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Agranular Stellate Cells (So-Called Follicular Cells) in Human Fetal and Adult Adenohypophysis and in Pituitary Adenoma

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Summary. Electron microscopic examination of the human fetal and adult adenohypophysis reveals a cellular network composed of epithelial stellate cells. These cells have no secretory granules. Their cytoplasm extends multiple processes and composes a supporting framework. The stellate cells are connected to each other or to adjacent granulated secretory cells by distinct junctional complexes. In the early stage of the fetus, these cells frequently form follicles and ductule-like intercellular spaces. In the adult such structures are rarely observed. The agranular stellate cells exhibit more cytological activity and possibly play some metabolic role. In two out of 18 cases of pituitary adenoma, these cells constitute a part of the tumor tissue. The stellate cells are interpreted as undifferentiated pituitary cells by some investigators. However, the marginal cells of Rathke's pouch and the primordial cells of the primitive adenohypophysis in the human embryo exhibit still more immature cytological characters and are thought to differentiate into granulated and agranular stellate cells.

Zusammenfassung. Elektronenoptische Untersuchungen der Adenohypophyse von menschlichen Foeten und Erwachsenen decken ein zelluläres Netzwerk auf, das von sternförmigen Epithelien, den sogenannten agranulären Sternzellen gebildet wird. Diese Zellen haben keine sekretorischen Granula, weisen jedoch multiple cytoplasmatische Fortsätze auf und formen auf diese Weise eine Art Stützgerüst. Die sternförmigen Zellen verbinden sich untereinander oder mit benachbarten granulierten sekretorischen Zellen mittels deutlichen Verbindungsapparaten. In foetalen Frühstadien bilden diese Zellen oft Follikel und Ductulus-ähnliche Intercellularräume. Beim Erwachsenen sind solche Strukturen jedoch selten. Die agranulären Sternzellen zeigen eine ausgeprägte celluläre Aktivität und haben möglicherweise eine gewisse metabolische Bedeutung. In zwei von 18 Fällen mit Hypophysenadenom bilden diese Zellen einen Teil des Tumorgewebes.

Die Sternzellen werden von einigen Autoren als undifferenzierte Hypophysenzellen angesehen. Die marginalen Zellen der Rathke'schen Tasche und die primordialen Zellen der primitiven Adenohypophyse menschlicher Embryonen weisen jedoch andere cytologisch unreife Strukturen auf. Wahrscheinlich können sich diese Zellen einerseits in granuläre sekretorische Hypophysenzellen und andererseits in agranuläre Sternzellen ausdifferenzieren.

Introduction

The utilization of the electron microscope and immunohistochemical methods made it possible to differentiate five types of hormone-producing cells in the adenohypophysis (Nakane, 1970). These cells have characteristic secretory granules in their cytoplasm and from their sizes, nature, and distribution one can identify each cell type. However, a small number of particular cells have no secretory

granules. Farquhar (1957) found agranular follicle-composing cells, so-called "follicular cells", in the adenohypophysis of the rat and considered them to be ACTH-secreting cells. Similar cells were recognized in various species of animals, such as the dog (Kagayama, 1965), dolphin (Harrison and Young, 1969), hamster (Dubois and Girod, 1970), and cat (Olivier, 1971), but systematic investigation was rarely performed on human pituitaries.

In the course of electron microscopic studies of normal and pathological tissues of human pituitaries, the author found some morphological characteristics of agranular cells, and comparative studies were performed between these and marginal cells of Rathke's pouch and primordial cells of primitive adenohypophysis.

Materials and Methods

Materials employed were the hypophyseal tissues of 2 human embryos belonging to Streeter's stage XIII (27 ± 2 days after ovulation) and stage XX (41 ± 1 days) (Streeter, 1945, 1951), of 4 fetuses obtained in the 16, 17, 24, and 28th weeks of gestation by spontaneous or legal abortion, and of 2 adults, resected from female patients with mammary carcinoma. The tumor tissues of 18 cases of pituitary adenoma were also examined. For the comparative morphology the pituitaries of two rhesus monkeys (macaca malutta) were investigated.

Small blocks of these tissues, taken within 10 minutes postmortem or immediately after resection, were fixed in a mixture of 2% glutaraldehyde and 2% formaldehyde and then in 1% osmium-tetroxide with sucrose added. The tissue was embedded in Epon 812 after Luft's technique or in Araldite. Ultrathin sections were doubly stained with uranyl acetate and Reynold's lead and observed with a JEM-100B electron microscope.

