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THE ULTRASTRUCTURAL LOCALIZATION OF ADENOSINE TRIPHOSPHATASE IN SEBACEOUS GLANDS AND ASSOCIATED EXTRA-SEBACEOUS STRUCTURES

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CHAPTER I

INTRODUCTION

Sebaceous glands in the human develop as one of the epidermal appendages during the third to the fifth month of human fetal life (Hamilton, et al., 1952; Montagna, 1962; Machida, 1964) and together with hair follicles form the pilosebaceous system (Tanaka, et al., 1965). There are, however, modified sebaceous glands which open directly to the surface of the skin without being associated with hair follicles (Ashley-Montague, 1943; Friderich and Schadel, 1949; Goeckerman, 1926; Kolliker, 1826; Perkins and Miller, 1926; Saalfeld, 1899). Aquatic environmental mammals such as the whale (Giacometti, 1967) and the dolphin do not possess sebaceous glands (Nicolaides and Kellum, 1965; Slijper, 1962a, 1962b).

Although the anatomical and physiological descriptions of sebaceous glands were defined by Eichhorn (1826) approximately one hundred and fifty

years ago, the process of sebum formation as the end product of the sebaceous glandular cells is still incompletely understood.

Virchow (1863) one hundred years ago suggested that the degenerative process in the sebaceous cells was similar to that in liver cells undergoing "fatty degeneration." He further introduced the "degeneration theory" in which there is destruction of the entire cell in the process of the formation of sebum. The theory of degeneration is generally accepted and the sebaceous glands are thought to be holocrine-type glands (Baker, 1949; Bizzozero and Vassale, 1887; Bowen, 1926, 1929; Brandes, et al., 1965; Charles, 1960; deDuve, 1959; Hoven, 1912; Klingman and Shelley, 1958; Palay, 1958; Regaud, 1911; Renault, 1911; and others). The process represents a general category of "physiologic or normal cell death" and is essential in developmental processes, in systemic functioning in some organs (deDuve, 1959) and in general body activity. However, this type of physiological cell death has not been studied extensively as a primary investigative objective.

The total spectrum of structural, chemical, and enzymatic changes in the active sebaceous gland is only little known. The present study, done at the ultrastructural level, is aimed at the correlation of adenosine triphosphatase (ATPase) activity with the structural alterations and transformation of the protoplasmic organelles of the sebaceous gland cells during the holocrine process of sebum formation.

CHAPTER II

MATERIALS AND METHODS

Materials

The sebaceous glands and other tissues used in this study were those occurring in the pinnae of King-Holtzman black male rats 9 to 12 months old.

Methods

Excision of Tissue

Tissue was obtained from animals anesthetized with sodium pentobarbital as follows:

1) The pinnae of the rat were cleaned thoroughly by washing with 70% ethyl alcohol and allowed to air dry.

2) Approximately 1/2 to 2/3 of the pinna was snipped off with one scissors' stroke.

3) The excised pinnae were placed immediately in appropriate fixing solutions for electron microscopic study and tissue enzyme study, respectively.

Tissue Processing for Morphologic Study by Electron Microscopy

Several types of osmic acid fixation mixtures (Mercer and Birbeck, 1961)

and newer aldehyde group mixtures (Sabatini, et al., 1963; Sabatini, et al., 1964) were tried. The best fixation of the tissue for both light and electron microscopic study was provided by a 0.1 M phosphate buffered (pH 7.4) 1% osmium tetroxide with 0.045 g sucrose per ml (Caufield, 1957) solution at 4°C. Its preparation is as follows:

Preparation of stock solutions, 0.2 M

Stock solution A — 5.52 g $NaH_2PO_4 \cdot H_2O$/200 ml Cutter's distilled water

Stock solution B — 11.36 g Na_2HOP_4/400 ml Cutter's distilled water

Preparation of working buffer solutions (pH 7.4), 0.1 M

19 ml of solution A

81 ml of solution B

100 ml of Cutter's distilled water

Preparation of final fixative

Combine 25 ml of working buffer solution, and 0.5 g of crystalline osmium tetroxide. Shake thoroughly and store overnight (12 to 24 hours) at 4°C until osmium is completely dissolved. Add another 25 ml of chilled working buffer solution to obtain final fixative consisting of a phosphate buffered (pH 7.4) 1% osmium tetroxide solution. To this was added 2.25 g sucrose (0.045 g sucrose per ml of fixative).

1) Immediately after excision, the tissue was dropped in cold fixative and cut into small pieces (approximately less than 1.0 cu mm) with oil-free and clean steel razor blades.

2) The minced tissue was then placed in fresh cold fixative and kept in the refrigerator for two hours. The specific schedule for further tissue processing (washing, dehydration, infiltration and embedding) was as follows:

Washing

45 minutes in 0.1 M phosphate buffer, pH 7.4 (3 changes)

Dehydration

(a) 35% C_2H_5OH for 15 minutes (2 changes)

(b) 50% C_2H_5OH for 15 minutes (2 changes)

(c) 80% C_2H_5OH for 15 minutes (2 changes)

(d) 95% C_2H_5OH for 15 minutes (2 changes)

(e) 100% C_2H_5OH for 30 minutes (3 changes)

Infiltration

(a) 1/2 propylene oxide and 1/2 C_2H_5OH mixtures for 30 minutes (2 changes)

(b) 100% propylene oxide and 1/2 Epon 812 (Luft, 1961) mixtures (mixture A - 62 ml of Epon 812 and 100 ml of dodecenyl succinic anhydride; mixture B - 100 ml of Epon 812 and 89 ml of methyl nadic anhydride) with 2,4,6-tridimethylaminomethylphenol (1.5%) as the catalyst overnight (approximately 15-20 hours) at room temperature

Embedding

Individual pieces of tissue were embedded in Epon 812 mixture containing catalyst in Beem plastic capsules and polymerized in the oven at 45°C for approximately 20 hours and at 60°C for approximately 48 hours until hardened sufficiently for cutting.

3) The blocks were cut into ultra-thin sections (approximately 400-500 A thick) utilizing MT-1, or MT-2 Porter-Blum ultra microtomes fitted with conventionally prepared glass knives or knives made by the LKB Knife-Maker.

4) Ultra-thin sections were mounted on uncoated copper 300 mesh specimen screens for electron microscopic study.

5) Ultra-thin sections were double stained with 2% aqueous uranyl acetate and aqueous lead hydroxide (Watson, 1958). The latter was preferred over other lead staining methods tried (Björkman and Hellström, 1965; Frasca and Parks, 1965; Reynolds, 1963; Venable and Coggeshall, 1965) in this study. Optimal results with 2% uranyl acetate solution were obtained when staining was done at room temperature (about 24°C) for 15 minutes, and washed by agitation in freshly prepared distilled water and air dried. After completely drying, the uranyl acetate stained sections were then stained with aqueous lead hydroxide solution at room temperature (about 24°C) for 25 to 30 minutes and handled by the filter paper technique described by Normann (1964).

6) The stained ultra-thin sections were examined with the RCA EMU-3F electron microscope at direct magnification ranging from 8500X through

17,500X and appropriate fields were photographed.

Tissue Processing for Enzyme Localization

The method used to study the localization of ATPase was that detailed by Wachstein and Meisel (1957a, 1957b). Freshly excised pinnae were processed for enzyme localization and subsequent microscopic visualization as follows:

1) Freshly excised tissue was fixed for enzyme study for 24 hours in 10% formalin containing 1% calcium chloride solution at $4^{\circ}C$. It was then cut into small pieces (approximately 1.0 cu mm or less) with oil-free and clean steel razor blades.

2) The minced tissue was then rinsed in cold Cutter's distilled water.

3) For positive test demonstration of ATPase the tissue was processed as follows:

(a) Incubated for 90 minutes at $37^{\circ}C$ in the following mixture made up with Cutter's distilled water:

20.0 ml of 0.125 g% ATP solution

20.0 ml of 0.2 M tris-maleat buffer solution pH 7.5*

3.0 ml of 2% lead nitrate solution

5.0 ml of 0.1 M magnesium nitrate solution

2.0 ml of Cutter's distilled water

*pH 7.5 at room temperature ($25^{\circ}C$) is pH 7.2 at $37^{\circ}C$

(b) One minute rinse in Cutter's distilled water (4 changes).

(c) Five minutes in diluted ammonium sulfide solution.

(d) A positive ATPase activity is indicated by a visible reaction product by converting the lead phosphate into lead sulfide.

4) For a control check of the method against false positive reactions, the tissue was similarly processed as above but with the basic mixture modified as follows to eliminate ATP substrate:

0.0 ml of 0.125 g% ATP solution

40.0 ml of 0.2 M tris-maleat buffer solution pH 7.5*

3.0 ml of 2% lead nitrate solution

5.0 ml of 0.1 M magnesium nitrate solution

2.0 ml of Cutter's distilled water

*pH 7.5 at room temperature (25°C) is pH 7.2 at 37°C

5) Further processing of tissue for enzyme localization at the light microscopic level was as follows:

(a) Washed momentarily in tap water and distilled water.

(b) Dehydrated in ascending order from 35%, 50%, 65%, 80%, 95%, 100% C_2H_5OH, then 1/2 100% C_2H_5OH and 1/2 100% $C_6H_5CH_3$ for 15 minutes each.

(c) Infiltrated sequentially in 3 paraffin (Tissuemat) baths 20 minutes each at 60°C.

(d) Embedded in paraffin (Tissuemat).

(e) Paraffin embedded tissue was sectioned at 8μ with the

A.O. rotary microtome.

(f) Sections were flattened on CO_2-free distilled water in a waterbath at $45^{o}C$.

(g) The sections were mounted on egg albumin-coated glass slides and allowed to dry at medium heat in the Lipshaw electric drier for 20 minutes.

(h) Stain: Remove paraffin in 3 changes of 100% $C_6H_5CH_3$, 5 minutes for each change; hydrated to Cutter's distilled water through graded changes of toluene and alcohol for 5 minutes each; and stained sections in aqueous Eosin solution (0.5%) momentarily.

(i) Dehydrated as above in graded alcohols and cleared in toluene and cover-slipped with permount medium.

6) Further processing of tissue for enzyme localization at the electron microscopic level was as follows:

(a) Formol-Calcium treated tissues were rinsed one minute in tap water and distilled water.

(b) Tissue post-fixed in sucrose isotonic phosphate buffered 1% osmic acid mixture (pH 7.4) for 2 hours.

(c) Washing, dehydration, infiltration, embedding, sectioning, staining of the time schedule given for tissue processing for morphologic study by electron microscopy.

(d) The finished ultra-thin sections were examined with the

RCA EMU-3F; RCA EMU-3G and RCA EMU-4A electron microscopes at direct magnifications ranging from 6000X through 17,500X and appropriate fields were photographed.

CHAPTER III

OBSERVATIONS

General Histology of the Pinna

The pinna shows a central supporting plate of elastic cartilage, the auricular cartilage as seen in Plate I, Fig. 1 in Appendix. (All Plates and Figures referred to in the dissertation will be found in the Appendix which begins on page 56). The meatal half of the pinna shows: (1) a dense irregular fibrous connective tissue zone in which the dermal and subcutaneous zones are indistinguishable and (2) an epidermal layer associated with hair follicles and sebaceous glands which may extend deep within the connective tissue layer to the supporting auricular cartilage. The outer surface of the pinna is structured generally like the meatal or inner surface but in addition shows portions of the auricular skeletal muscle inserted into the auricular cartilage as seen in Plate I, Fig. 1. Blood vascular and neural tissue components are present in the connective tissue zones. The vascular supply in particular to the sebaceous glands is limited and not closely associated with them, see Plate 1, Fig. 1. Neural elements (Droz, 1958; Jabonero, 1958) unmyelinated in type, occur in palisade-like arrangement in close association with the duct of the sebaceous gland (Dugan, 1965). In addition, axon-like structures (Bloom and Fawcett, 1962c; Breathnach, et al.,

1966; Hadek and Talso, 1967; Patton, 1960; Winkelmann, 1967) occur intercellularly in the supra-basal cell zone in the secretory portion of the sebaceous gland, see Plate XXVII, Fig. 54.

The General Ultrastructure of the Active Sebaceous Gland

The active sebaceous gland parenchyma is characterized by two apparently distinct cell types. The dominant cell type comprising the bulk of the sebaceous gland is the keratinocyte of basal cell or Malpighi-cell origin, see Plate II, Fig. 2, and Plate V, Fig. 5. It is also the cell type particularly involved in the holocrine process of sebum formation and is usually characterized by varied accumulations of lipid, Plate II, Fig. 2. In this process, all keratinocytes in a given sebaceous gland, except those comprising the basal cell layer, undergo degenerative changes (Epstein and Epstein, 1966) which end in their total destruction.

The second cell type in the sebaceous gland parenchyma, commonly known descriptively as the "Clear Cell" or "Langerhans' Cell" (Langerhans, 1868) is of undetermined cell lineage (Andrew and Andrew, 1949; Billingham, 1948; Billingham and Meadawar, 1953; Breathnach, 1964; Breathnach and Wyllie, 1965a, 1965b; Breathnach, et al., 1968; Dugan, 1965; Fan, et al., 1959; Ferreira-Marques, 1951; Masson, 1948; Meischer and Schaaf, 1935; Mishima, 1966; Niebauer, 1956; Niebauer and Sekido, 1965; Richter, 1956; Thies, 1962; Wolff, 1967b; Zelickson, 1965; Zelickson and Mottaz, 1968). It is present in exceedingly limited numbers and is restricted usually to a basal cell layer position or a slightly supra-basal cell layer position, as demonstrated in

Plate XXV, Figs. 51 and 52 and Plate XXVI, Fig. 53. It has not been possible to relate the "Clear Cell" to the process of sebum formation.

