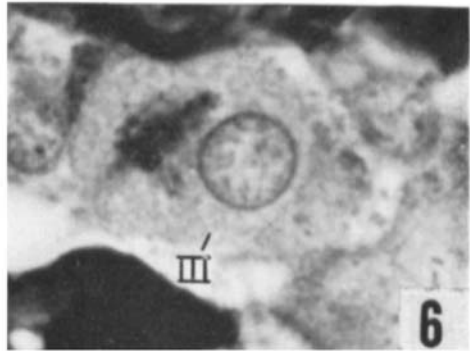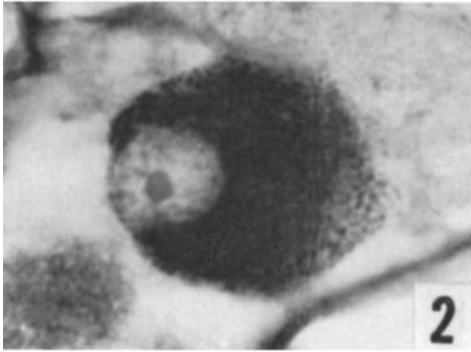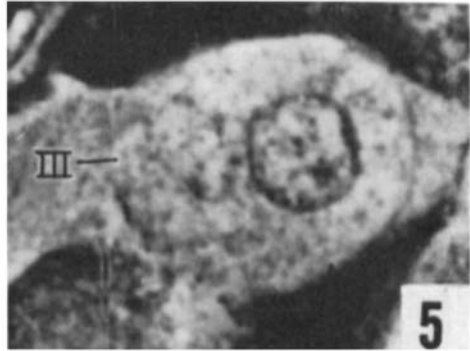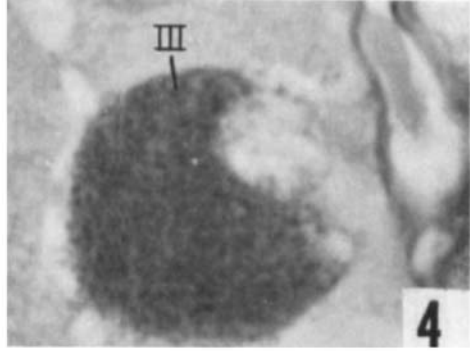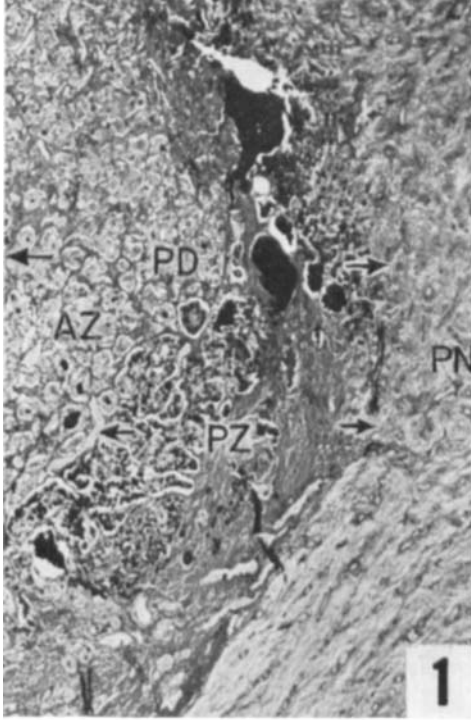
8 An intensely granulated, oval, type IV cell. Alcian blue-PAS-orange G. × 2000.

9 A round, type IV cell. Alcian blue-PAS-orange G. × 2000.

10 A cytoplasmic vesicle (V) in a type IV cell. Alcian blue-PAS-orange G. × 2000.

11 A field of type V cells. Note the differences in their appearance when contrasted with figures 12–14. Interference green filter. Aldehyde fuchsin and Masson. × 1600.

12 A type V cell exhibiting light cytoplasmic staining. Herlant tetrachrome. × 2000.

13 A type V cell. Note the coarse, darkly stained droplets or vesicles in the cytoplasm. Aldehyde thionin-PAS-orange G. × 2000.

14 A typical type V cell. Note the difference in the appearance of the cytoplasm when compared with the cell in figure 13. Alcian blue-PAS. × 2000.