Observations

Agranular Stellate Cells (So-Called Follicular Cells)

In the early stage of the fetus, follicles composed of agranular cells were frequently found in the adenohypophysis (Fig. 1). These cells were shaped like an elongated triangle and connected to each other by junctional complexes, such as zona occludens and zona adherens. Apart from these sealing structures of the follicles, several distinct desmosomes with tonofilaments were found between follicular cells and between follicular cells and adjacent granulated secretory cells. Multiple microvilli were projected from the free surfaces into the follicular lumen. The nuclei were round to oval and the scanty cytoplasmic rim contained a moderate amount of free ribosomes, tonofilaments, mitochondria, small amounts of endoplasmic reticulum, and lysosome-like membrane-bound particles. Golgi apparatus was generally indistinct.

Similar agranular spindle-shaped cells were distributed between the parenchymal cells around the follicles, projecting their narrow cytoplasmic processes towards the periphery. Between two or four of the cells, narrow intercellular ductule-like spaces were occasionally observed (Fig. 2). They were surrounded by microvilli and sealed up by junctional complexes. Although they looked like small follicles, no colloid-like materials nor any amorphous content was seen.

In the later stage of the fetus, follicles became inconspicuous and agranular cells formed a reticular framework between the granulated secretory cells. These cells were stellate in shape and were connected to each other or to adjacent granulated cells by distinct desmosomes (Fig. 3). Their nuclei were angular or wrinkled

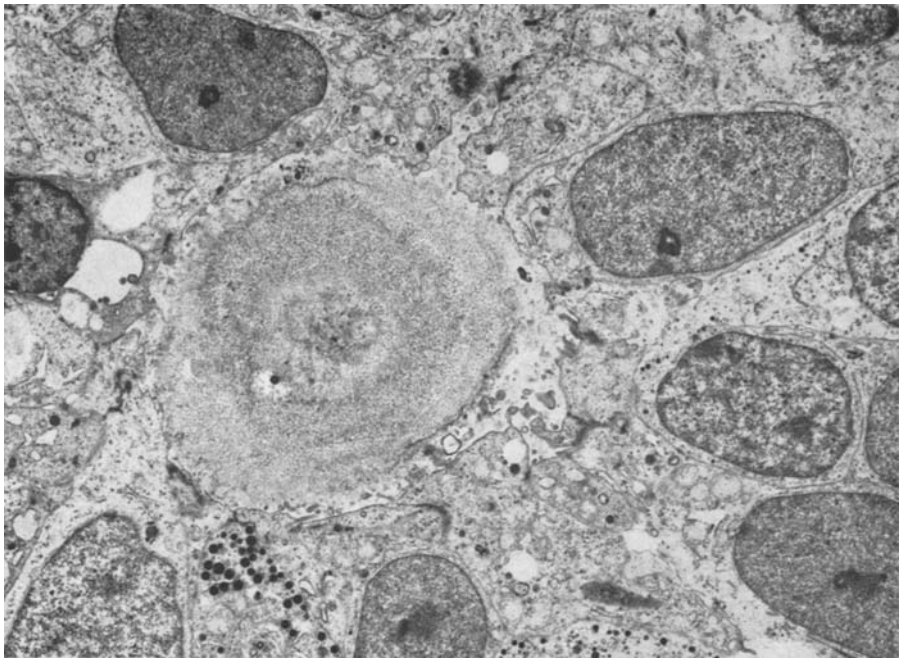

Fig. 1. A follicular cavity in the adenohypophysis of a human fetus, 16th week of gestation. The colloid-containing lumen is delineated by agranular cells, which are connected to each other by junctional complexes. × 5000