The Fine Structure of the "Clear Cell" of the Sebaceous Gland

The cytoplasm of the "Clear Cell" is strikingly electron-lucent when compared with the other sebaceous gland cells, as demonstrated in Plate XXV, Figs. 51 and 52. The general cell surface is comparatively smooth in contrast to the surrounding keratinocytes. The cell appears to be just "set in" (Birbeck, et al., 1961; Kiistala and Mustakallio, 1967). It possesses characteristic foot-like processes, as demonstrated in Plate XXV, Figs. 51 and 52, which vary in length from approximately 1500 A to 12,000 A. These processes do not interdigitate with comparable processes of adjacent keratinocytes, as demonstrated in Plate XXV, Figs. 51 and 52. The cell membrane has a varied thickness from 50-120 A, and shows no intercellular bridges or desmosomal structures (Dugan, 1965; Hirone, 1966; Wolff, 1967a; Wolff and Winkelmann, 1967; Zelickson, 1965, 1966) as demonstrated in Plate XXVI, Fig. 53. The "Clear Cells" are situated in a supra-basal cell layer position and the nucleus is convoluted (Dugan, 1965; Hirone, 1966; Jimbow, et al., 1969; Wolff, 1967a; Wolff and Sollereder, 1969; Wolff and Winkelmann, 1967; Zelickson and Mottaz, 1968) as seen in Plate XXV, Fig. 52 and Plate XXVI, Fig. 53; in those at the basal cell layer position, the nucleus is essentially monomorphic (Breathnach, 1965; Dugan, 1965) as demonstrated in Plate XXV, Fig. 51. It possesses a double nuclear membrante (Dugan, 1965) with

an intra-membrane space of 120-240 A thickness. The chromatin appears largely as clumps adherent to the inner nuclear membrane (Dugan, 1965). The karyoplasm shows exceedingly fine desoxyribose nucleic acid (DNA) granules (Bloom and Fawcett, 1962a; Copenhaver, 1964a; Moses, 1956) and very fine filaments (Dugan, 1965), see Plate XXVI, Fig. 53. Nucleolar structures have not been seen in this cell (Dugan, 1965). The cytoplasm shows a juxtanuclear Golgi complex (Wolff, 1967a; Zelickson, 1966), mitochondria, lysosome-like bodies (Zelickson, 1966) rough and smooth endoplasmic reticulum, centrioles (Breathnach, 1965; Heist and Mulvaney, 1968), abundant cytoskeletal reticulum (Dugan, 1965), and Birbeck's or Langerhans' cell granules (Breathnach, 1964; Birbeck, et al., 1961; Horiki, et al., 1966; Wolff and Sollereder, 1969; Zelickson, 1966, 1967). The fine ultrastructural features and variations characterizing the above protoplasmic organelles and inclusion of the "Clear Cell" (Basset and Furiaf, 1965; Basset, et al., 1965; Basset and Nezelof, 1966) are shown in Plate XXV, Figs. 51 and 52 and Plate XXVI, Fig. 53.

The Fine Structure of the Keratinocytes in the Active Sebaceous Gland

The final product of the active sebaceous gland is comprised of a special lipid component and, also, of substance of completely destroyed sebaceous gland cells or principally keratinocytes. A standardized descriptive terminology is introduced and used here to identify the severl phases which characterize the holocrine transformations of the keratinocytes during the elaboration of the final glandular product, sebum.

Phase I relates to cells comprising the basal cell layer in all sebaceous glands and to all other keratinocytes irrespective of position in the gland which show no evidence of lipid droplet formation. Phase I is an inactive or nonsecretory or presecretory phase, see Plate II, Fig. 2; Plate V, Fig. 5; Plate VI, Fig. 7; Plate VII, Fig. 9; Plate VIII, Fig. 11 and Plate IX, Fig. 13.

Phase II is an early or beginning phase of lipid formation or deposition and relates to keratinocytes irrespective of position in the gland which contain reduced numbers of small lipid inclusion droplets, see Plate X, Fig. 15 and Plate XXVIII, Fig. 56.

Phase III is a mid-secretory phase and relates to keratinocytes irrespective of position within the gland which are characterized by numerous small lipid inclusion droplets in varied degrees of coalescence as demonstrated in Plate II, Fig. 2; Plate XI, Fig. 17; Plate XII, Fig. 19; Plate XVI, Fig. 27; Plate XVII, Fig. 29; Plate XX, Fig. 35 and Plate XXI, Fig. 37.

Phase IV is a late secretory phase and relates to keratinocytes irrespective of their position within the gland which are characterized by numerous large, rounded lipid inclusion droplets and which are attended by marked general hypertrophy as shown in Plate II, Fig. 2; Plate XIII, Fig. 21 and Plate XXVIII, Fig. 56.

Phase V is a terminal secretory phase and relates to keratinocytes irrespective of position within the gland which are characterized by closely packed, large, angular surfaced lipid inclusion droplets that all but obliterate the cytoplasm, see Plate XIII, Fig. 21 and Plate XIV, Fig. 23.

Phase VI is the final or completely holocrine transformation phase and relates to structurally disrupted keratinocytes which are comprised only of unidentifiable protoplasmic remnants and debris dispersed among non-descript masses of coalesced lipid material.

Presecretory, Phase I, Keratinocytes

Keratinocytes in the inactive or presecretory phase (Phase I) have fine structural features which are directly comparable to those comprising the basal cell layer of the epidermis, generally. These cells grossly are ovoid, as seen in Plate V, Fig. 5; polygonal, see Plate V, Fig. 6; or flattened, as demonstrated in Plate XXVIII, Fig. 56; and may be arranged parallel to the basement membrane as shown in Plate VI, Fig. 7. Variations in cell shape are also reflected by corresponding changes in the shape of the nuclei, as demonstrated in Plate XXII, Figs. 39 and 40.

The nucleus is rather large (Dugan, 1965) and occupies about four-fifths of the total cell mass as shown in Plate V, Figs. 5 and 6. It may be spheroid (Callan, 1955), see Plate V, Fig. 5, elonaged, see Plate XXVIII, Fig. 56, or polygonal (Hibbs, 1962), as seen in Plate V, Fig. 6, depending on the shape of the entire cell. The nucleus possesses a double nuclear membrane with the space between the two varying from 120-240 A in width (Dugan, 1965), as seen in Plate V, Figs. 5 and 6. Numerous fine, electron-dense granules, supposedly DNA (Bloom and Fawcett, 1962a; Copenhaver, 1964a; Moses, 1956), and fine filaments are dispersed throughout the nucleus, as seen in

Plate V, Fig. 5 and Plate XXII, Fig. 39. The chromatin clumps, as seen in Plate XXII, Figs. 39 and 40, are arranged marginally and attached to the inner nuclear membrane as shown in Plate V, Figs. 5 and 6 and Plate XXII, Figs. 39 and 40. Nucleoli of varied size, shape and number are scattered within the nucleus. The nucleolus has features suggesting it to be an irregularly coiled coarse cable comprised of densely-packed fine granules as demonstrated in Plate XXII, Fig. 39.

The cytoplasm is relatively electron-dense, see Plate II, Fig. 2; Plate V, Fig. 5 and Plate XXV, Fig. 52, and is surface-delimited by a well-defined plasma membrane, see Plate V, Figs. 5 and 6. The latter is characterized by desmosomes (Bizzozero and Vassale, 1887; Giroud and Champetier, 1936; Ranvier, 1879) which consist of a localized thickening of apposing cell membranes (Dugan, 1965) where there is relatively electron-dense amorphic substance with a relatively electron-lucent (Komura and Ofuji, 1967) space between them. The thickening of the desmosomes appears to be a convergence of bundles of fine tonofilaments (Dugan, 1965).

The mitochondrial complement consists of short to long rod-shaped forms (Dugan, 1965; Sjostrand, 1953) as seen in Plate VI, Figs. 7 and 8. They have a double outer membrane (Dugan, 1965; Freeman, 1956; Low, 1956; Palade, 1952; Rouiller, 1960; Sjostrand, 1953; Sjostrand and Rhodin, 1953; Sjostrand and Hanzon, 1954) with an intramembrane space about 70 A thick, see Plate VI, Figs. 7 and 8. The internal membrane system (Dugan, 1965) which corresponds to "cristae mitochondriales" (Palade, 1952) or "cristae"

(Freeman, 1956), see Plate VI, Figs. 7 and 8, has an intramembrane space about 110 A wide (Dugan, 1965). The matrix of the mitochondria is usually electron-dense, see Plate VI, Fig. 7 and Plate XVIII, Fig. 31, and contains small electron-dense granules of about 60 A in diameter (Dugan, 1965), as shown in Plate VI, Figs. 7 and 8. The mitochondrial complement is well dispersed and appears to be comprised of a small number of units.

The Golgi apparatus is present in the form of smooth surface multivesiculate bodies and cisternae, as demonstrated in Plate V, Fig. 5 and Plate VII, Figs. 9 and 10. It is often found at juxtanuclear positions, see Plate VII, Figs. 9 and 10, and in association with the cytocentrum.

Centrioles (Amano, 1957; deHarven, 1968; deHarven and Barnhard, 1956; Rhodin, 1963a; Windhorst, et al., 1968) can be observed in the vicinity of the Golgi zone (deHarven and Bernhard, 1956), see Plate VII, Fig. 10 and Plate XXVIII, Fig. 56. The centriole canaliculi are covered with fine RNA-like granules (Amano, 1957; David-Ferreira, 1962; deHarven, 1968).

Granular endoplasmic reticulum is present in the form of narrow saccules (Ellis and Henrikson, 1963; Montagna, 1962; Zelickson, et al., 1963) with cisternal dilatations as seen in Plate VI, Figs. 7 and 8.

Tonofibrils and their component tonofilaments occur as both wavy, see Plate IX, Fig. 13, or relatively smooth, see Plate IX, Fig. 14, bundles throughout much of the cytoplasm. The length of these tonofilaments is undefined but the diameter is within 70 A. Often they are traced to the vicinity of desmosomes where they may converge and form the attachment plaques (Copenhaver, 1964b;

Dugan, 1965; Kallman and Wessells, 1967).

Lysosome-like bodies are present which have an electron-dense internum and which are delimited by a single smooth membrane (Asford and Porter, 1962; Dugan, 1965; Rouiller and Bernhard, 1956) are demonstrated in Plate VIII, Figs. 11 and 12. In some instances they appear to be mitochondrial in nature (Brandes, 1965; Gahan, 1967; Novikoff, 1961a; Novikoff and Essner, 1962). Its internum shows no conventional cristae, but the arrangement of small vesicles and granules therein are suggestive of modified cristae (Novikoff and Essner, 1962).

Lipid secretion droplets are not present in the inactive or presecretory (Phase I) keratinocyte, see Plate V, Figs. 5 and 6; Plate VI, Figs. 7 and 8, and Plate XXVIII, Fig. 56.

Keratinocytic Changes During the Active Holocrine Secretory Process (Phases II-VI)

Keratinocytes in the active holocrine secretory process (Phases II-VI) show a progressive and predictable characteristic series of fine structural alterations, which affect all parts and organelles characterizing the presecretory (Phase I) keratinocyte that result finally in their complete destruction and dissolution. In addition, these protoplasmic structural alterations are attended by a characteristic and progressive accumulation of intra-cellular lipid which becomes the dominant component and feature of terminal holocrine transformed cells.

Nuclear alterations. Characteristic progressive pyknotic nuclear changes occur during the entire sebaceous holocrine transformation that parallels

the accumulation of lipid droplets in the cytoplasm. These changes first appear in the presecretory phase and continue through the terminal phase, as illustrated in Plate XXII, Figs. 41 and 42; Plate XXIII, Figs. 43, 44, 45 and 46, and Plate XXIV, Figs. 47, 48, 49 and 50, to the final holocrine phase (Dugan, 1965). At the onset of the pyknotic nuclear change, the nucleus becomes progressively more polymorphic and misshaped by the progressive accumulation of lipid droplets. By the end of the late-secretory phase it is much reduced in size as illustrated in Plate II, Fig. 2 and Plate XXIV, Figs. 47 and 48. The inner and outer nuclear membranes persist until the late secretory phase, as seen in Plate XXII, Figs. 41 and 42; Plate XXIII, Figs. 43, 44, 45 and 46, and Plate XXIV, Figs. 47 and 48, when, coincident with the general disruption of the cytoplasmic constituents, the outer nuclear membrane is lost. During the terminal and final phases (Phases V and VI), see Plate XXIV, Figs. 49 and 50, the chromatin is transformed into small, dense masses. These are dispersed in disorderly array when the nucleus undergoes a final karyorrhexis (Copenhaver, 1964a; Dugan, 1965; Robbins, 1960), as seen in Plate XXIV, Figs. 49 and 50. A nucleolus is present up to the late secretory phase (Phase IV), but thereafter it cannot be recognized (Dugan, 1965).

Cell surface alterations. Presecretory and early secretory cells have similar surface structure and are characterized by desmosomes (Dugan, 1965). During the early phase (Phase II) the surface cytoplasm is progressively transformed into relatively short villous processes which interdigitate with those of contiguous cells. At the mid-secretory phase it shows microvilli projecting from

the surfaces of both cells into intercellular spaces, as demonstrated in Plate XII, Fig. 19 and Plate XIX, Fig. 33. As the intracellular accumulation of lipid droplets progresses there is a coincidental disappearance of the organized intercellular space and interdigitating cell processes, as demonstrated in Plate XIII, Figs. 21 and 22 and Plate XIV, Figs. 23 and 24, and a disruption of the limiting plasma membranes of contiguous cells (Dugan, 1965). Isolated desmosomes with tonofibrils (Neve, 1963) persist into the terminal secretory phase as seen in Plate XIV, Figs. 23 and 24 and Plate XV, Figs. 25 and 26. In the completely sebaceous transformed phase (Phase VI), no plasma membrane or desmosomes can be identified (Dugan, 1965). During the course of the mid- and late-secretary phases, it is often noted that cortical cytoplasm of contiguous cells following plasma membrane disruption is transformed into broad ambiguous zones (Palay, 1958; Weiss, 1955) containing ribosomal-like granules, glycogen-like granules, and cytoskeletal reticulum fibrils, see Plate XIII, Fig. 21. In the terminal phase, these areas are progressively reduced in number and size, and become electron dense zones, which enclose islands of lipid droplets (Dugan, 1965) as seen in Plate XIV, Fig. 23 and Plate XV, Fig. 25.

Mitochondrial alterations. In the early secretory phase (Phase II) the mitochondria show slightly irregular profiles and an increase in density of the matrix as illustrated in Plate X, Fig. 15. In the mid-secretory phase, the individual mitochondria exhibit a wide range of variation within the same cell, see Plate XI, Fig. 17; Plate XII, Fig. 19; Plate XVI, Fig. 27; Plate XVII, Fig. 29, and Plate XX, Fig. 35. Some have the structure of early secretory phase

mitochondria as illustrated in Plate XI, Fig. 17, but most appear swollen and show an enlargement of the intramembrane space. The majority of mitochondria in this stage show profiles which reflect the packing of lipid droplets. In some, the mitochondrial outer membrane of the external membrane system is broken and it appears to be associated with the smooth membrane of the endoplasmic reticulum or Golgi vesicles, see Plate XVI, Fig. 27. As it progresses to the late secretory phase, the electron density of the matrix decreases. Also during this time some mitochondria show electron-dense inclusions of unknown significance and origin. In this instance the cristae appear to be diffuse and appear as ambiguous shadows. In the terminal secretory phase, there are only occasional suggestive remnants of mitochondria noted among the lipid droplets, see Plate XIV, Fig. 23. No mitochondrial remnants can be identified in the completely holocrine transformed cell (Dugan, 1965).