15 Two oval type VI cells. Note the density and size of the cytoplasmic granules. A nuclear counterstain was not employed. Aldehyde thionin-PAS-orange G. × 2300.
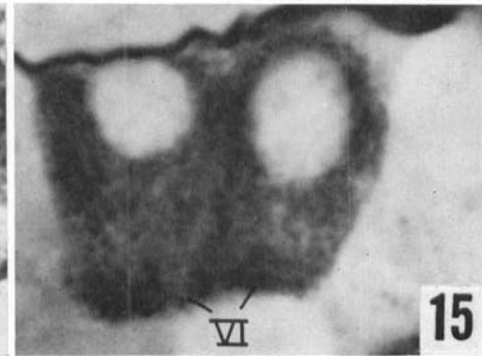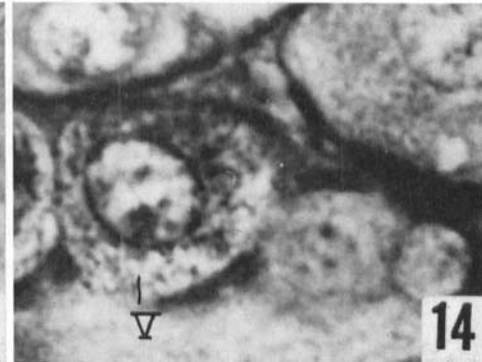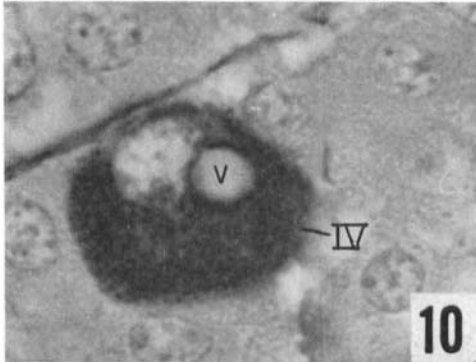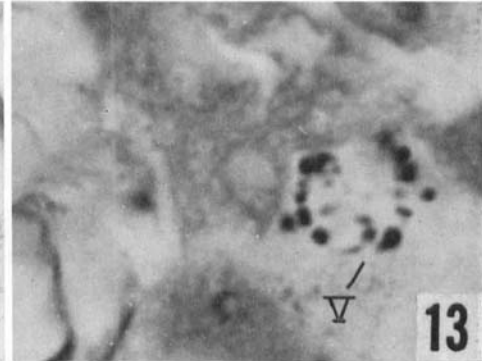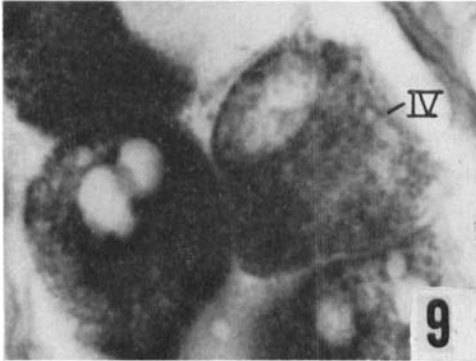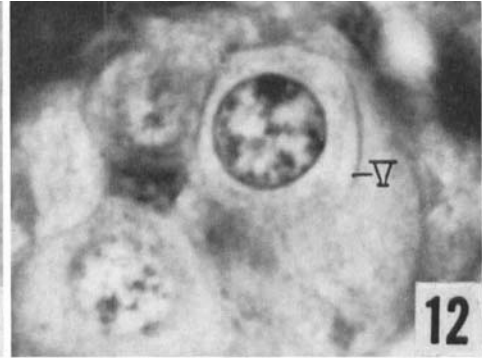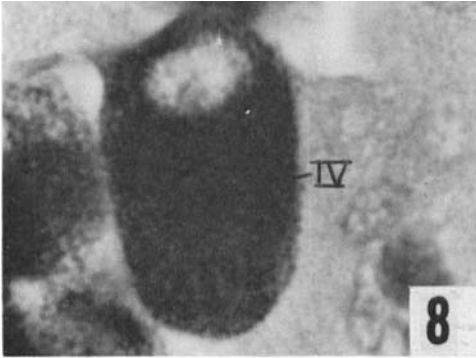
16 A round type VI cell. Aldehyde thionin-PAS-orange G. $\times$ 2300.

17 A vacuolated type VI cell. Interference green filter. Herlant tetra-chrome. $\times$ 2000.

18 A partially degranulated type VI cell. Note the centrioles in the juxtanuclear area. Herlant tetrachrome. $\times$ 2000.

19 Prominent, stained vesicles in a degranulated type VI cell. Aldehyde fuchsin and Masson. $\times$ 2300.

20 A mixture of cell types. Observe the weak staining of the type IV cell. Also note the intensity of staining and the variable morphology of the cells. Aldehyde thionin[1]-PAS-orange G. $\times$ 1400.