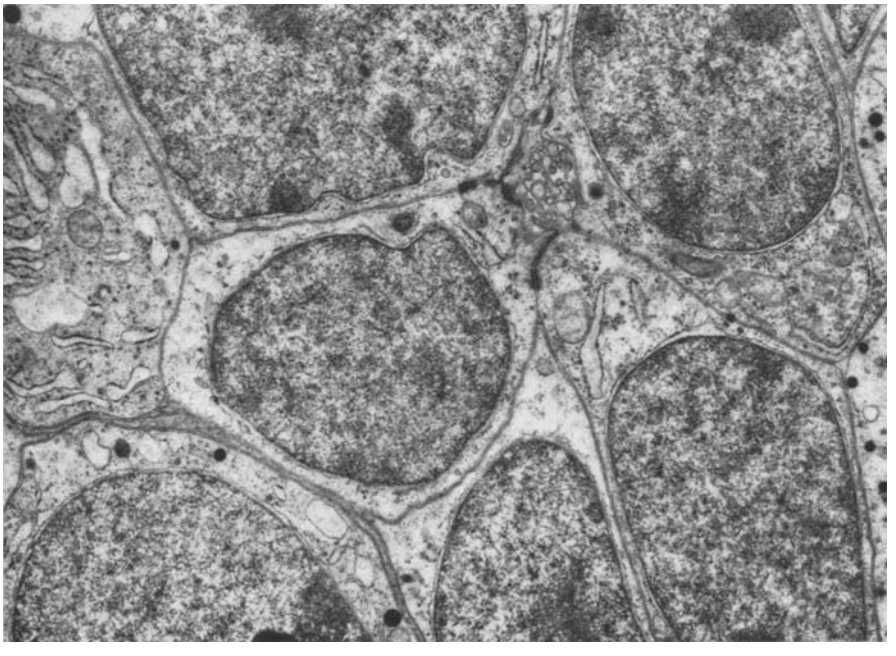

Fig. 2. A ductule-like intercellular space in the adenohypophysis of a human fetus, 16th week of gestation. The empty space is sealed by distinct junctional complexes and microvilli are protruded into the lumen. × 6200

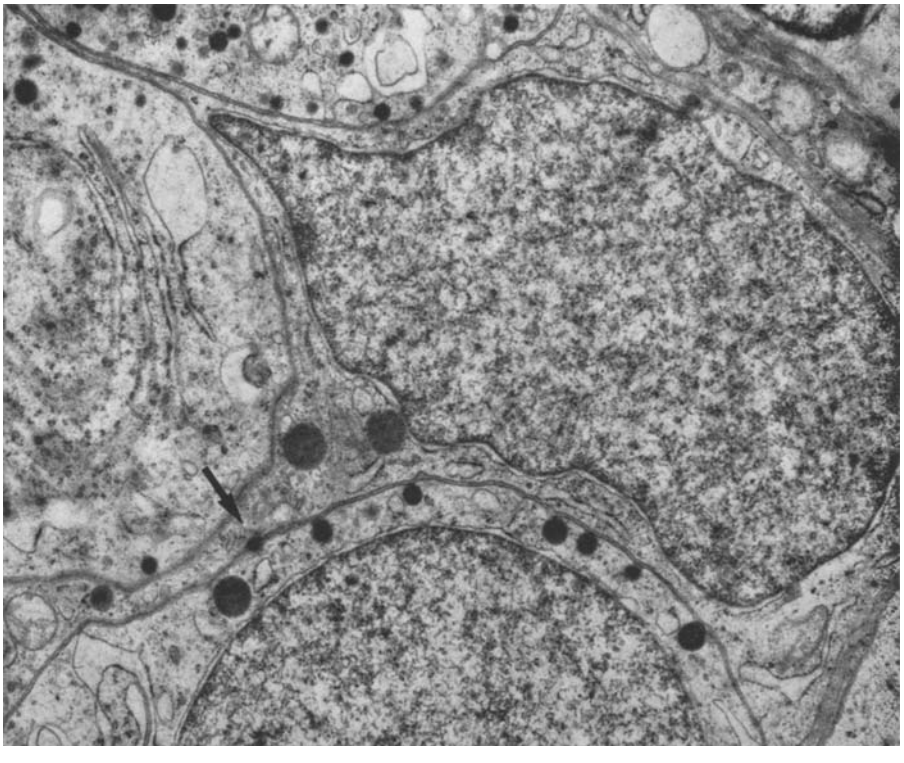

Fig. 3. An agranular stellate cell in the adenohypophysis of a human fetus, 24th week of gestation. The nucleus is angular and the cytoplasm extends narrow processes. The stellate cells are connected to adjacent granulated secretory cells by a distinct desmosome (arrow). × 16000

and their scanty cytoplasm extended multiple narrow processes between other parenchymal cells. Mitochondria, endoplasmic reticulum, and free ribosomes were more numerous than in the early stages, and several large vacuoles, lipid droplets, were found in the cytoplasm. Occasionally, a single cilium was observed in the cytoplasm without any connection to the lumen. At the periphery of the acini, narrow cytoplasmic processes or cytoplasmic bodies were in contact with basement membrane but no hemidesmosomes were seen.