Golgi complex alterations. In the earliest secretory phase (Phase II), the Golgi apparatus consists of extensive masses of flattened and spheroidal vesicles associated peripherally with the lipid droplets (Dugan, 1965; Ellis and Henrikson, 1963; Hibbs, 1962), as seen in Plate X, Fig. 16. In the mid-secretory phase (Phase III), the Golgi complex is widely distributed in the cytoplasm and consists of flattened and spheroidal vesicles, as demonstrated in Plate XVI, Figs. 27 and 28. In the late secretory phase (Phase IV) coalesence of the small lipid droplets is virtually complete and large ones show flattened membrane systems on their surfaces, see Plate XXI, Fig. 37. In the terminal secretory phase (Phase V), the Golgi membrane system is disrupted and is

recognized only as small fragments between the greatly enlarged and closely packed lipid droples (Dugan, 1965), see Plate XIV, Fig. 23. In the final holocrine phase (Phase VI) no recognizable membrane system can be found (Dugan, 1965).

Alterations in endoplasmic reticulum and glycogen. In the early and mid-secretory phases (Phases II and III) there is a progressive increase in the amount of glycogen-like granules (Dugan, 1965; Henrikson, 1965; Montagna, et al., 1951; Napolitano, 1963; Revel, 1964) see Plate XI, Fig. 17; Plate XVI, Fig. 27; Plate XVII, Fig. 29 and Plate XXIX, Fig. 33. These, in the early phase, are difficult to distinguish from the numerous free ribonucleoprotein (RNP) particles which characterize both presecretory (Phase I) and early secretory (Phase II) cells as demonstrated in Plate V, Fig. 5; Plate VI, Fig. 7, and Plate X, Fig. 15. In the mid-secretory phase (Phase III) glycogen granules (Dugan, 1965; Revel, 1964; Revel, et al., 1960) are topographically associated with agranular endoplasmic reticulum (Dugan, 1965; Porter, 1961) and lipid droplets, see Plate XVI, Fig. 27 and Plate XVII, Fig. 29. In the late, terminal and final secretory phases (Phases IV, V, and VI), the glycogen-like granules progressively disappear, as demonstrated in Plate XIII, Fig. 21 and Plate XIV, Fig. 23.

Conventional granular endoplasmic reticulum is discernible through the mid-secretory phase (Phase III). In the late, terminal and final phases (Phases IV, V, and VI) the endoplasmic reticulum is converted to agranular and dispersed forms which disappear completely in the final phase (Phase VI) as described by Dugan (1965).

Lysosomal alterations. Lysosome-like bodies increase progressively in number (Brandes, et al., 1965) through the mid-secretory phase (Phase III), see Plate XII, Fig. 19 and Plate XX, Fig. 35. All have a single limiting membrane (Novikoff, 1961b). Some show internal vesicles and granules suggestive of mitochondrial features (Novikoff and Essner, 1962), see Plate XX, Fig. 35, and others are filled with fine electron-dense granules (25-40 A in diameter) as seen in Plate XII, Fig. 19. In the late, terminal and final secretory phases (Phases IV, V, and VI) they become progressively reduced in number and ultimately disappear as formed elements among the protoplasmic remnants (Dugan, 1965), see Plate XIII, Fig. 21 and Plate XIV, Fig. 23.

Lipid accumulation. The lipid content during the course of the holocrine secretory process appears to increase in parallel with the progressive alteration, disruption and final dissolution of all the protoplasmic structural components. In the early secretory phase (Phase II) only occasional lipid inclusions are demonstrable (Montagna and Noback, 1947), as demonstrated in Plate X, Fig. 15 and Plate XXVIII, Fig. 56, and total protoplasmic structural features are near normal or unchanged from that characteristic of the presecretory phase (Phase I) cell. In the mid-secretory phase (Phase III) the entire cell is hypertrophic (or enlarged) and filled with various sized coalescing lipid droplets (Ellis, 1967), as seen in Plate XI, Fig. 17; Plate XII, Fig. 19; Plate XVI, Fig. 27 and Plate XVII, Fig. 29. The latter show electron-dense residue considered to be squalene (Ellis and Henrikson, 1963; Griesemer and Thomas, 1963; Nicholaides and Rothman, 1955), as shown in Plate XI, Fig. 17 and Plate XII, Fig. 19.

Other protoplasmic structural features show intrinsic alterations from those characterizing the normal or presecretory phase (Phase I) cells as illustrated in Plate V, Fig. 5 and Plate VI, Fig. 7. In the late secretory phase (Phase IV) numerous, rounded large lipid droplets are present; the majority show no evidence of residual squalene, as demonstrated in Plate XIII, Fig. 21; Plate XIV, Fig. 23 and Plate XV, Fig. 25. As noted in detail in previous paragraphs, all other protoplasmic structural features are markedly modified both in number and in their intrinsic structural order from the normal which characterize that of the presecretory phase (Phase I) cell, see Plate V, Fig. 5 and Plate VI, Fig. 7. In the terminal phase (Phase V) and final phase (Phase VI) there are only barely recognizable protoplasmic remnants or none at all and the lipid droplets are large and angular-shaped through closeness of packing and show no evidence of squalene residues, as demonstrated in Plate XIII, Fig. 21; Plate XIV, Fig. 23, and Plate XV, Fig. 25.

The Distribution of ATPase Enzyme Activity in the Active Sebaceous Gland

The histochemical method (Wachstein and Meisel, 1957a, 1957b) used in this study to test for the presence of adenosine triphosphatase (ATPase) under controlled conditions yields in the presence of adenosine triphosphate (ATP) a water insoluble precipitate, lead phosphate (Barka and Anderson, 1963; Burstone, 1962; Pearse, 1968b; Wachstein and Meisel, 1957a, 1957b). The reaction product, lead phosphate, is converted to lead sulfide in this method. It is electron dense and is demonstrable at both the light and electron microscopic

levels of observation. The presence of demonstrable reaction products is a positive indication of ATPase activity; the absence of demonstrable products indicates no ATPase activity (Barka and Anderson, 1963; Pearse, 1968b; Wachstein and Meisel, 1957a, 1957b). Although not clearly established, there appears to be several species of generic ATPase enzymes (Bradshaw, et al., 1963; Freiman and Kaplan, 1960; Padykula and Herman, 1955b). The method of Wachstein and Meisel (1957a, 1957b) which yielded reproducible results in this study, however, may or may not be sensitive to the three general types of ATPase that have been produced (Bradshaw, et al., 1963; Butschak, 1963). At the light microscopic level the reaction product may appear as irregular fine dark brownish-black granules or aggregates of granules, or as small diffuse poorly delimited areas of brownish-black coloration. At the electron microscopic level, the reaction product, while more clearly visualized, presents similar types of appearances. In its granular forms the product is present as round granules 115 to 350 A in diameter and may occur singly or in irregular aggregates. In its diffuse form it appears as small, poorly (or vaguely) delimited areas having relatively high electron density and sometimes showing a texturing of fine granules 30 to 50 A in diameter.

Control checks on the method per se, performed on tissue treated and incubated in the absence of ATP substrate, showed no evidence of a reaction product within the tissue. This established that the method of treatment in itself did not induce spurious or false-positive tests or other precipitates in the tissue.

Additional control checks on the general capability of the method

consisted of study of several cell types of markedly different lineage and function from those comprising the sebaceous gland parenchyma for ATPase activity. These included: (1) skeletal muscle fibers and (2) chondrocytes.

Keratinocytes in all phases of the holocrine secretory process were analyzed for ATPase activity. The "Clear Cell" or "Langerhans' Cell" also was examined for ATPase.

ATPase Activity in Non-Sebaceous Gland Cells

Skeletal Muscle Fibers

Muscle fibers of the voluntary auricular muscle showed excellent fine structure preservation (Bloom and Fawcett, 1962b; Rhodin, 1963b; Smith, 1962). ATPase reaction products occurred deep within the substance of the individual muscle fiber and were associated with the sarcotubule system (Gordon, et al., 1967; Padykula and Gauthier, 1963; Soulairac and Desclaux, 1949). The latter is an ordered system of tubules located between the individual myofibrils comprised of myofilaments as seen in Plate III, Fig. 3.

Chondrocytes

Chondrocytes within the auricular cartilage showed ATPase reaction products deposited in and on the cortical cytoplasmic zone (Zelickson and Mottaz, 1968). The latter is comprised of irregularly shaped and sized processes (Yasuzumi and Tsubo, 1966) with and without discrete plasma membranes which blend into the fibrous cartilaginous matrix, see Plate IV, Fig. 4. The fine structural features are consistent with those reported by others (Godman and

Porter, 1960; Lévai and Marx, 1969; Schenk, et al., 1967; Silver and Hart, 1967).

Nervous Cell Processes

Classically classified non-myelinated nerve cell processes show a diffuse distribution of ATPase reaction product in the general cortical cytoplasmic zone (Amamiya and Okazoe, 1960; Fried, 1965; Torack, 1964), as demonstrated in Plate XXVII, Fig. 55.

ATPase Activity in the Sebaceous Gland Cells

"The Clear Cell"

The "Clear Cells" of the sebaceous gland consistently showed no clear-cut evidence of ATPase activity in that no reaction products were formed nor had the sharp structural features, such as those developed in skeletal muscle fibers and chondrocytes. Possible reaction products, consisting of fine granules 40 to 50 A in diameter, occurred singly or as diffuse aggregates associated with the external membrane system of some but not all mitochondria, as demonstrated in Plate XXV, Figs. 51 and 52 which can be compared with Plate XXVI, Fig. 53. The other fine structural features of this cell, namely the intra-nuclear substance, the nuclear membranes, the Golgi complex, centriolar apparatus, cytoskeleton, Birbeck's granules, lysosomal-like bodies or cortical cytoplasm (Mustakallio, 1962) showed nothing comparable or equivalent to the fine granular material found associated with the mitochondria, see Plate XXVI, Fig. 53.

Keratinocytes

Discernible ATPase reaction product in the keratinocytes was comprised of individually dispersed fine electron-dense granules (40-50 A in diameter) or irregular small aggregates.

Reaction product was present in greatest amount in the inactive or pre-secretory phase (Phase I) keratinocytes. In this phase, the reaction product was associated with many of the mitochondria visualized. The product appeared either on the external mitochondrial membrane system or just outside the mitochondria in the cytoplasmic matrix. Some product appeared, too, to be distributed in limited areas in the cortical cytoplasm and near the plasma membrane, see Plate V, Fig. 6 and Plate XXVIII, Fig. 56. The reaction product could not be topographically related clearly to any other of the protoplasmic structural features, namely the nucleus, see Plate V, Fig. 6 and Plate XXVIII, Fig. 56; endoplasmic reticulum, see Plate VI, Fig. 8; Golgi complex, see Plate VII, Fig. 10; centriolar apparatus, see Plate VII, Fig. 10 and Plate XXVIII, Fig. 56; tonofibrils, see Plate IX, Fig. 14; or lysosome-like bodies, see Plate VIII, Fig. 12.

Keratinocytes in the active holocrine secretory process showed ATPase reaction product, in progressively diminishing amount, in the early (Phase II), mid (Phase III) and late (Phase IV) phases. The terminal (Phase V) and final (Phase VI) holocrine transformation phases showed no demonstrable ATPase product. The product appeared in fine granular aggregates which were directly comparable to those in the inactive phase (Phase I) cells. In addition, their distribution also

was characteristically limited to mitochondria of near normal structure, as seen in Plate XII, Fig. 20 and Plate XXVIII, Fig. 56.

In addition to mitochondrial localization, small, irregularly fine granular deposits occur near the surface of lipid inclusion droplets in the earliest formative phases. In the latter situation, the droplets are associated intimately with complexes of Golgi membranes; it is therefore not possible to establish whether these granular deposits relate primarly to the Golgi complex or to the lipid droplet. The granules have dimensions which are comparable to those noted in connection with the mitochondria and are not found in association with lipid droplets after the latter have matured beyond the "squalene phase" and they do not occur in sections of control untreated tissue, see Plate X, Fig. 16; Plate XI, Fig. 18; Plate XII, Fig. 20; Plate XIII, Fig. 22; Plate XIV, Fig. 24 and Plate XXVIII, Fig. 56.

ATPase reaction product was not found in association with any other protoplasmic components during their structural transformation in the active holocrine secretory process (Phases II-VI) that is, with the nuclear material, see Plate XXIII, Fig. 44; Plate XXIII, Fig. 46 and Plate XXIV, Figs. 48 and 50; endoplasmic reticulum, see Plate XII, Fig. 20; Plate XVIII, Fig. 32 and Plate XIX, Fig. 34; plasma membrane or desmosome, see Plate XIV, Fig. 24; Plate XV, Fig. 26; Plate XX, Fig. 36 and Plate XXI, Fig. 38; or lysosome-like bodies, see Plate XIII, Fig. 22; Plate XVII, Fig. 30; Plate XVIII, Fig. 32 and Plate XX, Fig. 36.

CHAPTER IV

DISCUSSION

General Chemical Considerations of the ATPase Histochemical Method

Most methods for the demonstration of ATPase activity in tissue possess some inherent defect(s) (Burstone, 1962; Pearse, 1968b; Seligman, 1964). Biochemical and histochemical evidence indicate that the activity of ATPase varies within a single tissue type and between different tissues (Padykula and Gauthier, 1963), and at different histogenetic stages (Hasegawa, 1966). Cytochemical data indicate that possibly as many as three types of ATPase may exist (Freiman and Kaplan, 1960; Padykula and Herman, 1955b). Their existence has been predicated on the basis of studies on ATPase activity under controlled conditions of pH and the presence of other substances, notably, -SH compounds. Many factors, temperature, salt concentration, substrate concentration, pH, and specific types of buffers (Bosch, et al., 1967; Padykula and Herman, 1955a; Pinset-Härström, 1968; Vethamany and Lazarus, 1967) have been reported to affect ATPase activity. In the situation of the tissue complex, chemical fixation or freeze-fixation becomes an additional factor which in some way influences ATPase activity (Butschak, 1963; Eränkö, 1952; Essner, et al., 1958; Lazarus and

Barden, 1964; Ohkura, 1962; Torack, 1965; Wachstein, et al., 1962). Present ATPase histochemical methods reveal only a small percentage of ATPase activity. The reasons for this are multiple: (1) fixation of tissue by whatever method may inactivate a portion of the enzyme; (2) the enzymatic cleavage products (adenosine diphosphate and orthophosphate) are water soluble and may diffuse into foreign sites within the tissue complex or actually be extracted by the fixative or incubation media (Burstone, 1962; Pears, 1968a); (3) the ATPase enzyme per se, may diffuse from its normal tissue site during the fixation and incubation periods (Eränkö, 1952; Goldfischer, et al., 1964); (4) the strength of the natural binding or fixation of ATPase in specific protoplasmic structures or sites is of varied stability (Gomori, 1951); (5) rates of diffusion of important components of the incubation medium, such as lead nitrate and magnesium nitrate, in tissue complexes are not known and could materially influence the activation of ATPase and, also, the formation of the insoluble reaction product, lead phosphate.

