Fine Structure of Trypsin-Dissociated Cells of the Rat Anterior Pituitary Gland[1] (36019)

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(Introduced by J. W. Craig)

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Portanova *et al.* (1) have described a trypsin technic for the dispersion of rat anterior pituitary tissue. The functional integrity of the isolated cells is indicated by their ability to retain ACTH against a large concentration gradient and to release ACTH into the incubation medium when exposed to corticotropin releasing factor (CRF). The structural integrity of these cells is the subject of the present report. Electron microscopic evidence is presented showing that trypsin-dissociated cells from the anterior pituitary are essentially intact and undamaged after 40 min of incubation, that five cell types are represented and that these cell types correspond to those seen *in situ*.

Materials and Methods. Suspensions of dissociated anterior pituitary cells from male rats were prepared by mechanical agitation of anterior pituitary quarters in Krebs–Ringer bicarbonate buffer (pH 7.4) containing glucose (KRBG) and 0.25% trypsin, as described by Portanova *et al.* (1). The cells were collected as a loose pellet by centrifugation at 100*g* for 30 min at 4° and samples of the unincubated cells were prepared for electron microscopy as described below. In those experiments where pituitary cells were incubated, the 100*g* pellet was resuspended to a concentration of 1 pituitary/ml in KRBG containing 0.5% bovine serum albumin and 0.3% lima bean trypsin inhibitor, at pH 7.4. One milliliter aliquots of the resuspended cells were pipetted into 10 ml Teflon beakers and incubated for 40 min at 37° under an atmosphere of 95% O_2:5% CO_2 in a Dubnoff metabolic shaker (66 oscillations/min). The incubated cells were collected by centrifugation at 600*g* for 5 min at room temperature.

Samples of the pellets from incubated or unincubated cell preparations were removed by microspatula and prepared for electron microscopy by methods similar to those previously described for trypsin-dissociated adrenal cells (2). Buffered 1% OsO_4 (3) was the fixative. Polyethylene microcentrifuge tubes were used to pack the samples into cohesive pellets (4). Epon 812 (5) was the embedding medium. Sections were doubly stained with uranyl acetate (6) and lead citrate (7), and then examined in the Philips 300 electron microscope.

Results. Figure 1 is a section through a group of unincubated anterior pituitary cells dissociated by the trypsin technic. Little clumping is seen even after the additional centrifugation step required for electron microscopic preparation. With few exceptions, these rounded or ovoid cells are intact and contain a rich array of cell organelles including secretory granules. Forty minutes of incubation was not associated with detectable necrosis or disruption of the plasma membrane. Examples of both incubated and unincubated cells are presented in the descriptions below.

We have been able to identify five different adenohypophyseal cell types (Figs. 1–5) in the suspensions. Our classification is based on properties of the cytoplasmic granules, which are summarized and contrasted in Table I. Each *in vitro* cell type is assigned a name according to the *in situ* type it resembles most closely (8, 9).

Figure 2 is a section through an incubated cell with granules of the smallest size encountered in our preparations, about