21 A binucleated type III cell and a small type VI cell. Note the similarity in the staining intensity of the granules in the two cells. Aldehyde thionin[2]-PAS-orange G. $\times$ 2000.
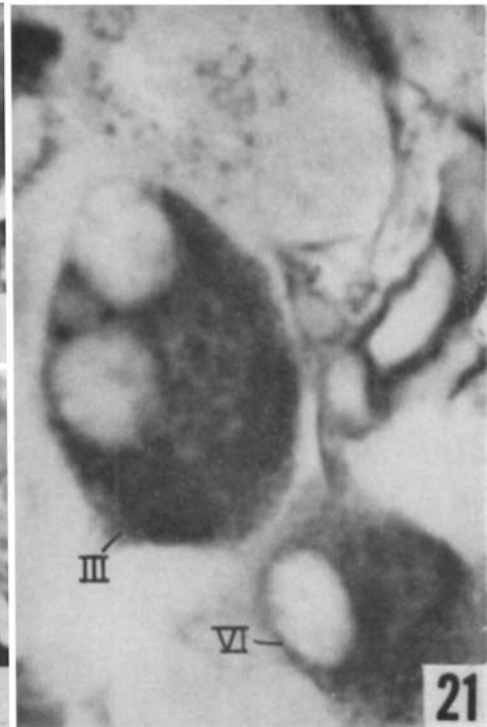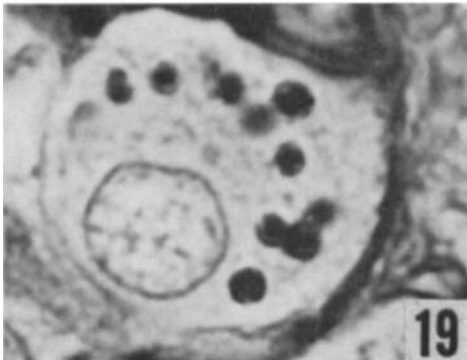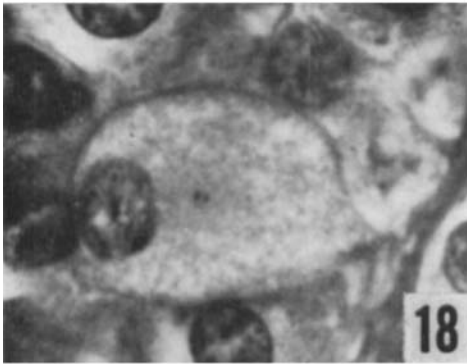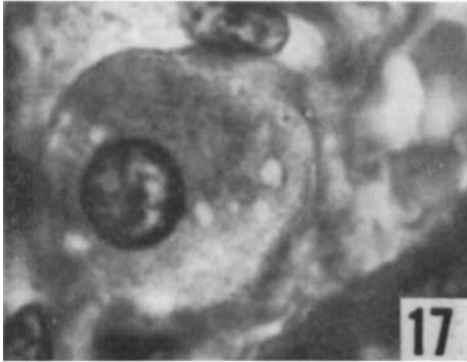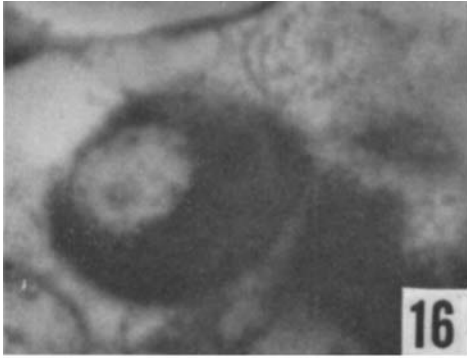
22   A variety of cell types. Note that the three different cell types exhibit slight differences in staining intensity while morphologic differences are not pronounced. Alcian blue-PAS-orange G. × 1600.

23   This field was photographed without a filter. Contrast with figure 25 which is of the same field. Differences in the staining of cytoplasmic granules are not apparent in this figure (23). Alcian blue-PAS-orange G. × 1600.

24   Cell types IV and VI. The use of a Wratten no. 22 filter illsutrates the difference between the granules of the two cell types. Alcian blue-PAS-orange G. × 1600.

25   Cell types III and VI photographed through a Wratten no. 22 filter. Note the contrast in the intensity of the granules in the two cell types. (See fig. 23.) Alcian blue-PAS-orange G. × 1600.