The combination of finely minced portions of tissue and an initial chemical fixation of the tissue in cold formol-calcium is presently considered as the most effective approach in the study of the tissue localization of ATPase (Barka and Anderson, 1963; Wachstein, et al., 1960).

There are three types of activity characterizing phosphatase (Padykula and Gauthier, 1963): (1) true ATPase which is generally considered to be -SH dependent or requires -SH groups; (2) a non-specific alkaline phosphatase which is inhibited by -SH groups; (3) a polyphosphatase whose activity is insensitive to the presence or absence of -SH groups. Generally each of these types of ATPase

cleaves ATP into adenosine diphosphate and orthophosphate (Padykula and Gauthier, 1963; Padykula and Herman, 1955b; Pearse, 1968b).

The histochemical method of Wachstein and Meisel (1957a, 1957b) used in this study has been reported to record all three types of phosphatase activity (Ashworth, et al., 1963; Wachstein and Meisel, 1957b; Wachstein, et al., 1960; van Lennep, 1968). At present, in the absence of precise biochemical data on these types of ATPase, it has been deduced from general biochemical data bearing on the distribution of -SH compounds in cell fraction and certain organ structures (Freiman and Kaplan, 1960; Padykula and Gauthier, 1963; Padykula and Herman, 1955b; Wachstein, et al., 1960) that (1) phosphatase enzyme activity associated with the mitochondria is indicative of the presence of type I (true) phosphatase; (2) phosphatase activity associated with Golgi membranes is usually considered to be indicative of acid phosphatase; and (3) that activity associated with cell surface or plasma membrane is considered to be indicative of type II (non-specific) alkaline phosphatase.

ATPase Localization

The status of ATPase activity in the epidermal cells of mammals is not clearly established. This has been relegated to species differences (Baillie, et al., 1966; Machida and Giacometti, 1967; Montagna, 1962; Nicolaides, 1965; Perkins, 1966; Perkins and Machida, 1967) to unknown intrinsic differences obtained in the skin from region to region in the same individual (Machida and Giacometti, 1967; Montagna, 1962; Nicolaides, 1965; Perkins, 1966;

Perkins and Machida, 1967) and to differences in methods used (Torack, 1965; Sabatini, et al., 1963; Seligman, 1964; Tormey, 1966).

In general, the data as recorded in this study on ATPase activity in the sebaceous gland and epidermis and associated stromal supporting and other tissues confirms and/or extends previously reported findings.

Light microscopic studies in general have demonstrated ATPase activity in the presecretory phase (Phase I) basal cell layer of the sebaceous gland proper and of the sebaceous gland duct, and in the equivalent basal cell layer of the epidermis generally (Bradshaw, et al., 1963; Raetallie and Levonen, 1963; Tormey, 1966; Wolff, 1963). One ultrastructural study on the localization of ATPase activity in an epidermal structure has been reported (Zelickson and Mottaz, 1968). Zelickson and Mottaz found that ATPase activity was primarily localized at the plasma membrane in both the "Clear Cell" type and basal cell type of the general epidermis; and that a diffuse minimal activity in the cytoplasm of the cells occurred which could not be related to any specific cytoplasmic organelle. The observations recorded here confirm that ATPase activity is associated with the cortical cytoplasm and cell surface regions of these cell type equivalents as they comprise a part of the sebaceous gland parenchyma, as seen in Plate V, Fig. 6 and Plate XXVIII, Fig. 56. In addition, mitochondrial associated (type I ATPase) ATPase activity has been demonstrated not only in the inactive (Phase I) basal cell but also in diminishing degrees in the early (Phase II), mid (Phase III) and late (Phase IV) phases of the holocrine secretory transformation, see Plate XII, Fig. 20; Plate XVII, Fig. 30 and Plate XXVIII, Fig. 56.

Further, the level of ATPase activity seems to be coincident with the presence of normally structured mitochondria as seen in Plate XII, Fig. 20 and Plate XXVIII, Fig. 56; no ATPase activity has been demonstrated in later holocrine phases (Phase V and VI) where the mitochondria are completely destroyed (Raetallie and Levonen, 1963; Wolff, 1963) as illustrated in Plate XIII, Fig. 22 and Plate XIV, Fig. 24. Inasmuch as ATPase activity is importantly involved in the Kreb cycle or respiratory enzyme cycle (Green, 1968; Harper, 1959) it is significant that studies by Montagna (1962) at the light microscopic level on succinic dehydrogenase showed that the activity of this respiratory enzyme in the sebaceous gland keratinocytes also paralleled the progressive decline in the mitochondrial complement during the holocrine transformation.

The possible occurrence of ATPase activity in connection with the early forming lipid inclusions (Phases II and III) which arise in close association with Golgi membrane complexes (Nasr, 1965) is of unknown significance, see Plate X, Fig. 16; Plate XI, Fig. 18; Plate XII, Fig. 20 and Plate XXVIII, Fig. 56. While specific roles are unknown, it seems certain that lipid synthesis involves jointly the participation of mitochondria, endoplasmic reticulum, lysosome, and Golgi activities (Brandes, et al., 1965; Parakkal and Matoltsy, 1964; Porter, 1961; Stein and Stein, 1967). Autoradiographic studies on lactating mouse mammary gland indicate some participation of the Golgi complex in the synthesis of triglyceride and the protein moiety of protein-bound lipid (Stein and Stein, 1967). The observations recorded here on the relation of the early lipid droplets to Golgi membranes (Nasr, 1965) in the early phases of the holocrine secretory

process are consistent with autoradiographic data (Stein and Stein, 1967) in that sebum under usual circumstances contains both triglyceride (Nicolaides, 1965) and lipo-protein components (Nasr, 1965). The ATPase activity noted in connection with the young-fat-droplet-Golgi complex association may not relate specifically to the Golgi membranes, although Nakai (1965) has reported ATPase activity in the Golgi membranes of neoplastic melanocytes of the epidermis of the hamster. It does seem significant though that ATPase activity is not noted in connection with the more sustained lipid droplets in late phases (Phases IV-VI) wherein they are unassociated with Golgi membranes or wherein there is a total destruction or absence of descernible Golgi membranes.

General Significance of Correlatable Structural and Enzyme Activity Changes in the Sebaceous Gland Parenchyma

While it seems certain the synthesis of lipids in cells generally involves the joint participation of mitochondrial, endoplasmic reticulum, Golgi and lysosomal activities (Brandes, et al., 1965; Parakkal and Matoltsy, 1964; Porter, 1961; Stein and Stein, 1967) their specific roles are not clearly defined. Lipid production in the sebaceous gland, through a progressive holocrine process, cannot be expected to be equivalent to that characterizing lipogenesis in the lipocyte, or that or partially comparable apocrine cells of the mammary gland inasmuch as in the two latter cases, normal protoplasmic function and structure is maintained. In the sebaceous gland, the total complement of enzymes and activities and enzyme machinery essential to the maintenance of the functional and structural integrity of the cells is non-operating. It is shown by the progressive retrograde structural

changes and final lysis of all parts of the sebaceous cells during Phases II-VI of the holocrine process. This is shown, too, by a parallel progressive reduction and loss of both succinic dehydrogenase activity (Montagna, 1962) and mitochondrial ATPase and Golgi-ATPase activity during the II-VI holocrine phases. The latter circumstances would indicate further that some metabolic energy is requisite to early aspects of the formation of the lipid product. Later aspects of lipid accumulation and formation may be dependent on lysosomal activity and particularly on their content of hydrolytic enzymes, such as acid phosphatase and beta-glucuronidase (Hayashi, 1967) which are dispersed throughout the cytoplasm in sebaceous cells during the IV-V phases (Brandes, et al., 1965). As shown here and by others (Brandes, et al., 1965; Helminen and Ericsson, 1968a, 1968b; Helminen, et al., 1968), lysosomes increase in abundance through the III-IV phases of the holocrine process; their structural change and disappearance is coincident with the disruption and lysis of all protoplasmic structures and with the appearance of fully differentiated or mature lipid inclusions, see Plate XIII, Fig. 22 and Plate XXVIII, Fig. 56. It seems permissible to suggest that hydrolytic lysosomal enzymes (Brandes, et al., 1965; deDuve, 1959, 1963) released after the mid-secretory phase (Phase III) of holocrine process are effective in the structural disruption of all the protoplasmic organelles (endoplasmic reticulum, Golgi, mitochondria, nuclear membrane, plasma membrane, etc.) and that they appear to be effective through their hydrolytic activity in the reclamation or salvaging of the lipid moiety of the lipo-proteins which represent a dominant molecular component of all the protoplasmic structural elements.

CHAPTER V

SUMMARY

The alterations in ultrastructure and in ATPase activity which accompany the production of sebum by an holocrine cytodegenerative process have been described in the sebaceous gland parenchymal cells of the rat.

The holocrine transformation of the keratinocytes of the sebaceous gland is an ordered and progressive process and is divisible into six structurally and functionally characterized phases: the inactive or presecretory (Phase I), early (Phase II), mid (Phase III), late (Phase IV), terminal (Phase V) and final (Phase VI).

The normal fine structuring which particularly characterizes the nucleus, endoplasmic reticulum, Golgi complex, cell surface and other features of the keratinocytes in the inactive or presecretory phase (Phase I) is progressively modified during the early through the terminal phases (Phases II through V). These progressive modifications include: (1) nuclear pyknosis and karyorrhexis; (2) a general lysis of cytoplasm; (3) specific structural disruption and disappearance of nuclear, mitochondrial, endoplasmic reticulum, Golgi, lysosomal and cell surface membrane systems; and (4) the disappearance of ribosomal particles and glycogen particles.

In the final phase (Phase VI) all of the intrinsic protoplasmic structures have been transformed into structurally unidentifiable protoplasmic debris.

In general, the progressive retrograde modification of intrinsic protoplasmic structure is paralleled by a progressive formation and deposition of particulate lipid material.

In general, the retrograde modification of intrinsic protoplasmic structures and the deposition of particulate lipid material are correlatable phase-wise with an increase in lysosomal-like bodies, during Phases II-III, and their structural disruption and disappearance during Phases IV through VI.

In general, ATPase activity is maximal in the presecretory or inactive phase (Phase I) and reduced in the II and III phases and is absent in the V-VI phases.

In general, the reduction and loss of ATPase activity is correlatable phase-wise with: (1) mitochondrial structural disruption and loss; (2) with the increase and disruption of lysosomal bodies; and (3) with the initial stage of lipid deposition and the terminal stage of lipid deposition.

The over-all indications of the data obatained are: (1) that initial stages in the lipogenic process in the sebaceous gland cells appear to require metabolic energy; (2) that the total enzymatic complex essential to the maintenance of normal structural and functional integrity of the cells (as in Phase I) is inoperable or becomes so during the holocrine transformation (Phases II-VI); and (3) that the structural disruption or lytic transformation of intrinsic protoplasmic structures during the III-VI holocrine transformation phases are referrable to the action of lysosomal hydrolytic enzymes.

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APPENDIX

FIGURE LEGEND

Figures are micrographs of sebaceous glands and their associated extra-sebaceous structures taken with the Zeiss Photomicroscope for the light micrograph and RCA Models EMU-3F, -3G and -4A for the electron micrographs.

List of Abbreviations

BG	Barbeck's granules
C	centriolar apparatus
C 1	basal (Phase I) cell
C 2	"Clear Cell" or "Langerhans cell" of unknown origin
C 3	presecretory (Phase I) cell
C 4	early secretory (Phase II) cell
C 5	mid-secretory (Phase III) cell
C 6	late secretory (Phase IV) cell
C 7	terminal sebaceous (Phase V) cell
AC	auricular cartilage
AM	auricular muscle
D	desmosome
ER	endoplasmic reticulum
F	foot-like process
G	Golgi complex
HF	hair follicle
IC	intercellular channel
IS	inner surface

L	lipid inclusion
M	mitochondria
MS	membrane system
MV	microvilli
N	nucleus
OS	outer surface
SG	sebaceous gland
SR	cytoskeleton reticulum
T	tonofibrils
Ax	axon-like structure
Gl	glycogen-like granules
INm	inner nuclear membrane
Ly	lysosome-like structure
Mf	myofibrils
NF	neurofilament-like fibers
NM	nuclear membrane
ONm	outer nuclear membrane
Pm	plasma membrane
ch	chromatin mass
cz	connective tissue zone
n	nucleolus
arrow	ATPase reaction product
double arrows	ATPase-like reaction deposit associated with lipid

PLATE I

Figure 1. A cross section of rat pinna showing its general histologic structuring. Inner surface of pinna (IS), outer surface of pinna (OS), auricular cartilage (AC), auricular muscle (AM), hair follicle (HF), sebaceous glands (SG), dense irregular connective tissue zone (cz). (Lillie Mayer's Iron hematoxylin and Eosin, X400).

