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FINE STRUCTURE OF ECCRINE SWEAT GLAND ADENOMA, CLEAR CELL TYPE

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INTRODUCTION

Eccrine sweat gland adenoma, clear cell type, (clear cell hidradenoma) was first described by Liu (1). By light microscopy the lesion has been characterized as a solid, primary, cutaneous tumor composed of clear epithelial cells which are focally arranged in glandular and cystic patterns. Of the cases reported to this date (2–12), only five have proved malignant. Kersting (6) has provided an excellent summary of the clinico-pathologic features of this neoplasm.

Controversy exists over the histogenesis of this lesion. The postulated sites of origin include the internal root sheath of hair follicles (1), the sweat gland (12), the apocrine myoepithelium (7), the eccrine sweat gland (13), the eccrine sweat duct (14, 15), the eccrine excretory unit (5) and the eccrine portal epithelium (5, 6). Recently, O'Hara et al (10) demonstrated histochemically that the mucin and enzyme properties of this lesion were closely related to the secretory unit of the eccrine sweat gland.

The present ultrastructural investigation was carried out on two of five lesions which were the subject of a previous histochemical study (10). The purpose of this paper is to present fine structural evidence which further relates this tumor to the clear cell of the eccrine secretory coil.

MATERIALS AND METHODS

The materials for the present study represent parts of two cases (cases 3, 4) that were reported in a previous histochemical investigation (10). The clinical histories are briefly as follows:

Case 1: A 68 year old Caucasian female complained of an enlarging blue nodule over the left upper lip of long duration. A 0.6 cm. lesion was excised and 5 months later two recurrent nodules were widely excised, measuring 0.5 and 0.2 cm.

Case 2: A 68 year old Caucasian male complained of a 1.5 cm. pebbly surfaced nodule above the outer third of the left eyebrow. A biopsy was performed and one month later the entire lesion was excised.

Approximately one third of each lesion was immediately fixed in 3.5% cold buffered glutaraldehyde (16), transferred into a buffered wash solution, later postfixed in osmium tetroxide (17), embedded in Epon (18) or Maraglas (19), and sectioned on a LKB 4800A Ultrotome. Electron microscopic studies were carried out on sections stained with lead salts (20). Sections one micron thick were stained with toluidine blue for examination and orientation with the light microscope.

LIGHT MICROSCOPY

Both lesions were predominantly solid intradermal tumors composed largely of clear epithelial cells. The tumor nodule in case 2 replaced the overlying epidermis in its central portion and merged imperceptibly with an adjacent acanthotic epidermis; the lesion in Case 1 was located entirely within the dermis and subcutaneous fat.

The round or polygonal clear tumor cells contained a small round or ovoid, moderately chromatic nucleus which was centrally located and contained one or two nucleoli. These cells sometimes exhibited fine eosinophilic cytoplasmic granules or septa. Intercellular spaces were evident in thin sections.

In addition to the predominant clear tumor cell, a few compact epithelial cells were present, characterized by a round or oval vesicular nucleus surrounded by faintly eosinophilic or amphophilic, finely granular cytoplasm. Foci of squamous metaplasia were encountered in the superficial portion of the tumor nodule in Case 2.

Small duct-like structures and larger cystic spaces which were seen in paraffin embedded material (10) were not present in the numerous thin sections examined.

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ELECTRON MICROSCOPY

The two lesions presented in almost identical fine structural pattern and will be described together. The tumor was predominately composed of large polygonal or round cells with a relatively large nucleus. The latter was often indented with recesses extending deeply into the nucleoplasm. (Fig. 1). All nuclei had at least one or more prominent nucleoli. The distribution of nuclear chromatin followed a normal pattern. Mitoses were not encountered.

The striking feature of all cells was an abundance of glycogen in the cytoplasm (Figs. 1, 2). The glycogen containing zones were usually irregular in outline and ranged in size from a fraction of a micron to many microns in diameter. The pattern of glycogen distribution appeared to be random, but in those cells containing large amounts of glycogen the nucleus was completely surrounded by this material (Fig. 2). On higher magnification one could see the lead-stained glycogen rosettes in these areas outlined well against a very electron transparent background (Fig. 4). At the periphery of the glycogen-rich zones one could often see an accumulation of mitochondria and electron opaque lysosome-like structures which were usually circular and surrounded by a unit membrane (Figs. 2, 3, 4). The mitochondria were structurally classical as regards size, outer double membranes, cristae and their distribution.

A small number of the cells showed areas which in a fashion resembled the glycogen-rich zones described above. Typical glycogen rosettes were never seen in these zones which were remarkably electron translucent (Figs. 6, 7), relatively poorly circumscribed and never surrounded by a unit membrane. These electron translucent zones were sometimes adjacent to the previously described glycogen containing areas (Fig. 6). At higher magnification one could see that the matrix of these zones was not uniform in that areas of electron translucency alternated with areas of greater electron opacity. Present in these zones were mitochondria which were usually randomly distributed (Fig. 7). Occasional spherical electron opaque bodies, apparently lysosomal structures, were also seen.

Little rough endoplasmic reticulum was encountered in these tumor cells, the majority being of the smooth type. A close association of the latter type of endoplasmic reticulum with the mitochondria was noted. The Golgi complexes were very prominent in all of the cells and usually located in nuclear indentations (Fig. 1). At higher magnifications small vescicles of varying size were found near canalicular surfaces (Figs. 4, 5).