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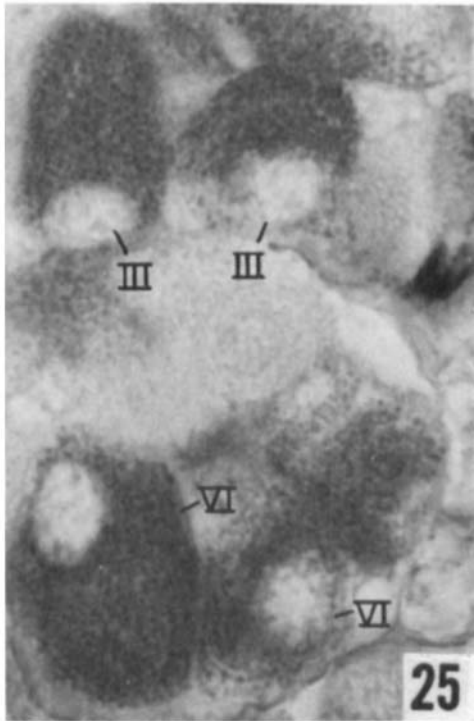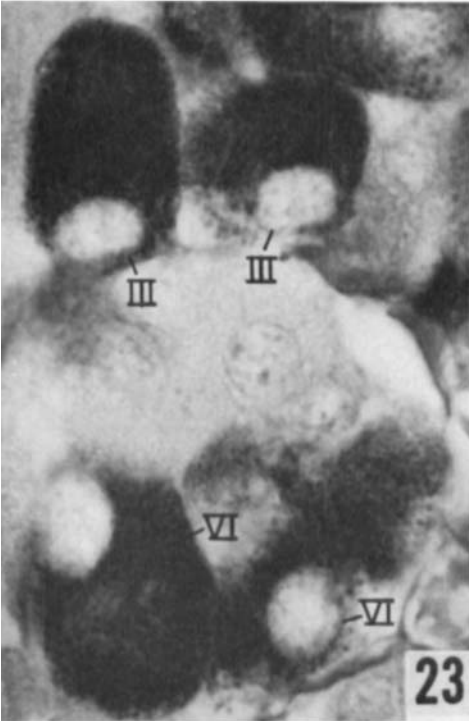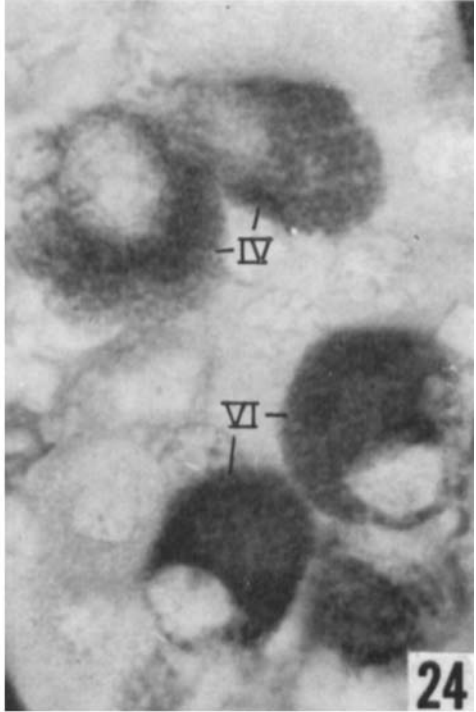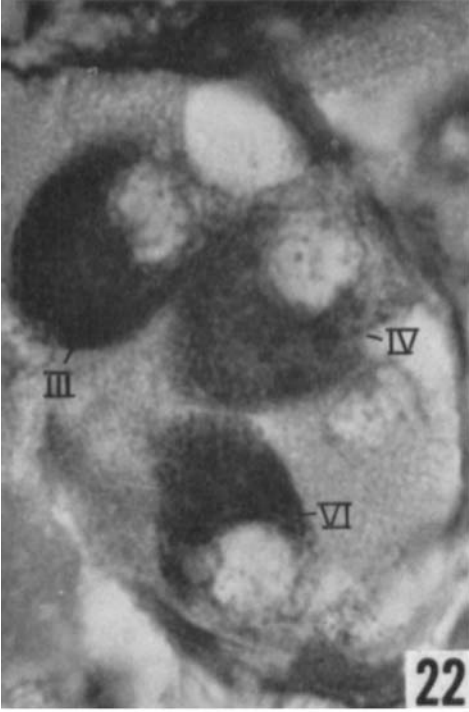PLATE 5

All figures are of cells in the posterior zone of the *pars distalis*.

26 The type III cell is degranulated. Contrast the type IX cell in this and figures 27–28. The poor cellular detail is the result of the poor cellular preservation in this area. Aldehyde fuchsin and Masson. × 1400.

27 A mixture of cell types. The more intense staining of the type IX cell is apparent in this field. Alcian blue-PAS-orange G. × 1600.

28 The type IV cell is a modified type. Aldehyde thionin-PAS-orange G. × 1600.

29 The type III cell is a modified type. Aldehyde fuchsin and Masson. × 2300.