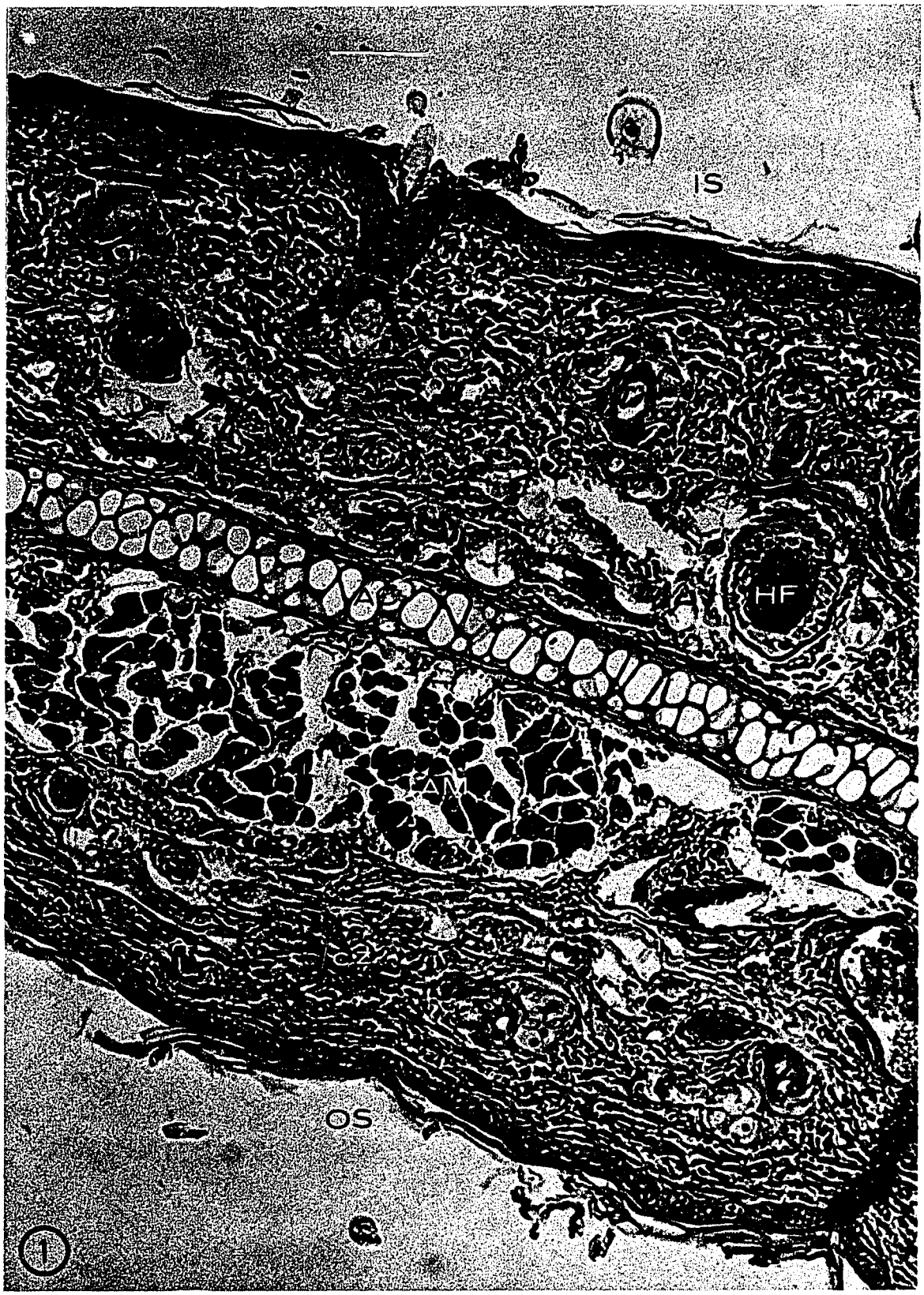

IS
HF
AM
OS
1

PLATE II

Figure 2. Longitudinal section through the neck and tip of a sebaceous gland showing its general organization and constituent cell layers with the electron microscope. Note the following in the orientation photograph: the basal (Phase I) cell (C 1), the mid-secretory (Phase III) cell (C 5), and the late secretoary (Phase IV) cell (C 6) at the center of the gland. (Stained with aqueous lead hydroxide, approximately X61,000).

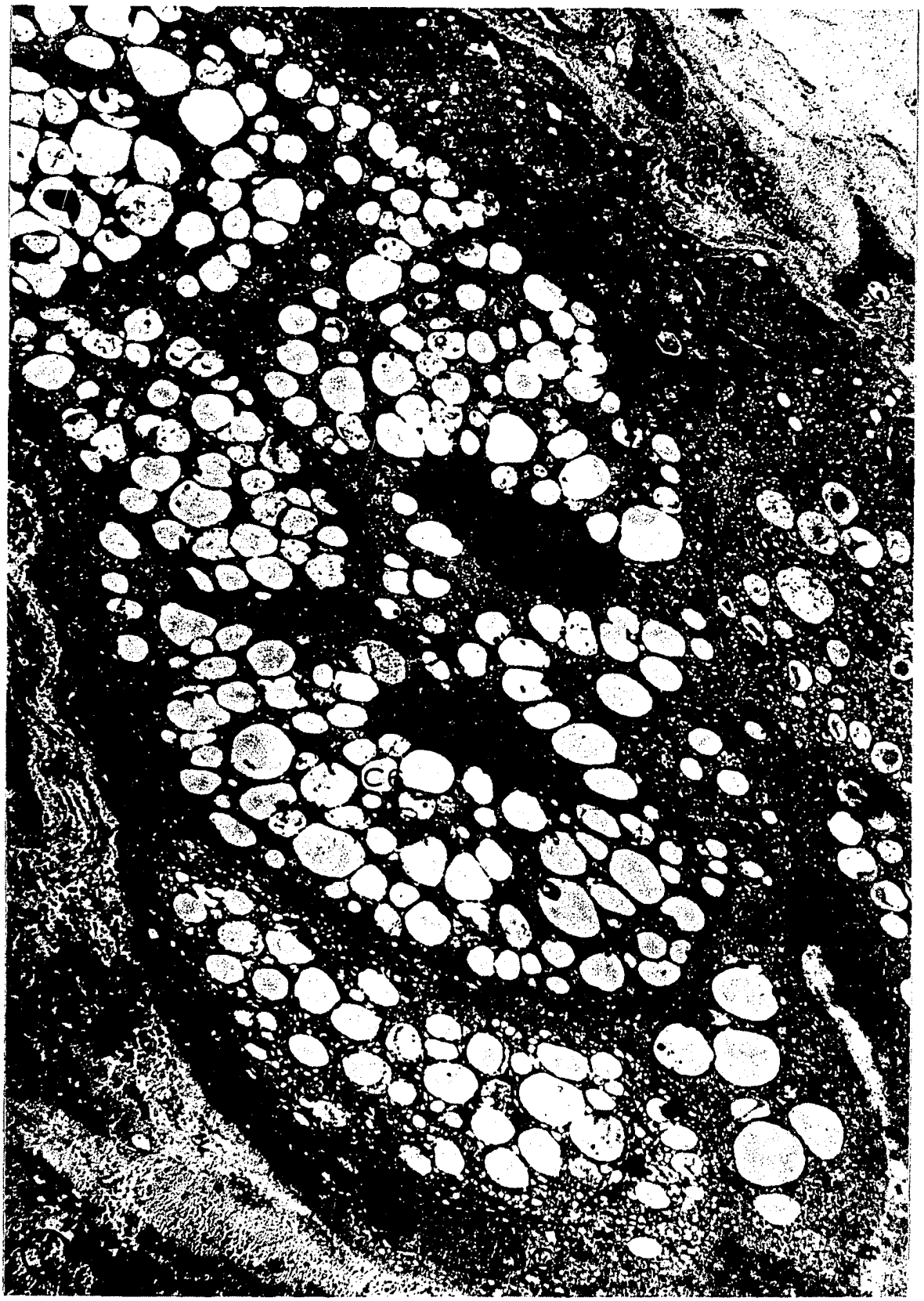


PLATE III

Figure 3. Cross section through the auricular muscle, showing ATPase localization. It demonstrates strong positive ATPase reaction product (arrow) within the sarcotubules which form a continuous system around and between individual myofibrils (Mf). (Stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X44,000).


Mt
3

PLATE IV

Figure 4. Section showing ATPase localization in chondrocyte of the peripheral portion of the auricular cartilage (see Figure 1). ATPase reaction product (arrow) is deposited in numerous, short cytoplasmic processes. Observe: numerous mitochondria (M) and lipid inclusion (L). (Stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X28,000).

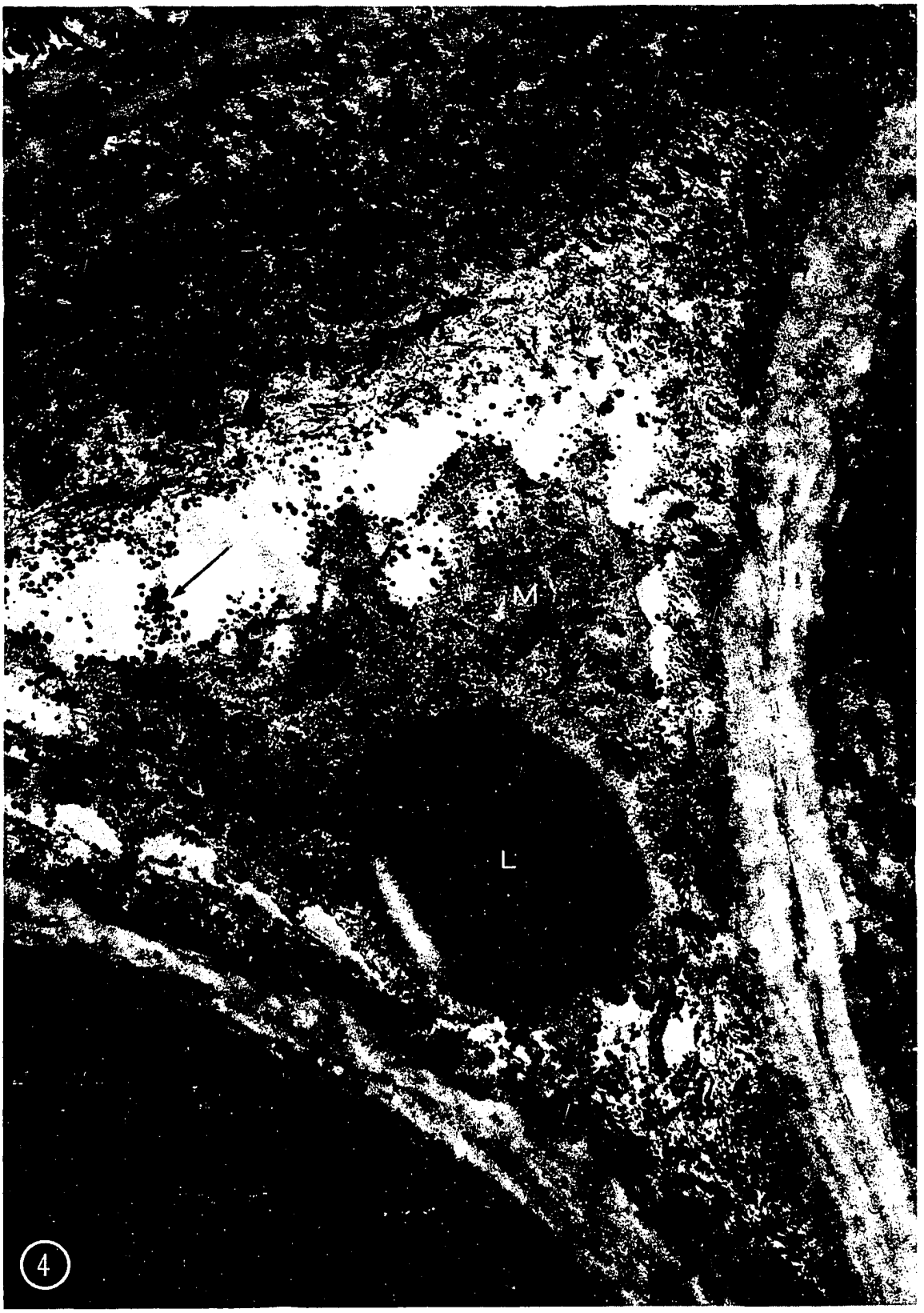
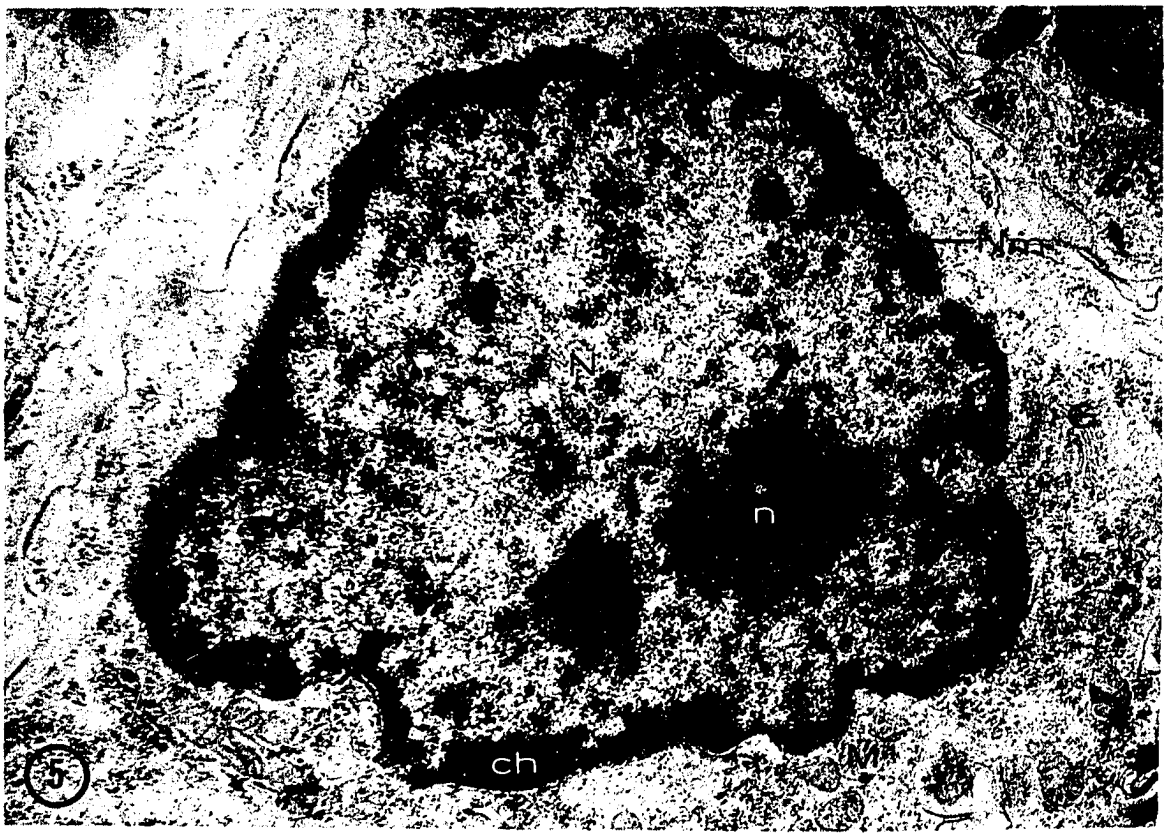

M
L
4

PLATE V

Figure 5. Control preparation of basal cell (Phase I).

Figure 6. Basal cell (Phase I) showing positive ATPase reaction product (arrow) in general cortical surface of cell.