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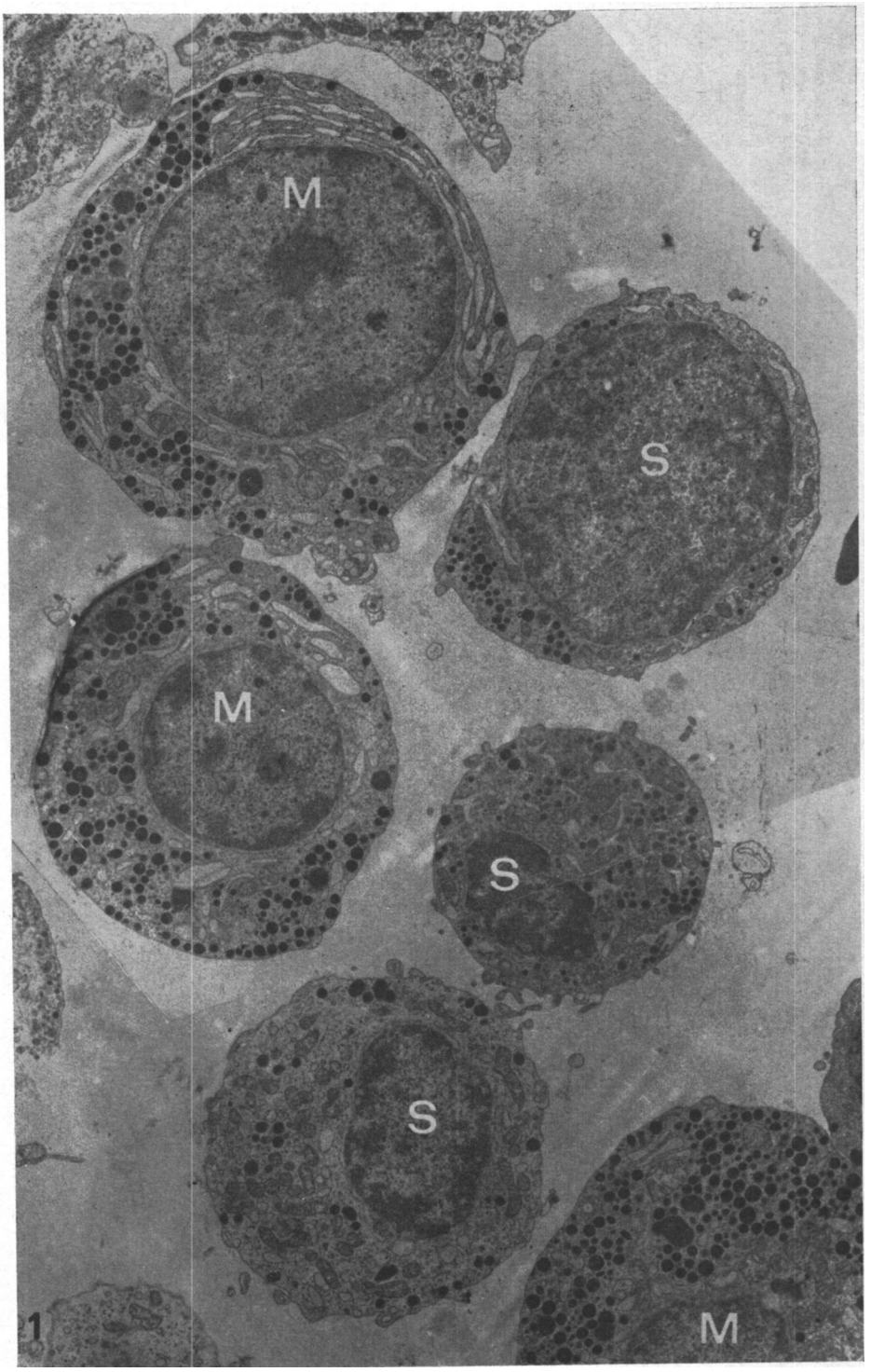

FIG. 1. Trypsin-dissociated cells from the rat anterior pituitary: Because of their granules, these cells in suspension resemble somatotrophs (S) or Mammotrophs (M) *in situ;* ×6300.