In the adults, follicles were more inconspicuous but agranular stellate cells with junctional complexes were easily recognized (Fig. 4). They were connected to each other by desmosomes or zona adherens and sometimes showed intimate interdigitation (Fig. 5, 6). Desmosomal connections with adjacent granulated secretory cells, however, were rare and rudimentary in nature. The nuclei were extremely wrinkled, and it was easy to differentiate these cells from granulated secretory cells. The cytoplasm was more abundant in organelles than in the fetal stages. Golgi apparatus became distinct and polyribosomes, small cisternae of endoplasmic reticulum, and numerous minute vesicles up to 150 mμ in diameter occupied the whole cytoplasm. Multivesicular bodies and large lysosome-like

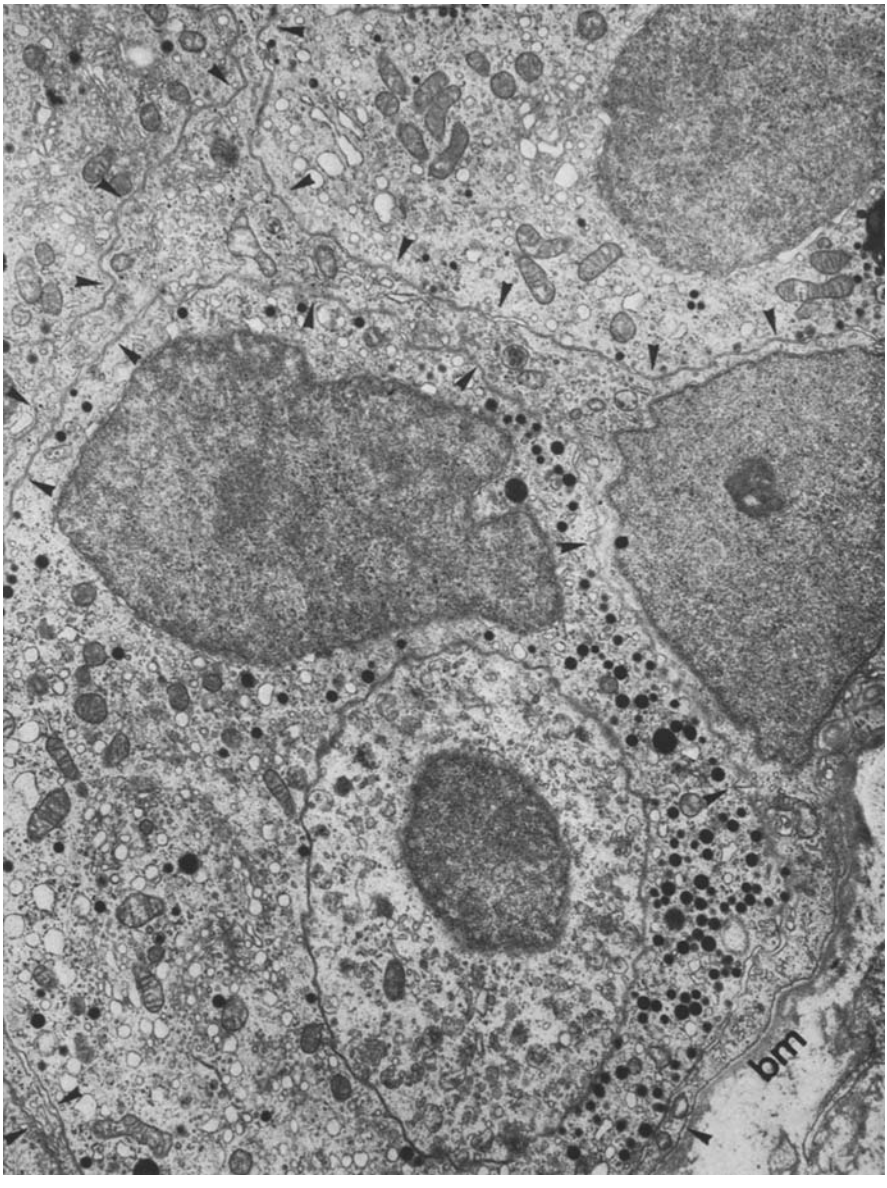

Fig. 4. An agranular stellate cell (surrounded by arrows) in the adenohypophysis of a human adult. The cytoplasm is abundant with organelles and a cytoplasmic process makes contact with the basement membrane (*bm*). × 14000

granules were more frequently observed. These features seemed to indicate that in adults, agranular stellate cells in the hypophysis play a more active metabolic role than in the fetal stages.