Note: nucleus (N), nucleolus (n), chromatin masses (ch), nuclear membrane (Nm), Golgi complex (G), mitochondria (M). (Stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X24,000).


N
n
ch
M

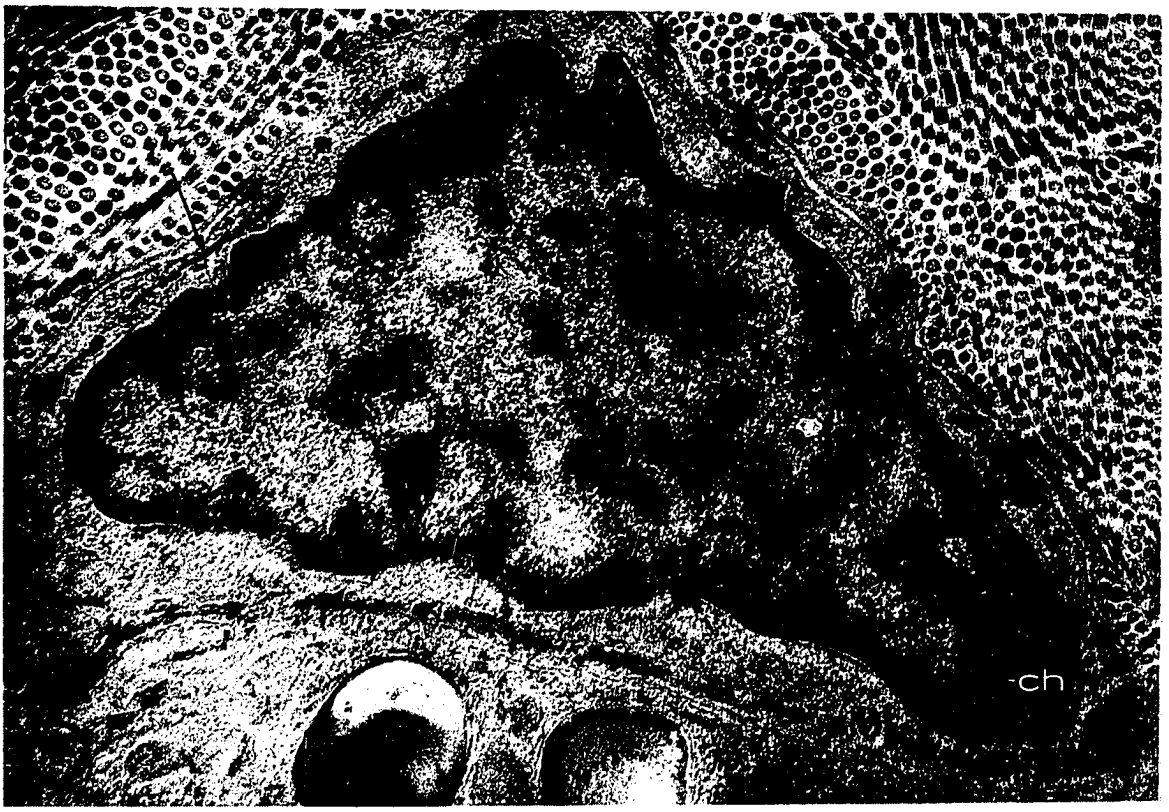

ch

PLATE VI

Figure 7. Control preparation of basal (Phase I) cell (C 1) and slightly advanced mid-secretory (Phase III) cell (C 5).

Figure 8. Basal cell (Phase I) showing no ATPase reaction product.

Note: mitochondria (M), endoplasmic reticulum (ER) with ribosomal granules. (Stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X31,000).

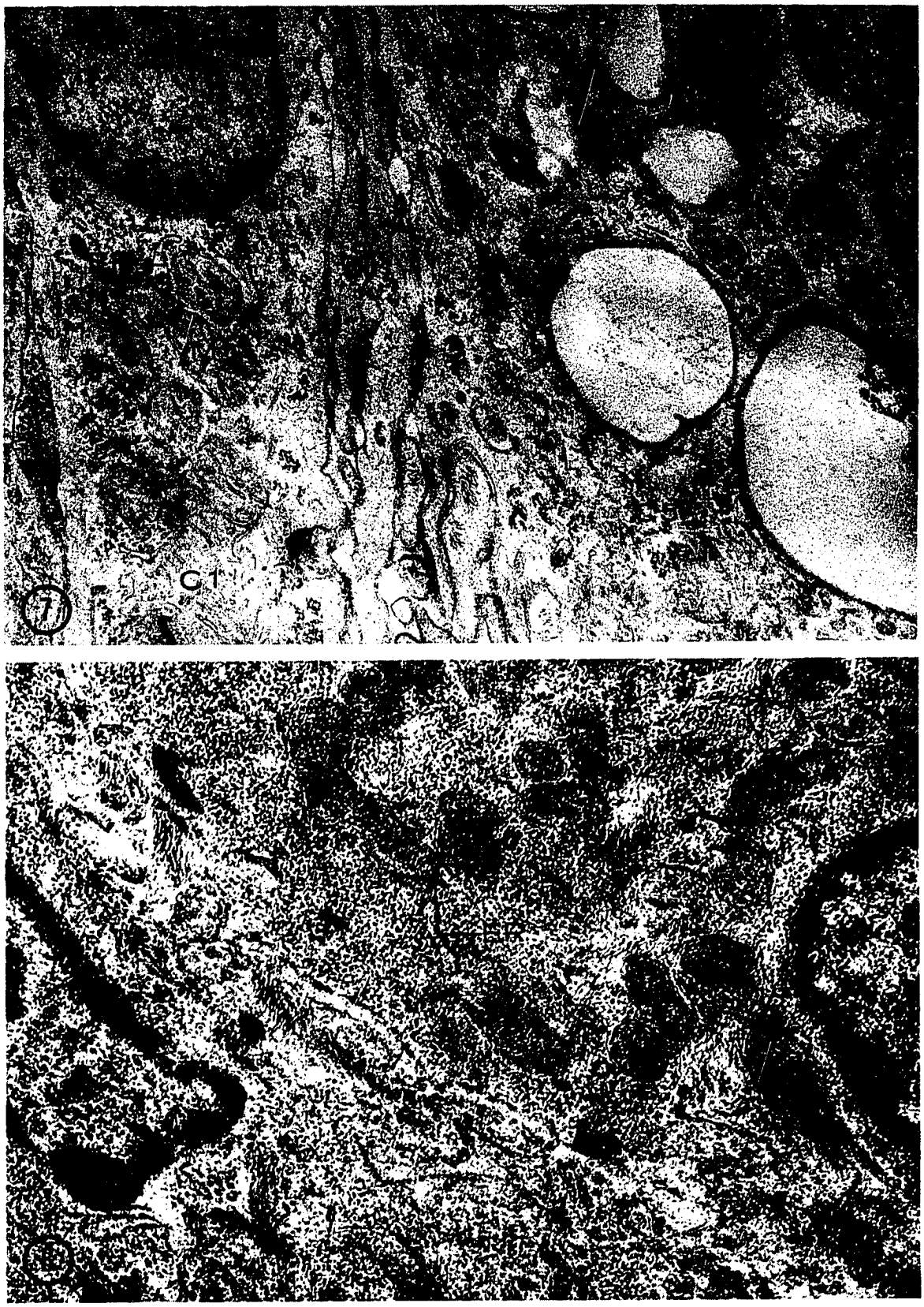
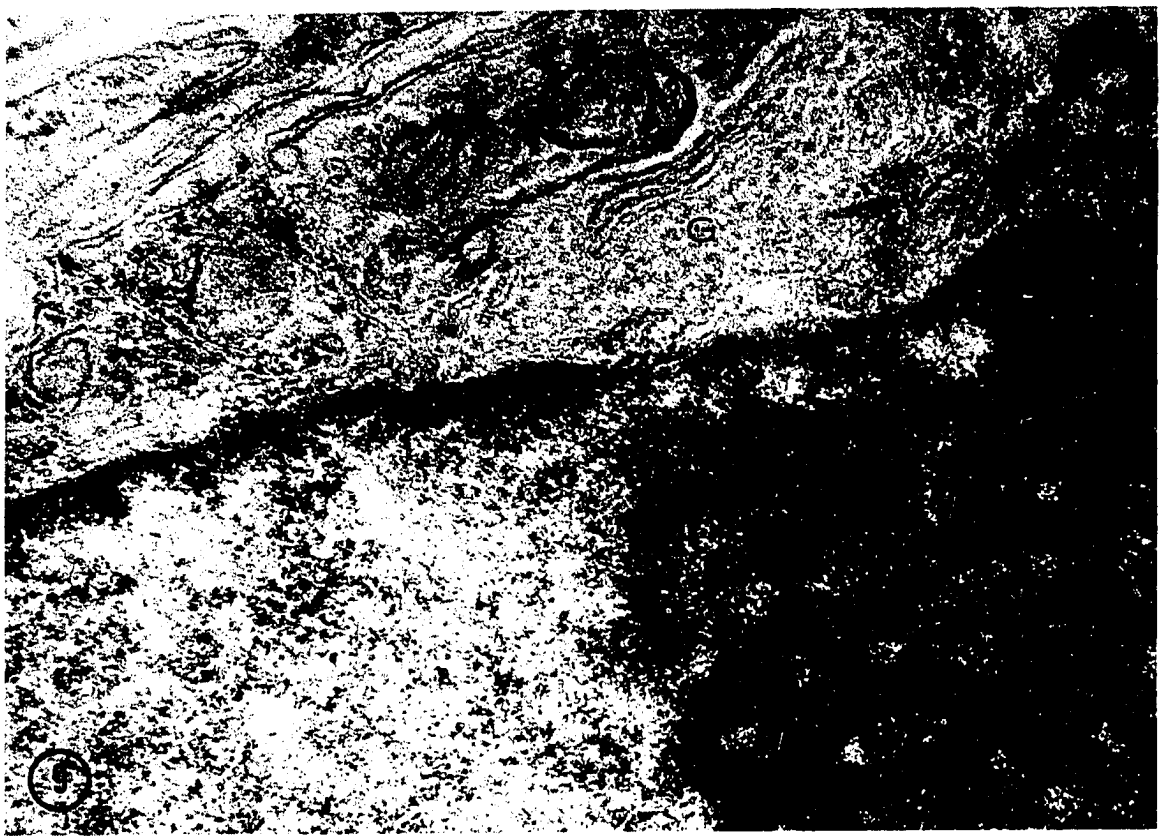

7
C1

PLATE VII

Figure 9. Control preparation of basal cell (Phase I).

Figure 10. Basal cell (Phase I) showing no ATPase reaction product.

Note: Golgi complex (G), and centriolar apparatus (C). (Stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X31,000).


9

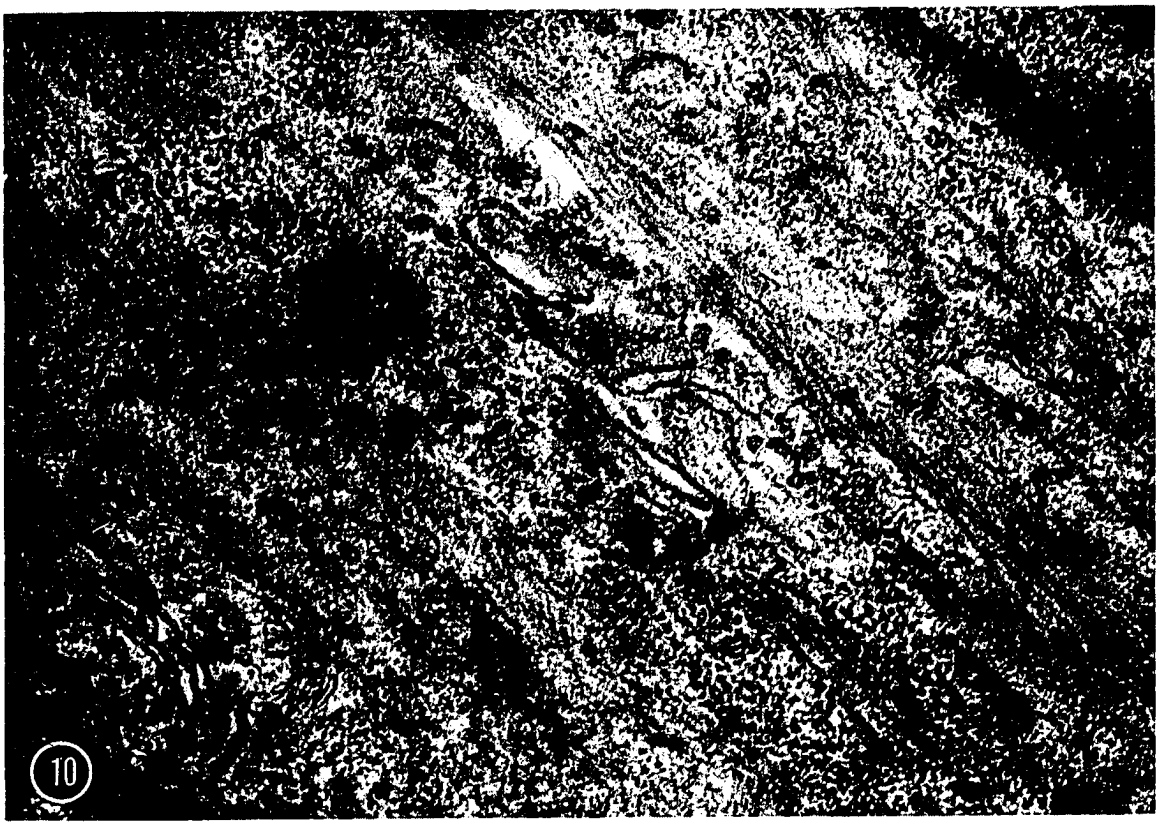
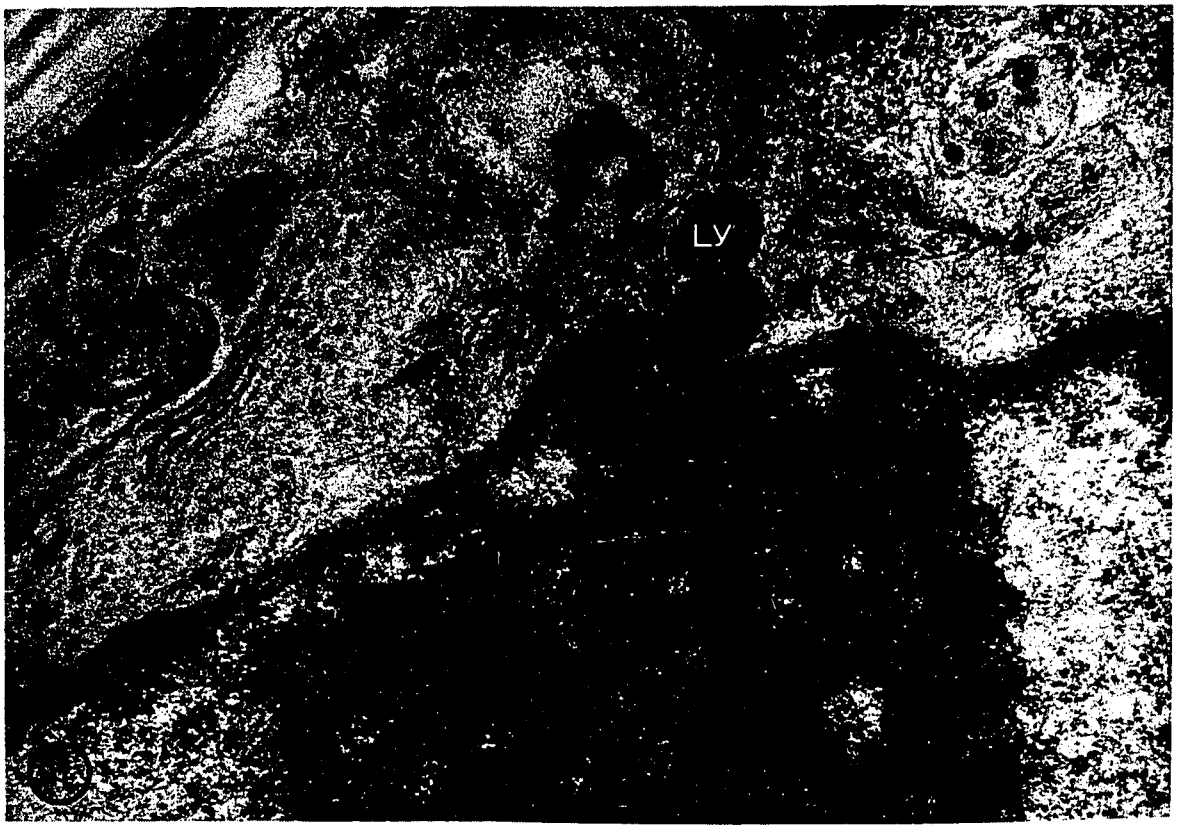