The typical tumor cell was attached to its neighbor by desmosomes which bridged the large number of cytoplasmic imbrications present (Figs. 1, 2, 3, 4, 5). The complex intercellular spaces were narrow as a rule but when larger intercellular clefts were encountered, finely amorphous material of low electron opacity was encountered. The latter was usually attached to the outer cell wall (Fig. 5). A small percentage of the tumor cells contained distinct tonofilaments which could be seen to terminate in desmosomes (Figs. 1, 6). This type of cell organelle was usually encountered in tumor cells in the vicinity of foci of squamous metaplasia. The most prominent features of the cells in the foci of squamous metaplasia (Fig. 8) were the presence of large numbers of tonofibrils, broad intercellular bridges with prominent desmosomes, and an absence of glycogen containing areas and the areas of electron translucency described above.

At the border of the tumor there was usually a very distinct basement membrane around the nests of tumor cells. The tumor stroma was composed of collagen, fibroblasts and small capillaries. Myoepithelial cells were never found in the tumor. No true glands or ducts were encountered in sections examined with the electron microscope.

DISCUSSION

Recent histochemical and fine structural investigations of the fetal and adult cutaneous systems have greatly contributed to the biology of cutaneous adnexal neoplasms. The human adult eccrine secretory acinus has been shown by others to be composed of two basic cell types, "clear" cells and "dark" cells. This terminology, coined by light microscopists, has been well defined in the notable works of Montagna (21, 22). The cytoplasm of "dark" cells stains avidly with basophilic dyes while that of "clear" cells contains small acidophilic granules. A mixture of acidophilic and

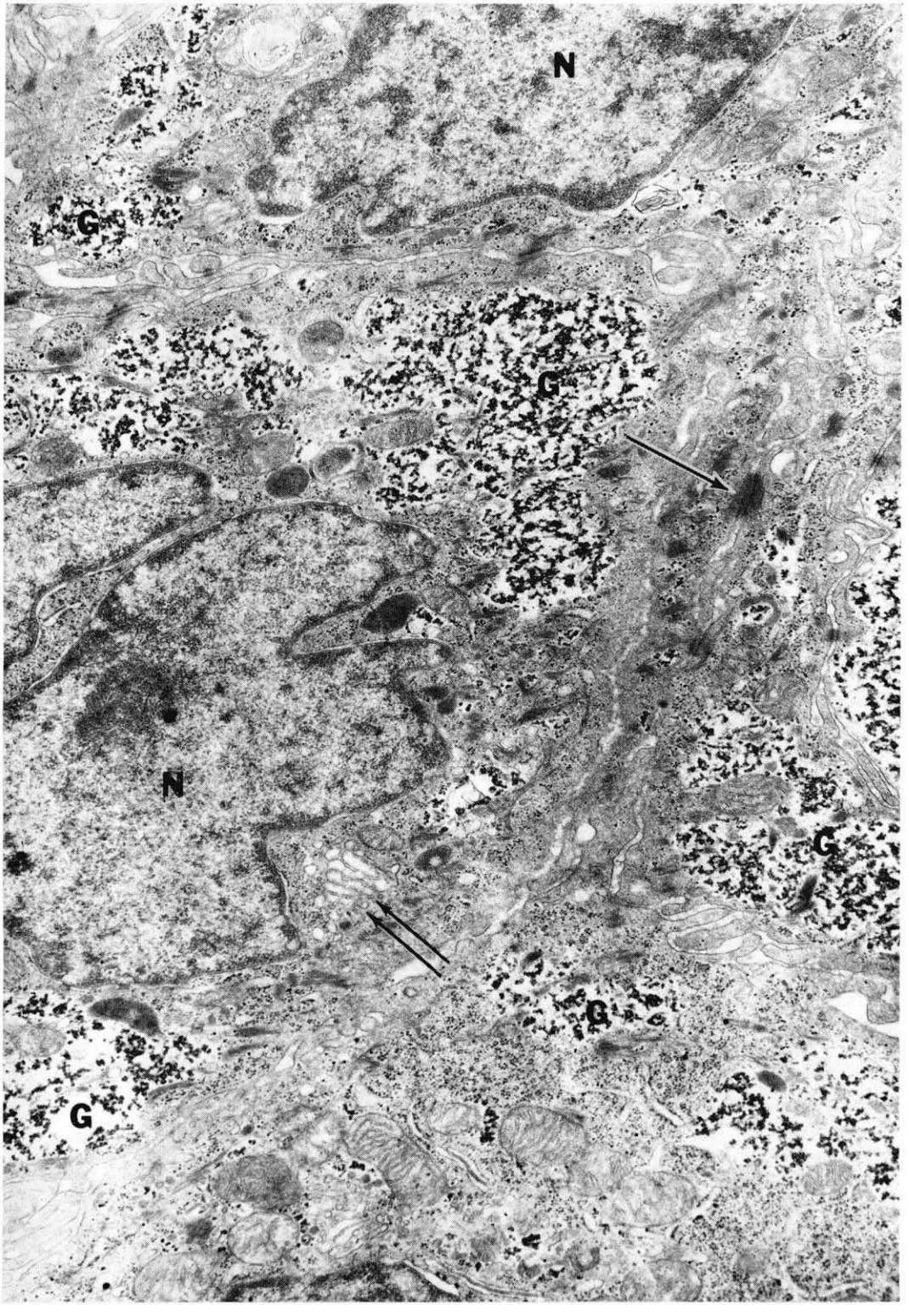

FIG. 1. Electron micrograph showing typical appearance of several tumor cells, each with a relatively large lobulated nucleus (N), prominent Golgi zone (double arrows) and numerous foci of glycogen collections (G) in the cytoplasm. Note the presence of intercellular attachments (desmosomes) (arrow). Case 1, Mag. 16,200×.