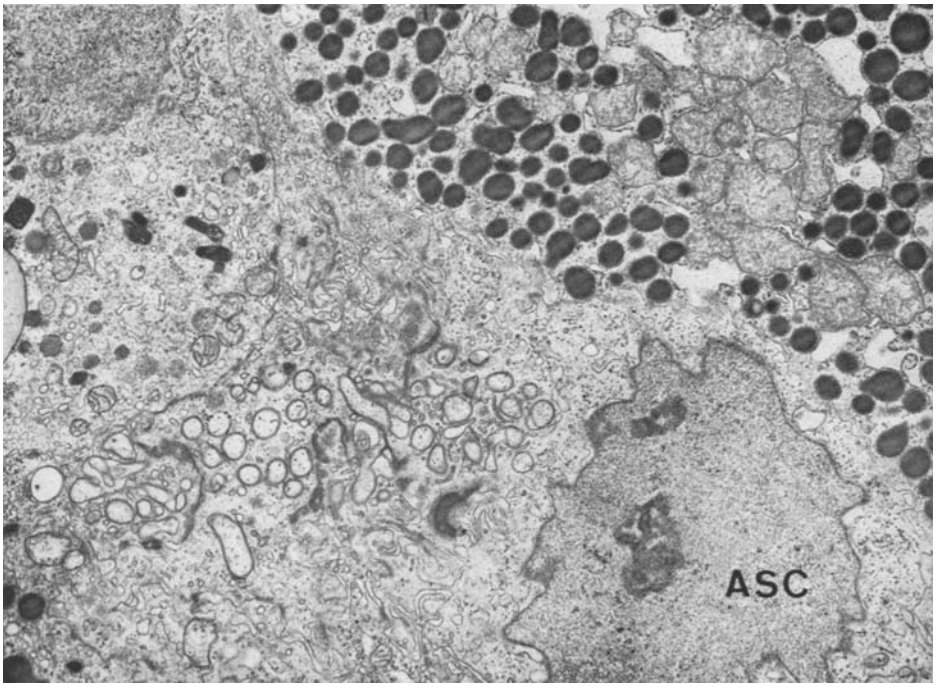

Fig. 5. Agranular stellate cells (*ASC*) in the adenohypophysis of a human adult. They exhibit prominent interdigitation and compose distinct junctional complexes with each other. × 12000

In only two out of 18 cases of chromophobe adenoma of the pituitary gland, similar agranular stellate cells, roughly 30% and 10% of the cell population respectively, were intermingled among the granulated tumor cells (Fig. 7). These cells had wrinkled nuclei and a narrow cytoplasmic rim extending multiple processes. They were connected to each other or to adjacent granulated tumor cells by distinct desmosomes. The organelles were scarcer than in normal cells but abundant with free ribosomes and tonofilaments. The stellate cells were somewhat atypical, and binucleated giant cells were occasionally encountered.

In the adult rhesus monkeys, agranular stellate cells similar to those of human adults were more frequently encountered than in the human fetus. They formed intercellular spaces, surrounded by microvilli and sealed up by junctional complexes. Even among primates, there are big differences between animal and human pituitaries, animals having more distinct follicles or intercellular spaces.

Marginal Cells of Rathke's Pouch

At Streeter's stage XIII (27 ± 2 days after ovulation), Rathke's pouch was composed of three to four layers of spindle-shaped cells. The cells directly lining the lumen, so called "marginal cells", projected irregular microvilli and a few cilia from their free surface and were attached to each other by junctional complexes at the vicinity of the lumen (Fig. 8). The other cells of the outer portion