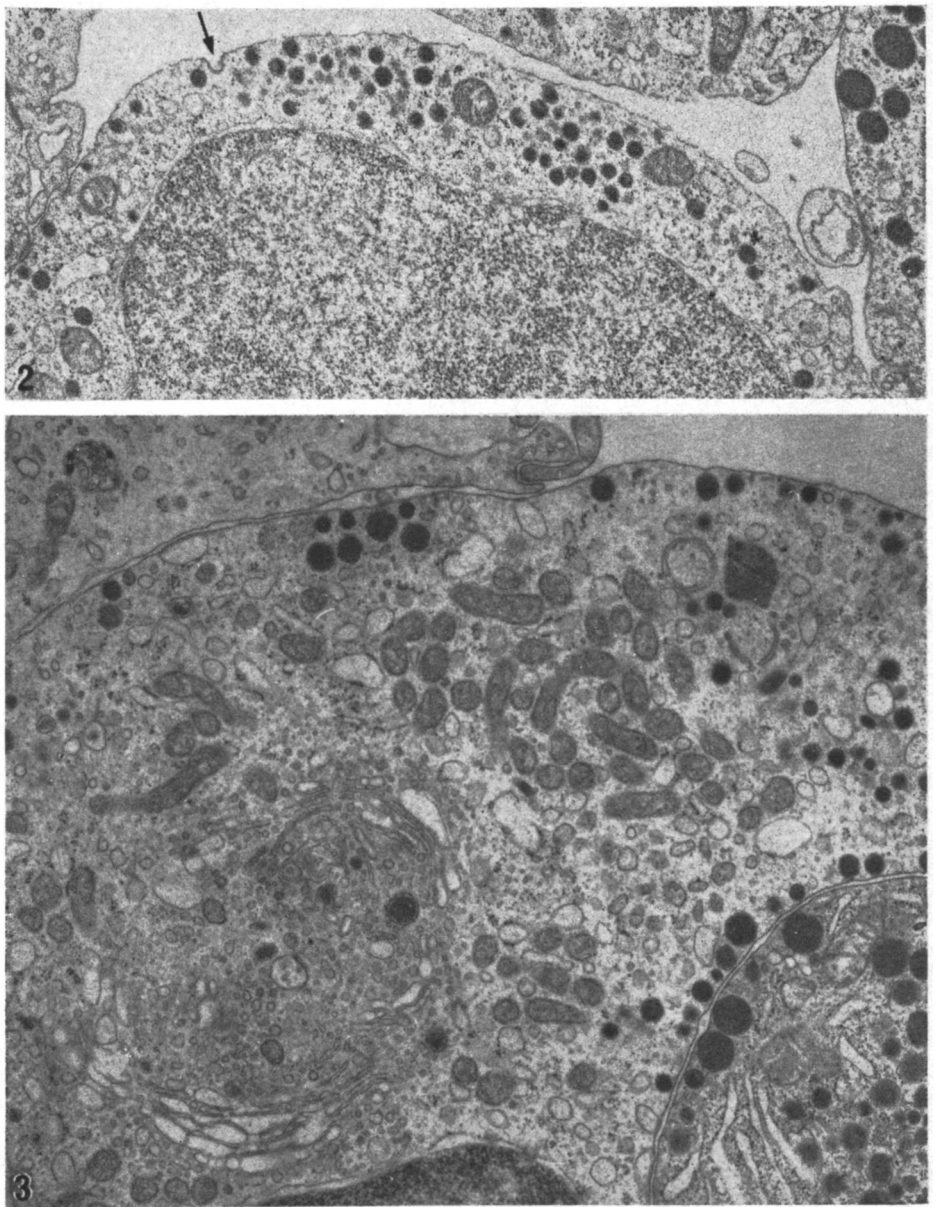

FIG. 2. Trypsin-dissociated thyrotroph from the rat anterior pituitary: The maximal diameter of the granules is about 1500 Å, and many are at least partially membrane-bound. A coated, inward fold of the plasma membrane is shown at the arrow; ×17,500.

FIG. 3. Trypsin-dissociated corticotroph from the rat anterior pituitary: Almost all the membrane-bound granules of about 2000 Å maximal diameter appear in the periphery of the cytoplasm near the plasma membrane. A small portion of a somatotroph is also shown (cf. Figs. 1 and 5); ×17,500.

1500 Å maximal diameter. The cell resembles those designated as thyrotrophs *in situ* (8). Although it is difficult to discern at the magnification of Fig. 2 (17,500), many of the granules appear to be at least partly bounded by membranes. A coated invagination of the plasma membrane shown in the upper left quadrant suggests bulk transport across the

TABLE I. Characteristics of the Cytoplasmic Granules of Trypsin-Dissociated Cells of the Rat Anterior Pituitary.