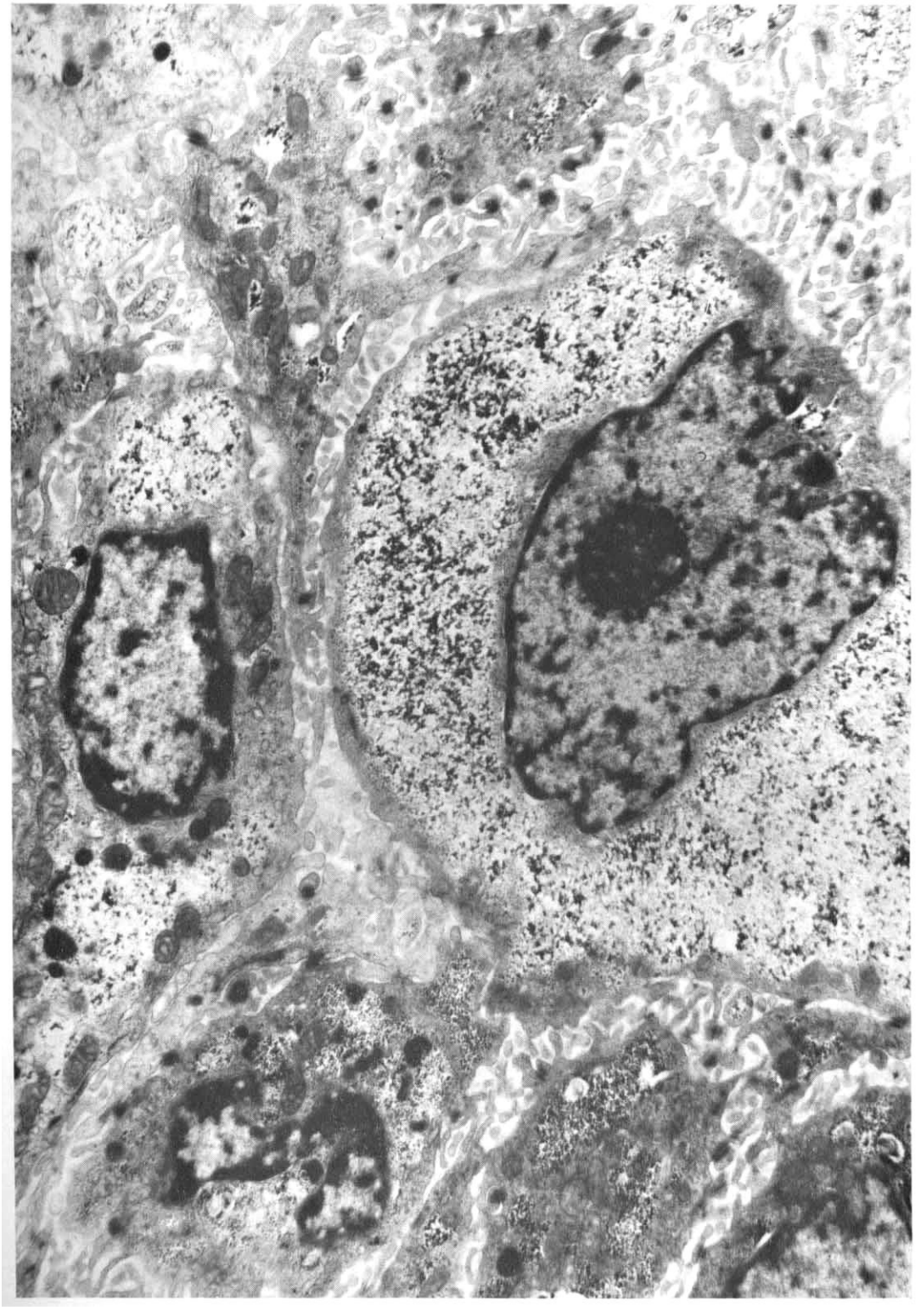

FIG. 2. Not infrequently the perinuclear cytoplasm consisted of glycogen (top right). Note the prominent nucleolus in this cell. The interdigitations and desmosomes are very well demonstrated in this area of tumor. Case 1, Mag. 11,500×.