10

PLATE VIII

Figure 11. Control preparation of basal cell (Phase I).

Figure 12. Basal cell (Phase I) showing no ATPase reaction product.

Note: lysosome-like (Ly) structure. (Stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X44,000).


LY

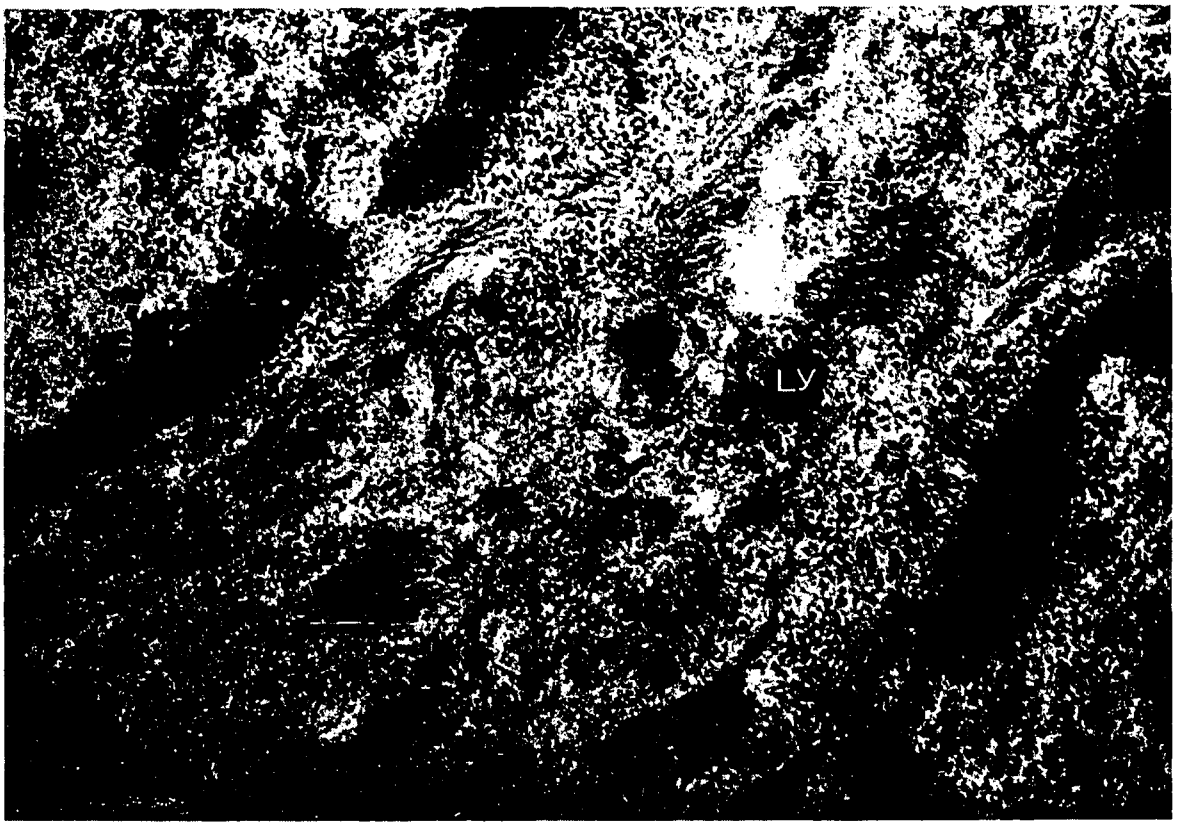
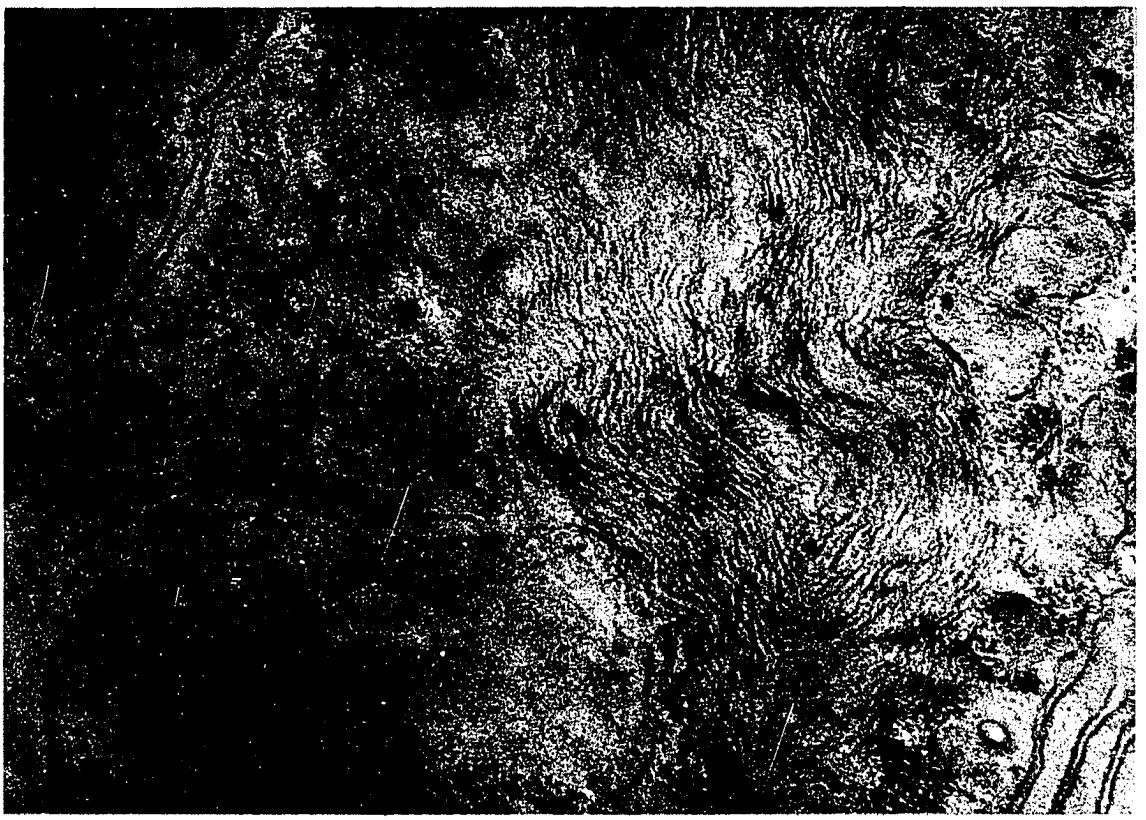

LY

PLATE IX

Figure 13. Control preparation of basal cell (Phase I).

Figure 14. Basal cell (Phase I) showing no ATPase reaction product.

Note: tonofibrils (T). (Stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X44,000).



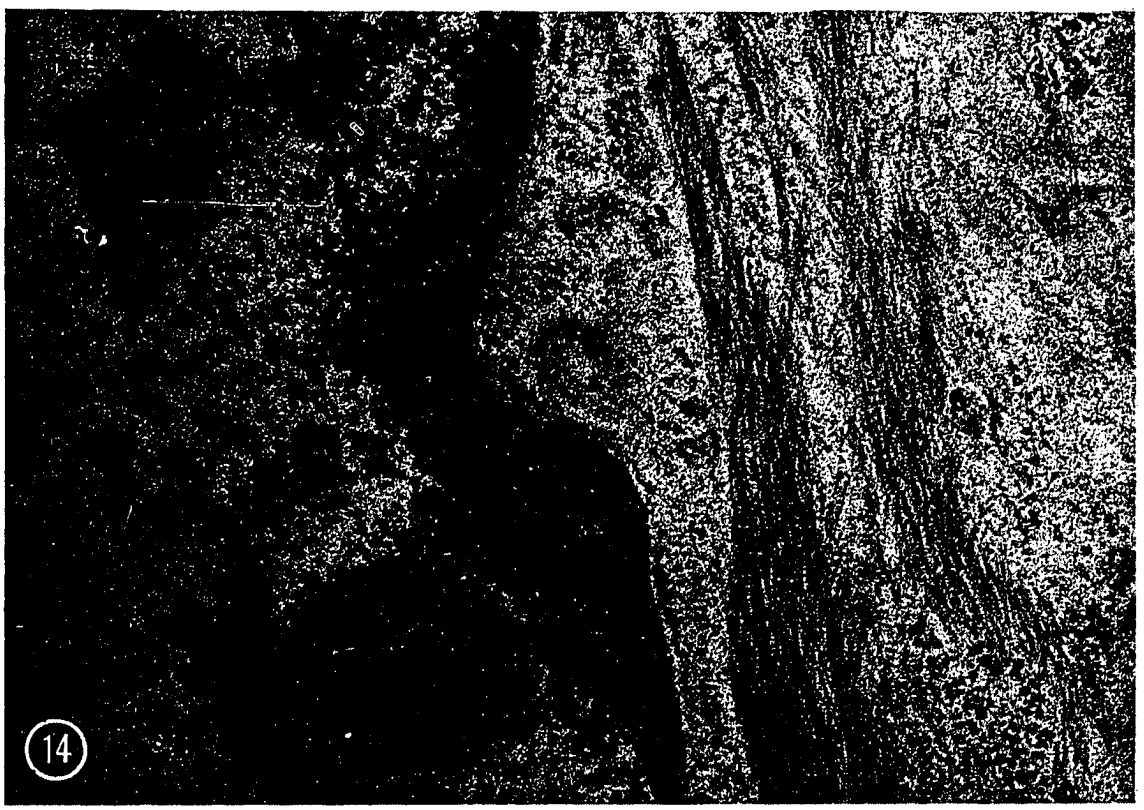

14

PLATE X

Figure 15. Control preparation of slightly advanced early secretory cell (Phase II).

Figure 16. Slightly advanced early secretory cell (Phase II) showing ATPase-like reaction deposit (double arrows) within the lipid inclusion (L).

Note: lipid inclusion (L) with electron-dense cloudy and clear areas. (Stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X44,000).