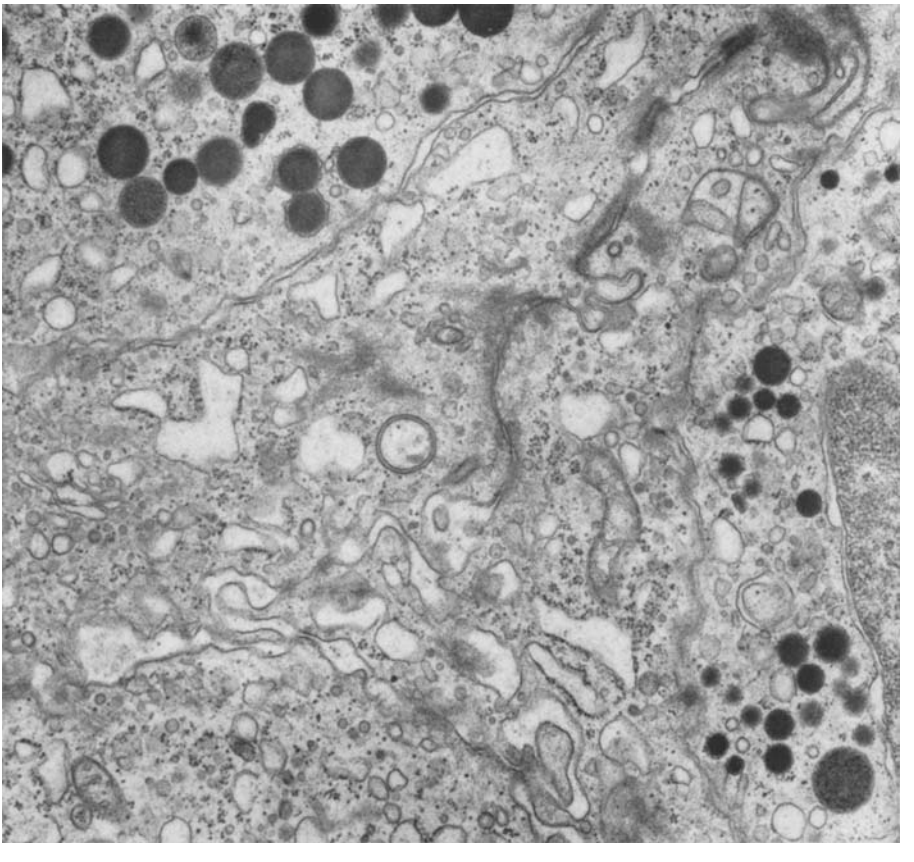

Fig. 6. Agranular stellate cells in the adenohypophysis of a human adult. The cytoplasm is abundant with vesicles, free ribosomes and tonofilaments. Distinct desmosomes are found between two adjacent cell membranes. × 22000

were polygonal or spindle in shape and triangular intercellular spaces of various sizes were formed between two or three of them. No sealing structure nor microvilli were observed in these spaces although desmosomes were occasionally seen between two adjacent cells These cells had large oval nuclei with one or two distinct nucleoli and the scanty cytoplasm contained numerous free ribosomes, singly or in rosettes, and a moderate amount of tonofilaments, minute vesicles, and lysosomal granules of various sizes. Golgi apparatus was relatively distinct and lipid droplets and multivesicular bodies were occasionally observed.

Primordial Cells of the Primitive Adenohypophysis

At Streeter's stage XX (41 ± 1 days) contact of the primitive adenohypophysis and neurohypophysis became distinct but still no granulated cells were observed, although marginal cells as well as other primordial cells exhibited more active cytological features (Fig. 9). Intercellular spaces were no longer observed.

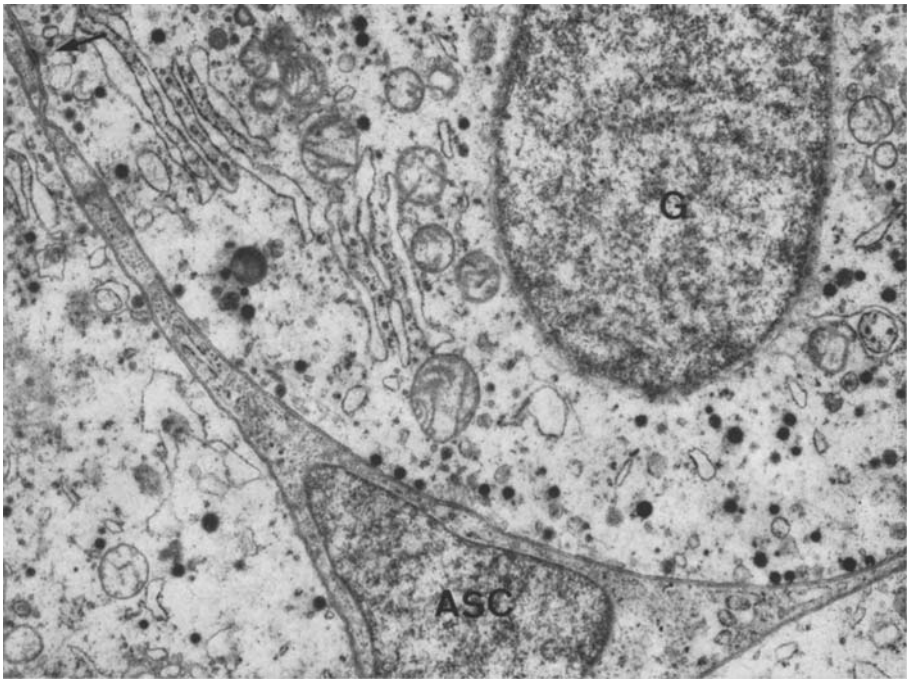

Fig. 7. Two types of tumor cells found in a case of pituitary adenoma. One tumor cell (*G*) contains granules of 50 to 100 mμ in diameter in the cytoplasm, but the other cell (*ASC*) resembles an agranular stellate cell and composes a desmosomal connection with the other. × 12000

The nuclei were oval and in the cytoplasm the number of mitochondria was greatly increased in the supranuclear region. The mitochondrial cristae tended to be dilated forming a clear intracristal space. Free ribosomes, particularly polysomes, increased their size and number. Golgi apparatus became more prominent and were composed of flattened cisternae and minute vesicles. The cisternae of the rough surfaced endoplasmic reticulum became elongated and paired cisternae, which seemed to be a characteristic feature of the fetal cells (Fukuda, 1972), appeared at this stage. The most distinct feature was the presence of glycogen granules aggregated in certain areas of the cytoplasm. These cytological features seemed to be quite different from those of agranular stellate cells.