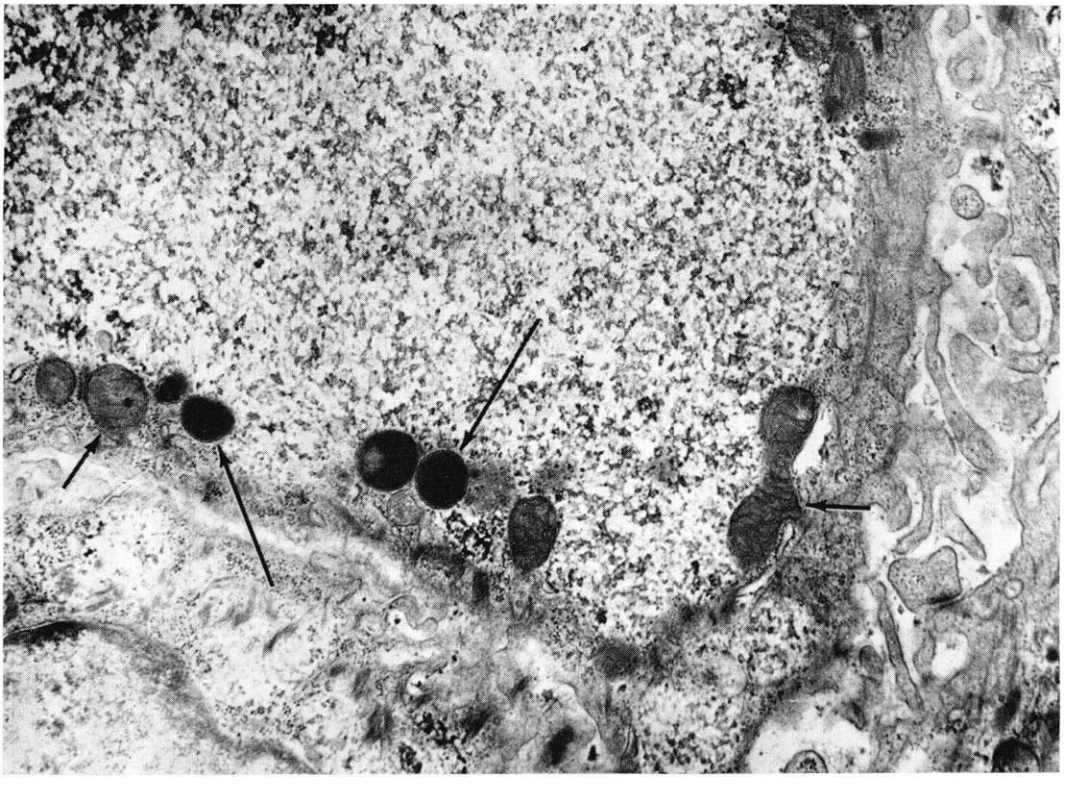

FIG. 3. Higher magnification of glycogen-rich tumor cell illustrating displacement of the usual intra-cytoplasmic organelles to the periphery. In addition to a normal complement of mitochondria (short arrow), several lysosomes can be identified (long arrows). Case 1, Mag. 19,000×.

basophilic granules can be demonstrated in both cell types with Giemsa and eosinmethylene blue stains; both cell types contain ribonuclease-labile granules metachromatic to toluidine blue. It has been found that the two cell types can be further delineated by the electron microscope (21, 23, 24, 25). Somewhat paradoxically the "clear" cell as defined by light microscopic methods actually appears darker under the electron microscope due to the relative electron opacity of the glycogen-rich cytoplasm of this cell. For purpose of convention, the terms "clear" and "dark" cell used in this paper will refer to their well-established light microscopic appearance.

By fine structural methods "clear" cells are characterized by large irregular nuclei and a glycogen-rich cytoplasm which also contains numerous small vesicles of varying size and density. Rough endoplasmic reticulum, which is plentiful in "dark" cells, is sparse while smooth endoplasmic reticulum is more abundant especially in glycogen-rich areas. The Golgi zone of the "clear" cell is usually small and located near the intercellular canaliculus. Mitochondria are randomly scattered throughout the cytoplasm with no preferential orientation. Occasional lysosomal structures are seen in both cell types.

The most remarkable feature of the "clear" cell is the extraordinary modification of its surface membrane. The surfaces of adjacent "clear" cells are marked by an extensive system of interdigitations forming a complex and irregular intercellular space. Occasional desmosomes join adjacent "clear" cells. The complex surfaces of adjacent "clear" cells also form the intercellular canalicular system. This system is separated from the remainder of the intercellular space by terminal bars (21). The canalicular surface is studded with short microvilli. The canaliculi begin near the base of the