Approx maximal diam (Å)	Cytoplasmic distribution	Shape	Suggested *in situ* cell types	Fig. no.
1500	General or peripheral	Round or ovoid	Thyrotroph	2
2000	Peripheral	Round or ovoid	Corticotroph	3
2000	General	Round or ovoid	Gonadotroph[a]	4
4000	General	Round or ovoid	Somatotroph	1, 3, 5
7500	General	Irregular	Mammotroph	1

[a] An additional distinguishing feature is the presence of other granular bodies. These are pale spheroids of about 7000 Å maximal diameter, and show peripheral electron translucent halos within incomplete membranes.

membrane.

An unincubated cell containing membrane-bound granules of approximately 2000 Å maximal diameter occupies most of Fig. 3. These granules are peripherally distributed, a feature characteristic of the corticotroph (10, 11).

The unincubated cell in Fig. 4 is representative of another type which contains membrane-bound granules of 2000 Å maximal diameter. This cell type differs from that shown in Fig. 3 in that the granules are of general rather than of peripheral cytoplasmic distribution. Furthermore, pale spheroidal bodies of about 7000 Å maximal diameter, that are incompletely bound by membranes [similar to those of Fig. 5 in Ref. (12)], are found among the smaller, darker granules. These features are characteristic of a cell type that has been referred to as a gonadotroph (8).

Cells with membrane-bound granules of about 4000 Å maximal diameter are especially abundant in the suspensions. These cells, *in situ*, have been termed somatotrophs (8). An incubated example of this type of cell is seen in Fig. 5. (Unincubated examples are seen in Figs. 1 and 3.) A naked granule appears outside the cell but largely within a fuzzy coated invagination of the plasma membrane. Elsewhere on the periphery of the same cell, another indentation of the plasma membrane occurs, this one containing only some amorphous, fine stuff, which extends

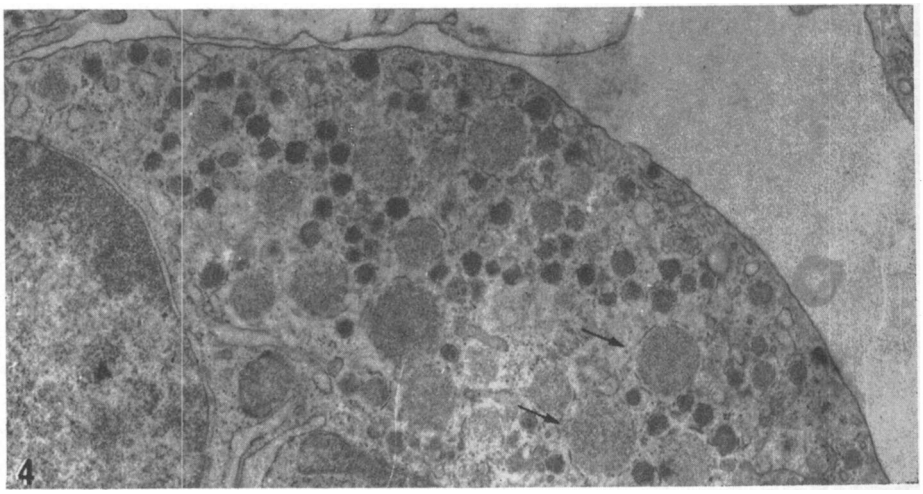

FIG. 4. Trypsin-dissociated gonadotroph from the rat anterior pituitary: Membrane-bound granules of about 2000 Å maximal diameter appear throughout the cytoplasm. Another feature distinguishing the gonadotroph from the corticotroph (Fig. 3) is the presence of pale spheroidal bodies (arrows) of about 7000 Å maximal diameter; ×17,500.