Discussion

In human pituitary glands one can identify particular cells which are different from other granulated secretory cells. They compose follicles in the early fetal stages, but with progressing differentiation they become stellate and make a reticular framework throughout the adenohypophysis. They are connected to each other or to adjacent granulated cells by distinct junctional complexes, but no such connections are observed between the granulated secretory cells. Follicles, intercellular spaces, and perivascular channels which were described in dolphins (Harrison and Young, 1969) were not observed in the hypophysis of human adults.

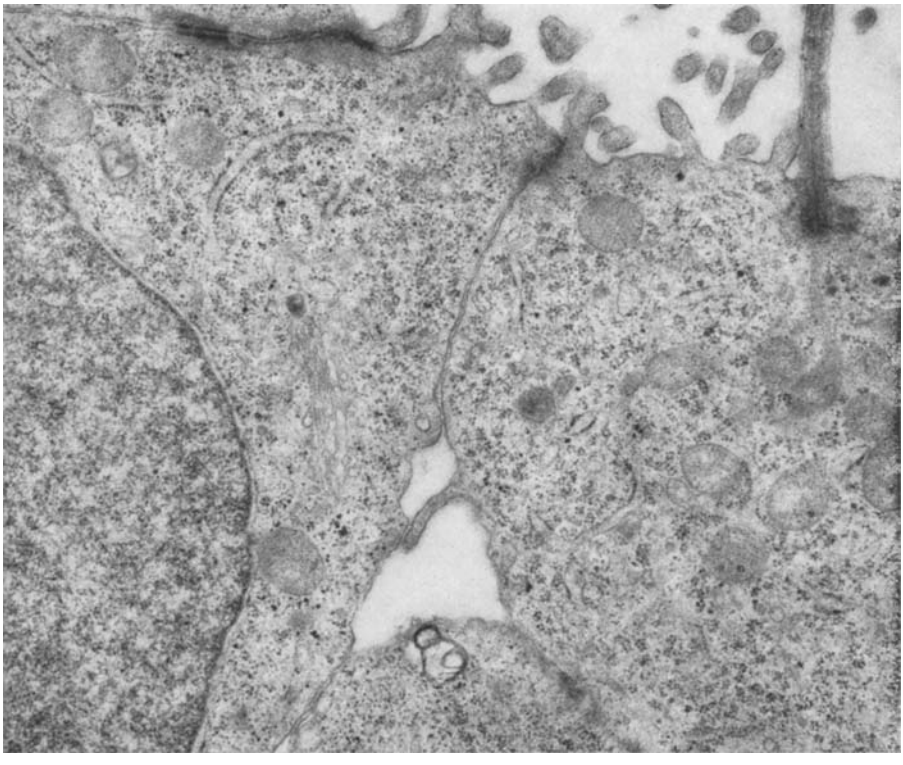

Fig. 8. The marginal cells of Rathke's pouch in a human embryo of Streeter's stage XIII. Irregular microvilli and a cilium extend from the free surfaces. The cytoplasm is abundant with free ribosomes. × 25000

It therefore seems better to call these cells agranular stellate cells, at least in the human pituitary.

In two out of 18 human pituitary adenomas, tumor cells quite similar to agranular stellate cells of the fetal stage were found. In normal adults these cells do not form distinct junctional complexes with granulated secretory cells, but in tumors desmosomes were frequently observed between agranular and granulated cells as in fetal pituitaries.

The agranular stellate cells somewhat resemble palisade and type II cells described in the intermediate lobe of the cat and ferret by Bargmann et al (1967) and Vincent and Kumar (1969). Palisade cells form follicles but no network. There are no junctional complexes between palisade cells and granulated cells.

Since Farquhar's (1957) first description, these agranular stellate cells of the adenohypophysis were investigated in various species of animals and their existence seems to be confirmed.