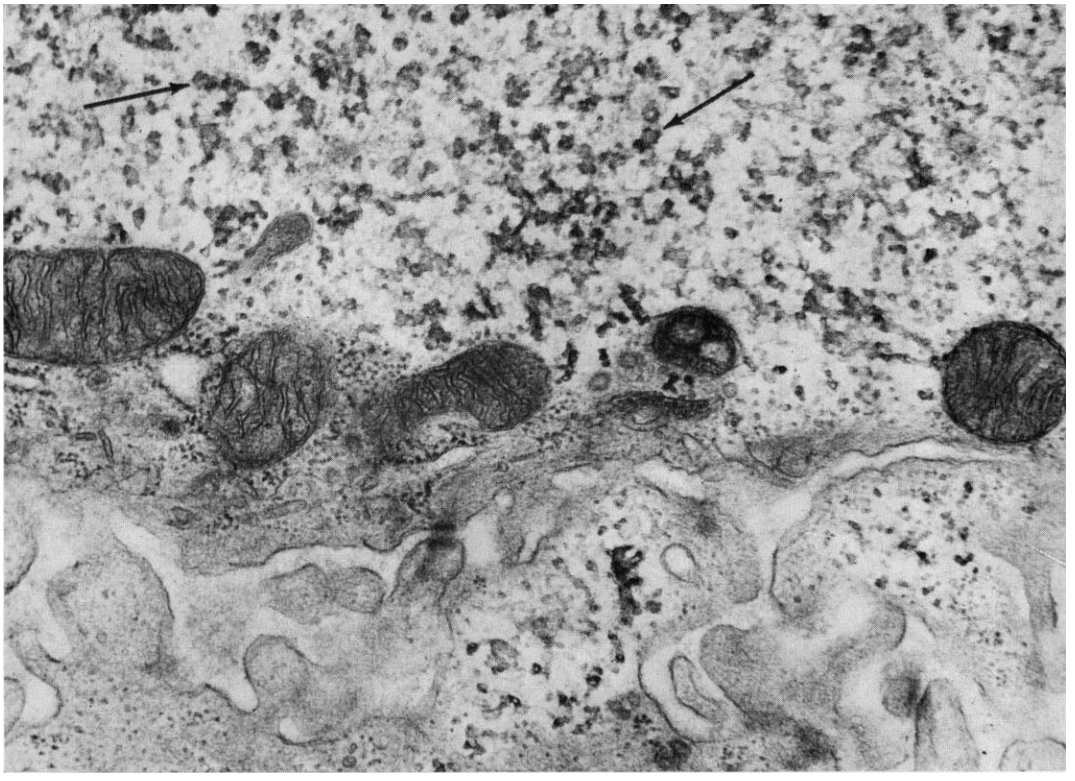

FIG. 4. At higher magnification the electron-opaque rosettes (arrows) typical of glycogen are prominent against a very electron-translucent background. Note the rough endoplasmic reticulum, free ribosomes and mitochondria at the cell periphery. Case 1, Mag. 34,500×.

gland and course irregularly to terminate in the gland lumen. It should be noted that the canaliculus is always formed between adjacent "clear" cells; when "clear" cells and "dark" cells are in apposition, the intercellular space is always fashioned in a much less complex manner. The membrane of the "clear" cell as it abuts on the central lumen of the gland is fashioned in much the same manner as is the canalicular surface, indicating that both might serve a similar physiologic function.

The "dark" cell is characterized by a smaller irregularly shaped nucleus and cytoplasm which is packed with both smooth and rough endoplasmic reticulum. The apical portion of the "dark" cell contains an extensive Golgi complex and numerous secretory vacuoles, 1 to 2 micra in diameter; the latter appear to contain a mucoid substance. The surface membranes of adjacent "dark" cells are less elaborate than their "clear" cell counterpart and their junction is characterized by a very regular "corrugated union" (21). The "dark" cells appear well bonded along these surfaces and there are no intercellular spaces. The apical surface of the "dark" cell bears blunt, irregularly distributed microvilli.

It is evident from the description of the fine structural characteristics of the eccrine secretory coil that the tumor cells of the clear cell sweat gland adenoma (clear cell hidradenoma) closely replicate the ultrastructural cytology of the normal eccrine "clear" cell. In addition to the high glycogen content in the normal and neoplastic "clear" cell, close parallels can be drawn between their systems of intracellular organelles, distribution and number of mitochondria, cytoplasmic vesicles and Golgi zones. However, the marked degree to which the clear tumor cells replicate the complex intercellular membrane system seen between adjacent "clear" cells of the eccrine coil