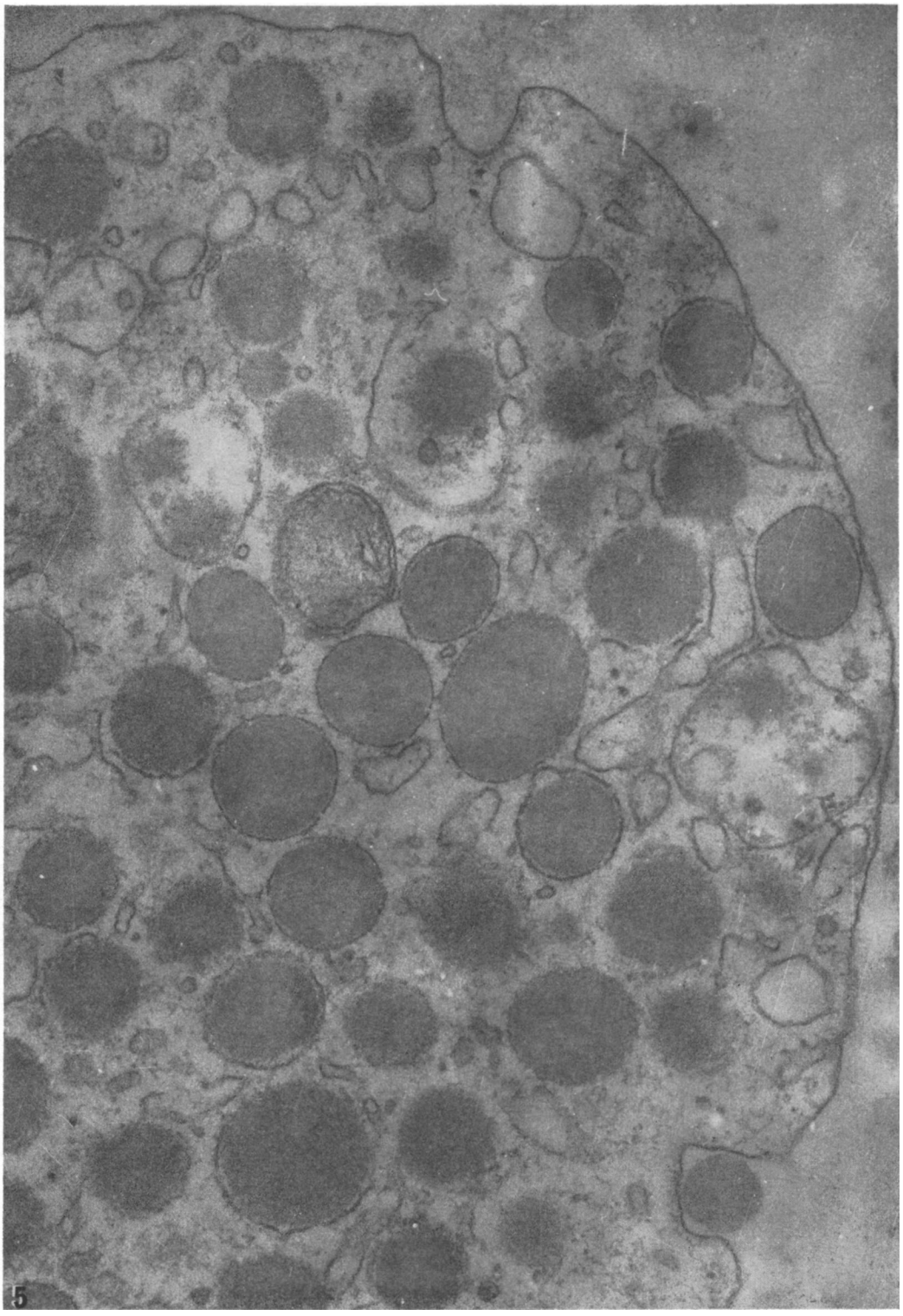

FIG. 5. Trypsin-dissociated somatotroph from the rat anterior pituitary: Granules of approximately 4000 Å maximal diameter. A granule without a membrane appears outside the

beyond the surface of the cell.

The largest granules shown are irregular in shape, perhaps due to coalescence of smaller granules. Such granules are peculiar to cells identified as mammotrophs, *in situ* (8). Figure 1 shows examples of unincubated cells of this type, containing membrane-bound granules of about 7500 Å maximal diameter.