Because of the particular distribution and the absence of secretory granules the stellate cells were interpreted as follows:

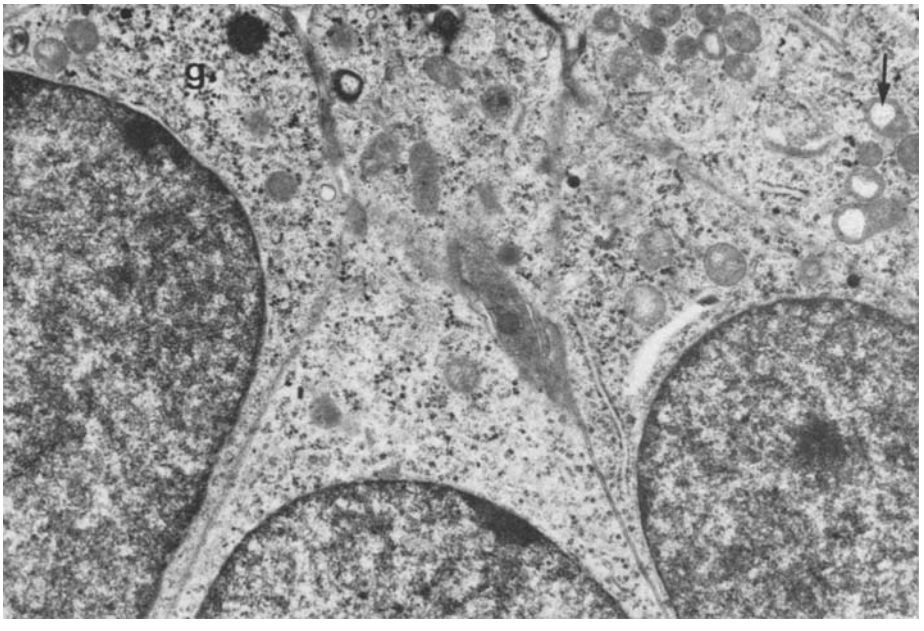

Fig. 9. Primordial cells of the primitive adenohypophysis in a human embryo of Streeter's stage XX. The cytoplasm is abundant with glycogen particles (*g*). The mitochondrial cristae tend to be dilated, forming a clear intracristal space (arrow). × 16000

1. ACTH-Secreting Cells (Farquhar, 1957; Foncin and Le Beau, 1964; Bergland and Torack, 1969). Using the peroxidase-labelled antibody method and electron microscopy, Nakane (1970) confirmed that ACTH-secreting cells have a moderate amount of secretory granules with a diameter of about 200 mμ at the periphery of the cytoplasm. The anti-ACTH antibodies were attached only to such granules of a particular type of cells but not to any other granules; therefore, only one type of cells was considered to produce ACTH. In experiments of bilateral adrenalectomy, Siperstein (1970) failed to demonstrate cytological changes in agranular stellate cells. However, another type of granulated cells which was considered to be ACTH-secreting cells showed distinct changes. He called them adrenalectomy cells. Therefore, agranular stellate cells are probably not responsible for ACTH production.

2. Immature or Undifferentiated Cells. Dubois and Girod (1969, 1970) considered these agranular cells to be residue of embryonic cells and Kurosumi (1968) and Yoshimura (1965) considered them to be immature or undifferentiated cells that can differentiate to ACTH-secreting cells whenever ACTH is in demand. However, the agranular stellate cells are more differentiated cytologically compared with primordial cells of the primitive adenohypophysis or marginal cells of Rathke's pouch. The primordial cells show an extremely high nucleocytoplasmic ratio and have more abundant polysomes than agranular stellate cells. Glycogen granules which are abundant in primordial cells are lacking in the stellate cells. The pri-

mordial cells of the primitive adenohypophysis seem to differentiate not only to granulated secretory cells but also to agranular stellate cells.

3. Colloid Secreting Cells. In most animals, the agranular stellate cells frequently form colloid follicles, and Kagayama (1965) found several small granules of 150 to 200 mμ in diameter subjacent to the follicular surface considering them to be probably discharged into the follicular lumen. Except in an early stage of the fetus, colloid follicles were not observed in our human pituitaries and no secretory granules were encountered in agranular stellate cells. The perivascular channels described in dolphins (Harrison and Young, 1969) could not be demonstrated in human pituitaries.

4. Supporting Cells (Kagayama, 1965; Salazar, 1968). As a rare exceptional finding in epithelial tissues, there are no junctional complexes between the granulated secretory cells of the adenohypophysis; however, agranular stellate cells are connected to each other or to adjacent secretory cells so that secretory cells seem to be fixed by these supporting cells. Such a relationship between two kinds of epithelial cells in an endocrine gland corresponds to that of Sertoli cells and spermatogonias in the testicle.

Apart from these supporting functions, the stellate cells probably have some metabolic role. Cytoplasmic organelles are more abundant in the stellate cell of the adult than in the corresponding cells of the fetus. In addition, lysosomes, particu larly phagosomes, are found under various conditions (Yamashita, 1972).

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