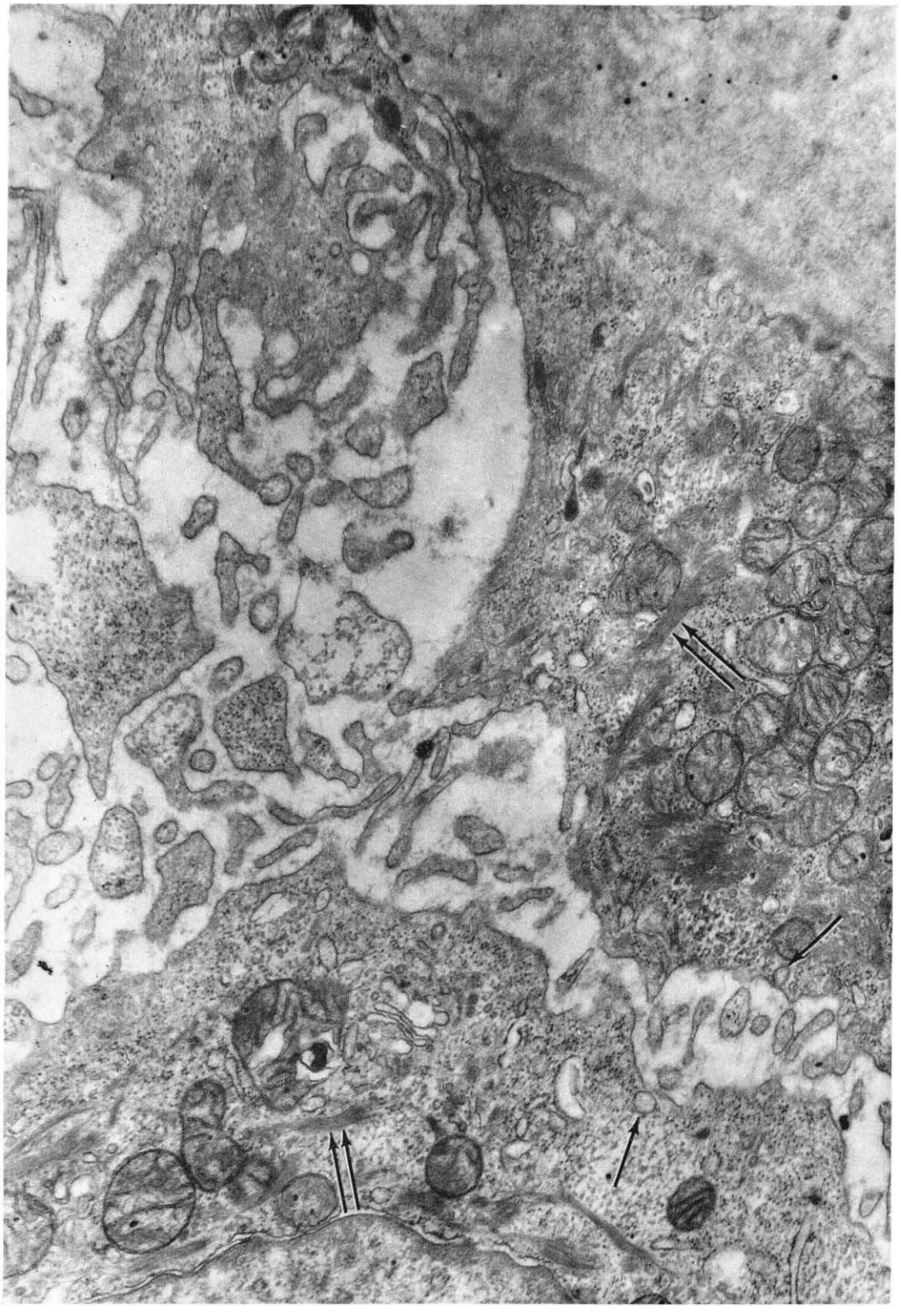

FIG. 5. Parts of both tumors showed a network of larger intercellular spaces partly filled with cytoplasmic processes. The cytoplasm bordering these spaces contains small vesicles (arrow). Note the bundles of tonofilaments coursing through the cytoplasm (double arrows). Case 1, Mag. 25,000×.

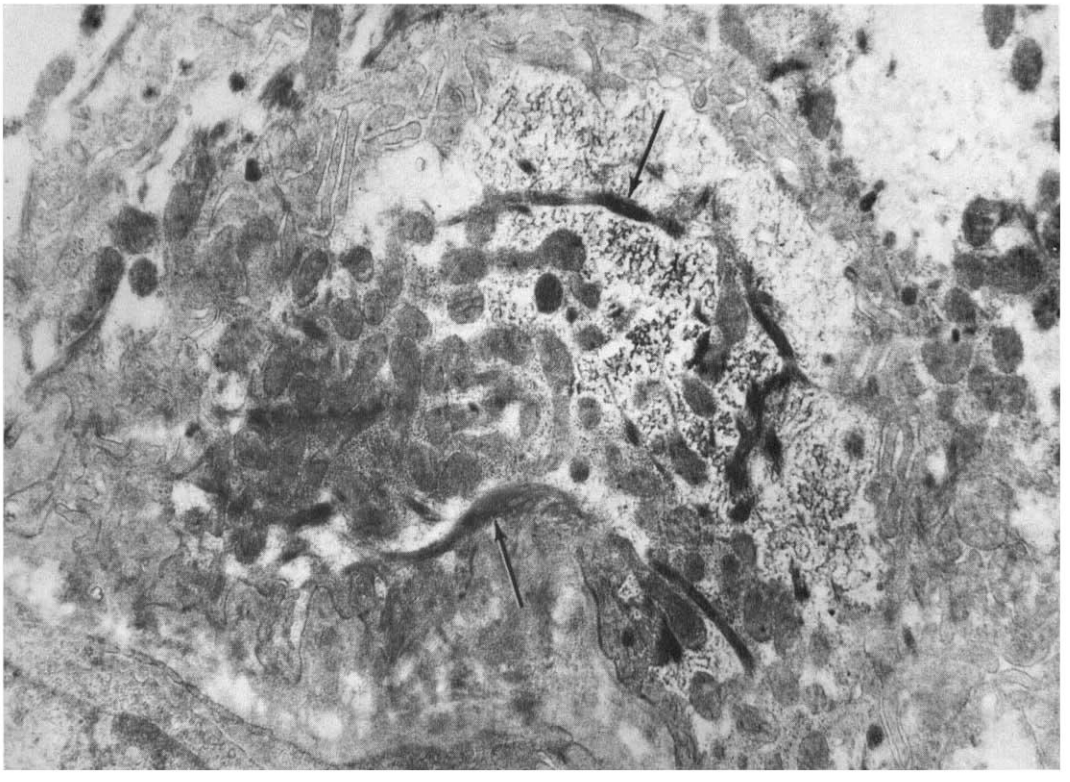

FIG. 6. This illustrates numerous bundles of tonofilaments (arrows), several of which are present in glycogen-rich areas. Case 2, Mag. 17,800×.

seems to present the most conclusive evidence that these cells share a close biologic relationship.

In the present study, well formed canaliculi defined by terminal bars were not encountered. In other respects, the complex system of intercellular imbrications, including the presence of desmosomal plates, were highly characteristic. In many areas of the tumor, as illustrated in Figs. 2 and 5, the broadened intercellular spaces, partially occupied by short irregular microvilli, bore marked resemblance to the structure of the intercellular canaliculus.