Discussion. As a result of the correlation of morphological findings with increasing functional information, each of the anterior pituitary hormones has been assigned to a discrete cell type [corticotroph, thyrotroph, etc. (13)]. These designations stem largely from studies in which morphological changes in the anterior pituitary have been noted following a maneuver designed to alter hormone biosynthesis and/or release. Such maneuvers have included adrenalectomy, gonadectomy, thyroidectomy, administration of cortisol, of estrogen, or of thyroid hormone. The assumption has been made that the maneuver alters the metabolism of a specific anterior pituitary hormone and that this alteration is reflected in morphological changes in the cell and only in the cell responsible for the synthesis and release of that hormone. Such evidence is indirect and circumstantial. A direct approach is the peroxidase-labeled antibody technic of Nakane (14).

Our own work, also direct in approach, is aimed at separation of isolated cells of a suspension into relatively pure morphological populations, subsequent assay for the various tropic hormones and correlation of cell type with hormone content. The purpose of this paper is to present evidence that isolated anterior pituitary cells prepared by the trypsin technic are good starting material for separation into cell types. The cells appear structurally intact after 40 min of incubation. Furthermore, at least five discrete cell types appear that correspond to *in situ* cell types. Ishikawa (15) and Hymer and Evans (16) have demonstrated that a mixed population of dissociated anterior pituitary cells can be separated into groups distinguished by their morphological properties. However, hormone assays of these cells were not reported; therefore, it is not possible to determine if functionally distinct populations of cells were separated.

It should be noted that the identification of the rat corticotroph *in vitro* is more tentative than that of the other cell types because of controversy about its characteristics *in situ*. For example, although some workers (10, 11) consider the peripheral distribution of the cytoplasmic granules a distinctive feature of corticotrophs, Nakane (14) disagrees with this view.

The functional integrity of typsin-dissociated cells from the anterior pituitary has been demonstrated previously in several ways. Drs. G. Mozafforian and O. Pearson of the Department of Medicine, Case Western Reserve University, have shown that the cells contain a large amount of growth hormone. The cells also contain a relatively large amount of ACTH, a very small fraction of which escapes into the medium upon incubation. For example, in the case of the suspensions whose morphological characteristics are reported here, 100,000 cells contained approximately 3500 μU; whereas the medium contained only 91 $\pm$ 8 μU (mean $\pm$ one standard deviation) of ACTH after 40 min incubation. They released ACTH to the medium upon the addition of acid extracts of hypothalamic-medium eminence (HME) tissue. Upon addition of an extract equivalent to 0.1 of an HME, the medium contained 255 $\pm$ 42 μU of ACTH/100,000 cells. The release of hormone is inhibited when calcium ion or glucose is omitted from, or when physiological concentrations of corticosterone are added to, the incubation medium (1, 17, 18).

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cell in the lower right. Although external to the cell, the naked granule is largely surrounded by an invagination of the plasma membrane that has a fuzzy coat on its cytoplasmic side. The plasma membrane appears darker about the granule than it does elsewhere. At the upper center a similar fuzzy coated pocket of the plasma membrane appears, but devoid of any content other than a diffusion of very fine stuff some of which spreads beyond the pocket; $\times$60,000.

Other evidence of activity by these cells is indicated by the plasma membrane invaginations shown in the thyrotroph and somatotroph of Figs. 2 and 5, respectively. One of these contains a denuded granule. These examples suggest bulk transport of secretory granule material in isolated cells that is strikingly similar to that shown *in situ* in connection with release of anterior pituitary hormones (19, 20, 21). Although the possibility cannot be excluded, it seems unlikely that the extracellular granule shown in Fig. 5 was released *in situ* and then retained in a recess of the cell surface through all the cell dissociation and other procedures prior to visualization in the electron microscope.

Summary. Trypsin-dissociated cells from the rat anterior pituitary appeared essentially intact and undamaged in the electron microscope. Suspensions contained five cell types which correspond closely to those seen *in situ*: thyrotroph (with cytoplasmic granules 1500 Å approximate maximal diam), corticotroph (2000 Å), gonadotroph (2000 Å), somatotroph (4000 Å), and mammotroph (7500 Å).

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