Many sections of the tumor were examined in the hope of finding the glandular and cystic spaces which were observed in the paraffin-embedded material. However, none were encountered. In Case 2 this probably was due to random distribution of these structures which were encountered more frequently in deep portions of the tumor nodule when the paraffin-embedded material was studied with the light microscope. In Case 1, these structures were extremely rare in the sample studied by the light microscope. Therefore the mechanism of formation of the glandular and cystic structures within these tumors is left open to question. This is particularly relevant in view of the recent work of Hashimoto et al (26, 27) in studying the mechanism of formation of the intradermal and intraepidermal eccrine gland lumen in human fetal skin. These investigators have found that the formation of the lumen in the intradermal segment of fetal anlage is initiated by fracture of desmosomal plates; in contrast, the intraepidermal lumen is formed by partial autolytic cavitation of apposing cells. Both mechanisms present attractive hypotheses in the present study in view of light microscopic data (10) from earlier work. The cystic spaces in the previous study were shown to contain nuclear and cytoplasmic profiles and a hyaluronic acid-like substance in addition to a wide spectrum of other mucosaccharides. In the tissues

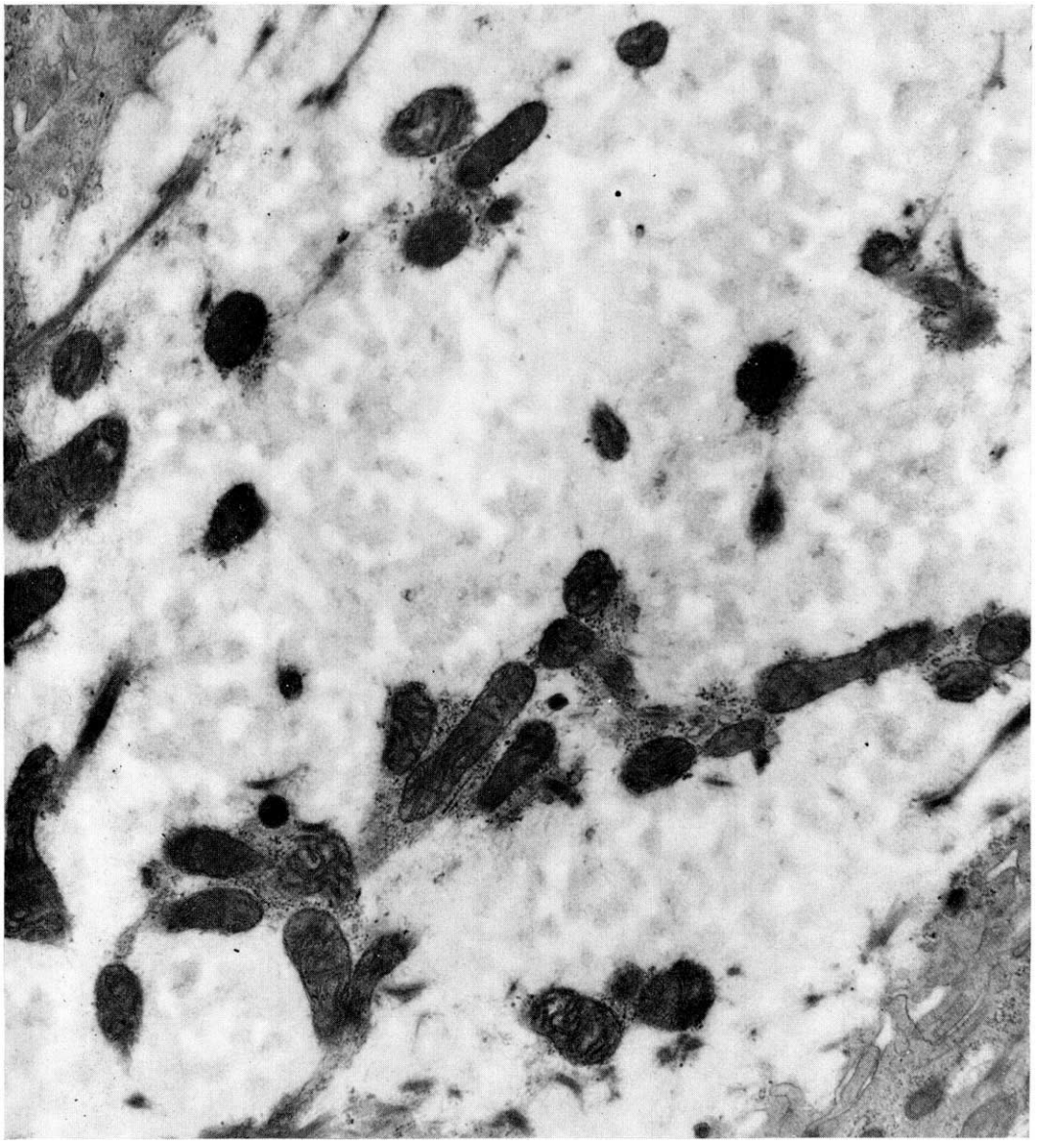

FIG. 7. An occasional tumor cell showed absence of glycogen rosettes in zones of electron-translucent perinuclear cytoplasm. These areas showed at high magnification randomly distributed mitochondria and strands of hyaloplasm against a background of varying electron translucency. Case 2, Mag. 23,500×.

studied by electron microscopy, lysosomes were prominent in some of the tumor cells and could conceivably contribute to autolysis. The demonstrated incomplete formation of the intercellular canalicular system might further contribute to an autolytic process due to increased pressure caused by incomplete removal of secretory products in these metabolically active cells.

A small percentage of the tumor cells in the present study resemble the fine and light microscopic structural characteristics of the cells of the upper dermal and lower intraepidermal eccrine duct (23, 24, 28, 29, 30, 31).

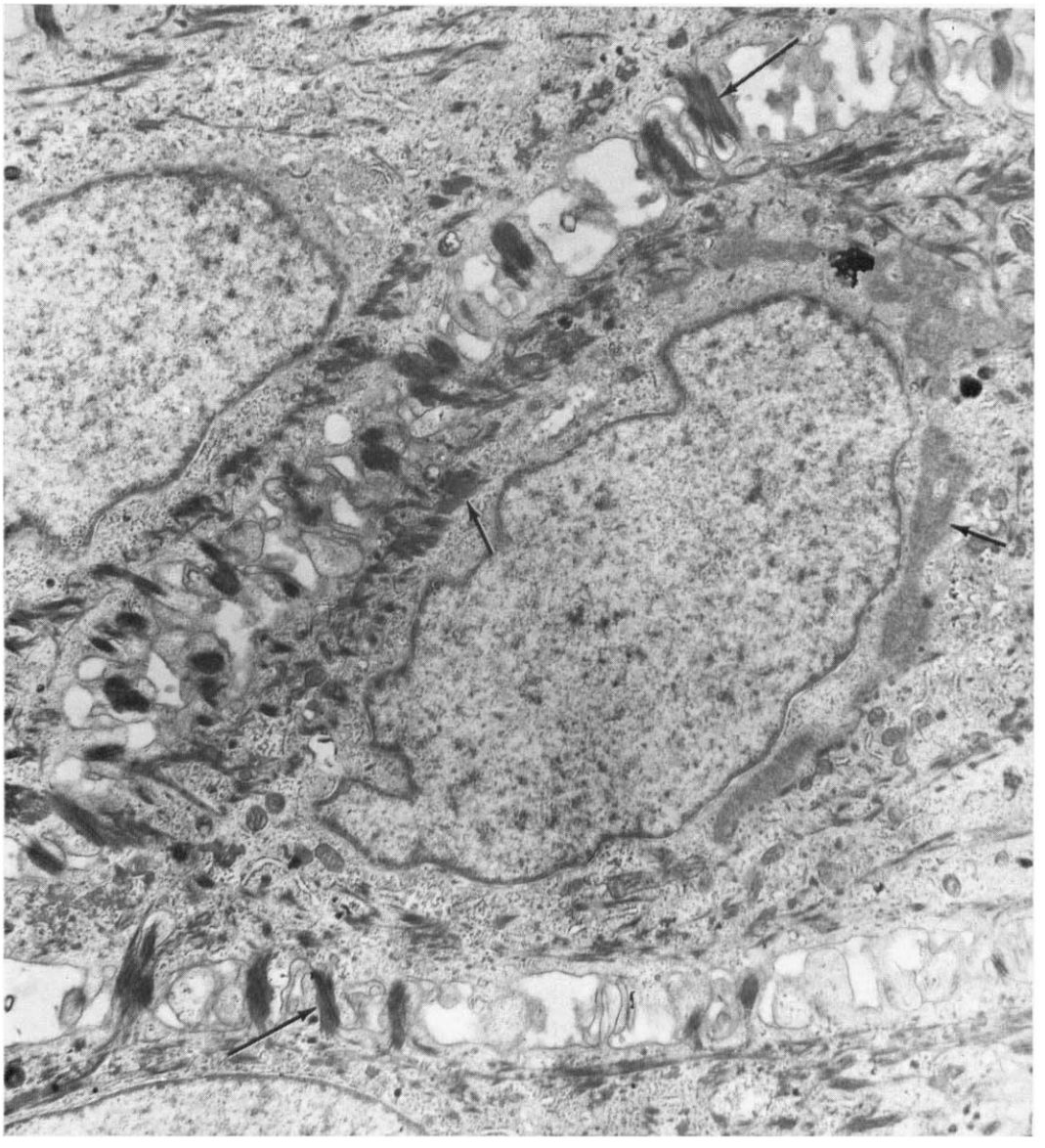

FIG. 8. A small percentage of tumor cells in the vicinity of foci of squamous metaplasia contained abundant tonofibrils (short arrows) which terminated in demosomes (long arrows). Case 2, Mag. 11,500×.

This is not surprising in view of the well known pluripotential capacity of the embryonic and adult cutaneous system (7). Complete replication of the intraepidermal poral unit was not seen.

A peculiar feature of a small percentage of the clear tumor cells was the presence of relatively electron translucent, poorly circumscribed areas which in a fashion resembled the matrix of glycogen-rich areas frequently encountered. Mitochondria were often randomly distributed through these zones along with apparent lysosomal structures (Fig. 7). Unit membranes never delineated the periphery of these zones. We were unable to find parallels to this fine structural pattern in the literature pertinent to the cutaneous system.

It should also be emphasized that myoepi-

thelial cells were not encountered among the tumor cells in the present study. To our knowledge, of the several adnexal tumors of the cutaneous system which have been extensively studied (32, 33, 34, 35, 36, 37), only syringoma (38) has been demonstrated to possess these highly characteristic cells.

SUMMARY

The ultrastructural features of two clear cell eccrine sweat gland adenomas are described. The clear tumor cells are shown to closely replicate the fine structure of the "clear" cells of the eccrine secretory coil. Each has a high glycogen content and a similar system and distribution of intracellular organelles. However, the most conclusive evidence that the clear tumor cell is biologically related to the eccrine secretory "clear" cell is provided by the marked similarity of their complex intercellular membrane systems.

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