L

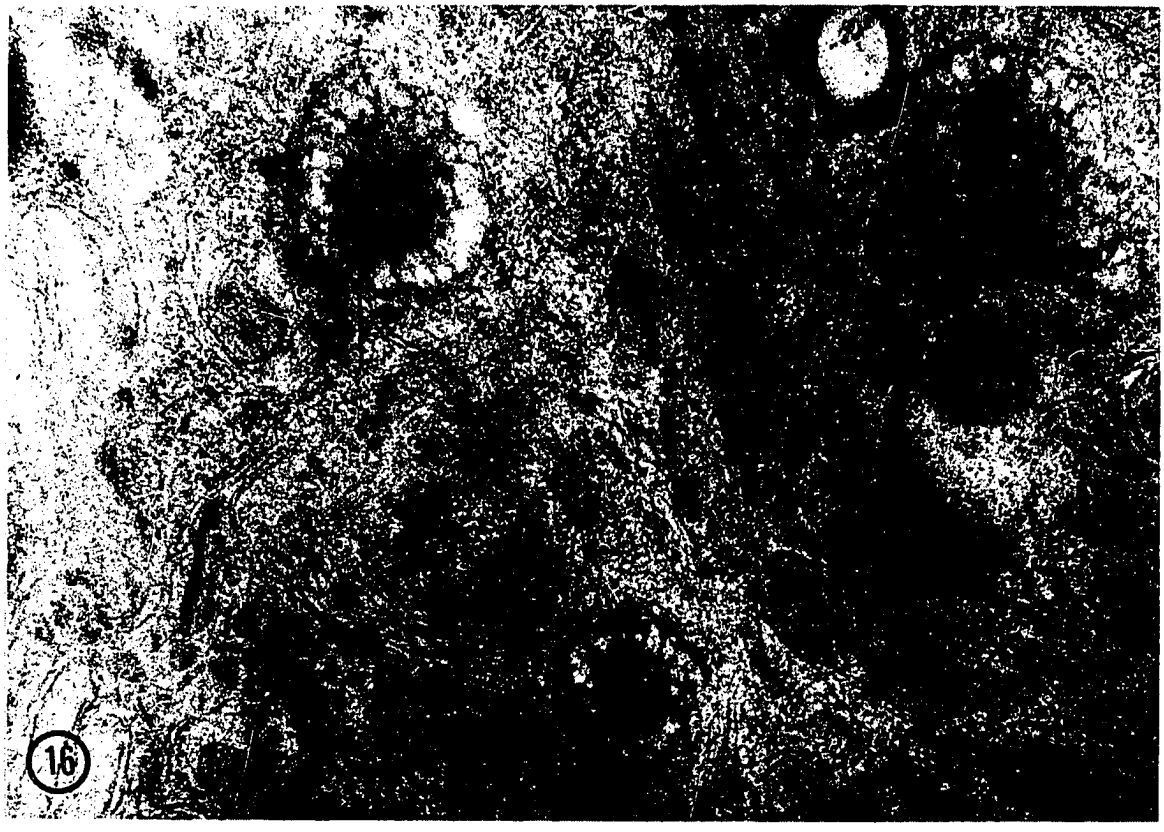
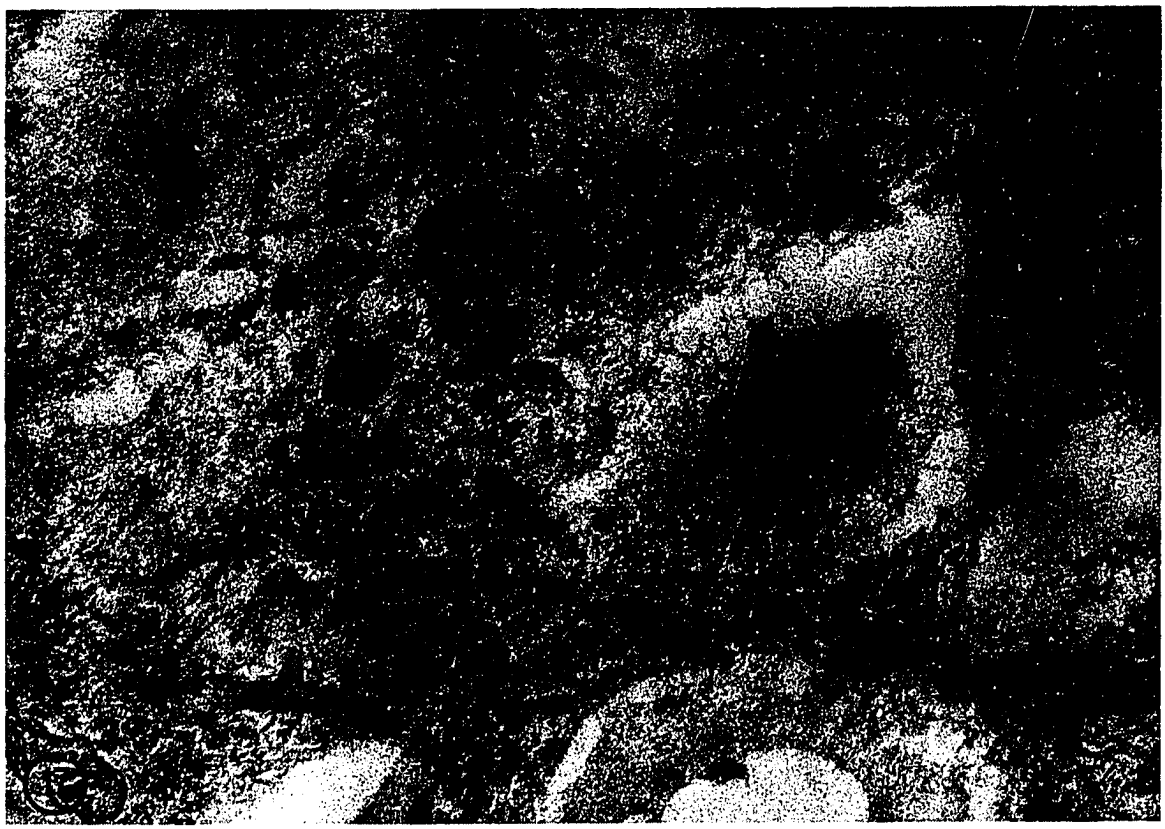

16

PLATE XI

Figure 17. Control preparation of mid-secretory cell (Phase III).

Figure 18. Mid-secretory cell (Phase III) showing ATPase-like reaction deposit (double arrows) associated with electron-dense area of the lipid inclusion (L).

Note: mitochondria (M) exhibit a wide range of variation. Some of them still appear like an early phase, but most of them appear to be swollen. (Stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X53,000).



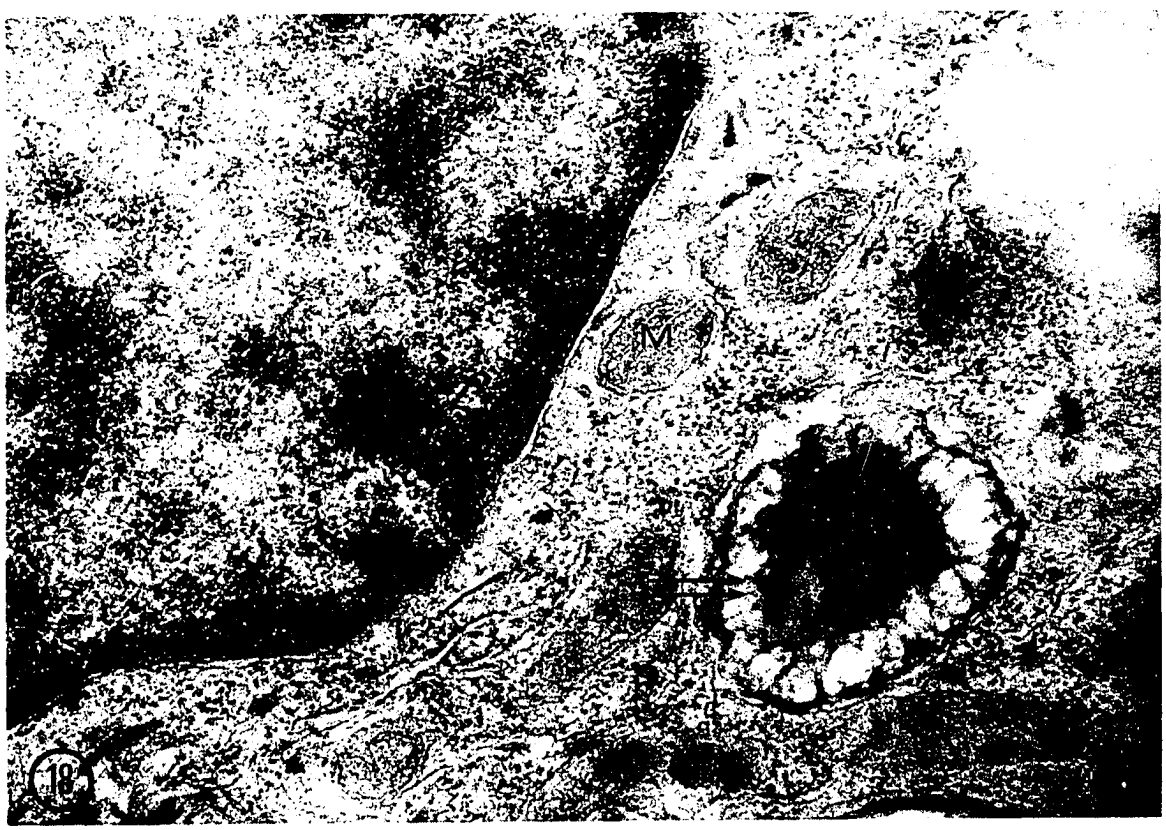

M

PLATE XII

Figure 19. Control preparation in mid-secretory cell (Phase III).

Figure 20. Mid-secretory cell (Phase III) showing positive ATPase reaction product (arrow) associated with external membrane system of mitochondria (M). Also finely granular ATPase-like reaction deposit (double arrows) associated with complex of Golgi (G) membranes especially with the electron-dense cloudy area of lipid inclusion (L).

(Stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X44,000).


L
M
19

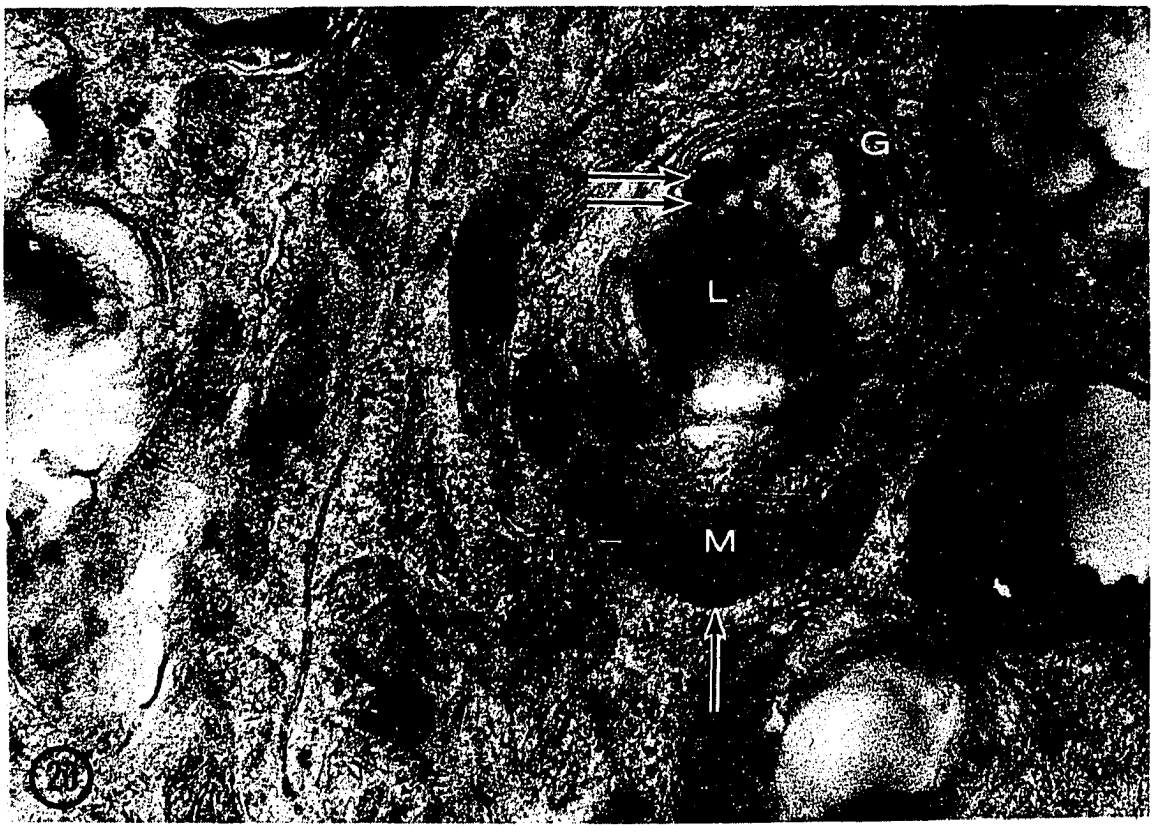

G
L
M
20

PLATE XIII

Figure 21. Control preparation of late secretory (Phase IV) cell (C 6) and terminal (Phase V) cell (C 7).

Figure 22. Late secretory (Phase IV) cell (C 6) and terminal (Phase V) cell (C 7) showing no ATPase reaction product.

Note: the lipid inclusions (L). (Stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X34,000).


L
C7
L
21

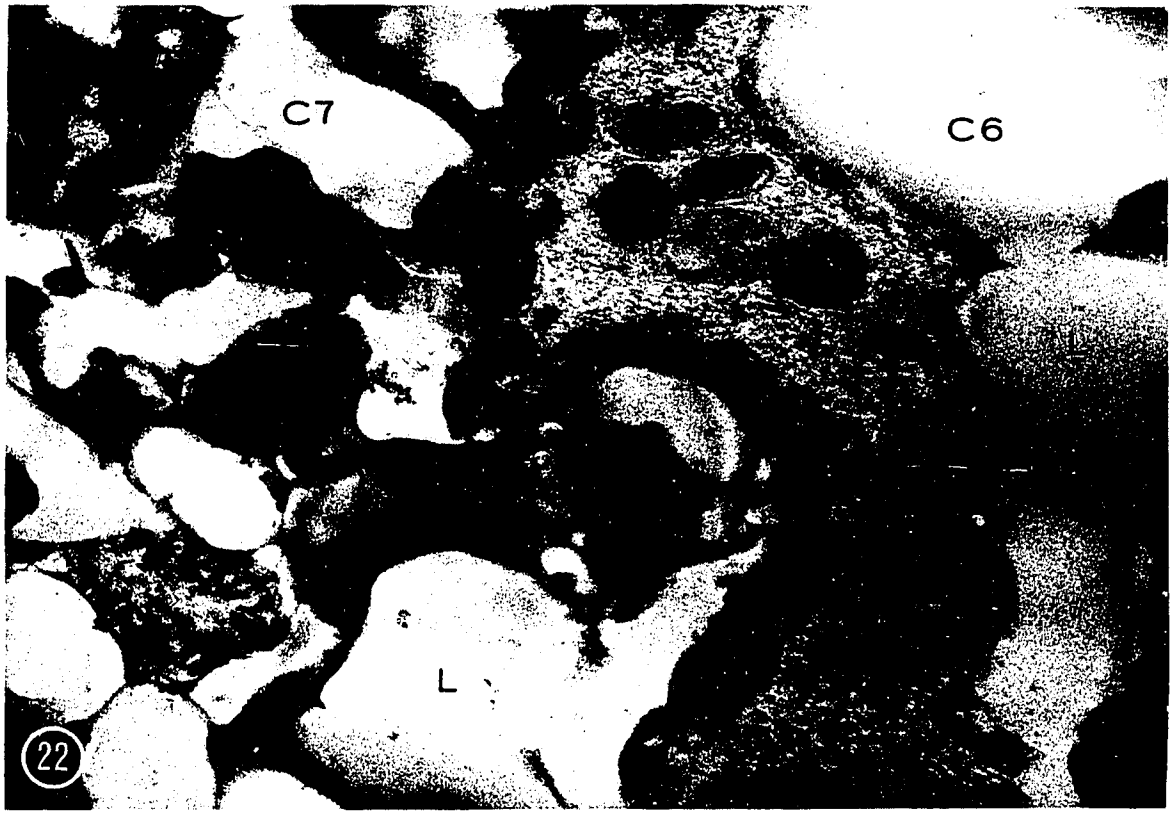

C7
C6
L
22

PLATE XIV

Figure 23. Control preparation of terminal (Phase V) cell.

Figure 24. Terminal (Phase V) cell showing no ATPase reaction product.

Note: angular shaped lipid inclusions (L) and only occasional suggestive remnants of mitochondria and lysosome-like bodies. (Stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X25,000).

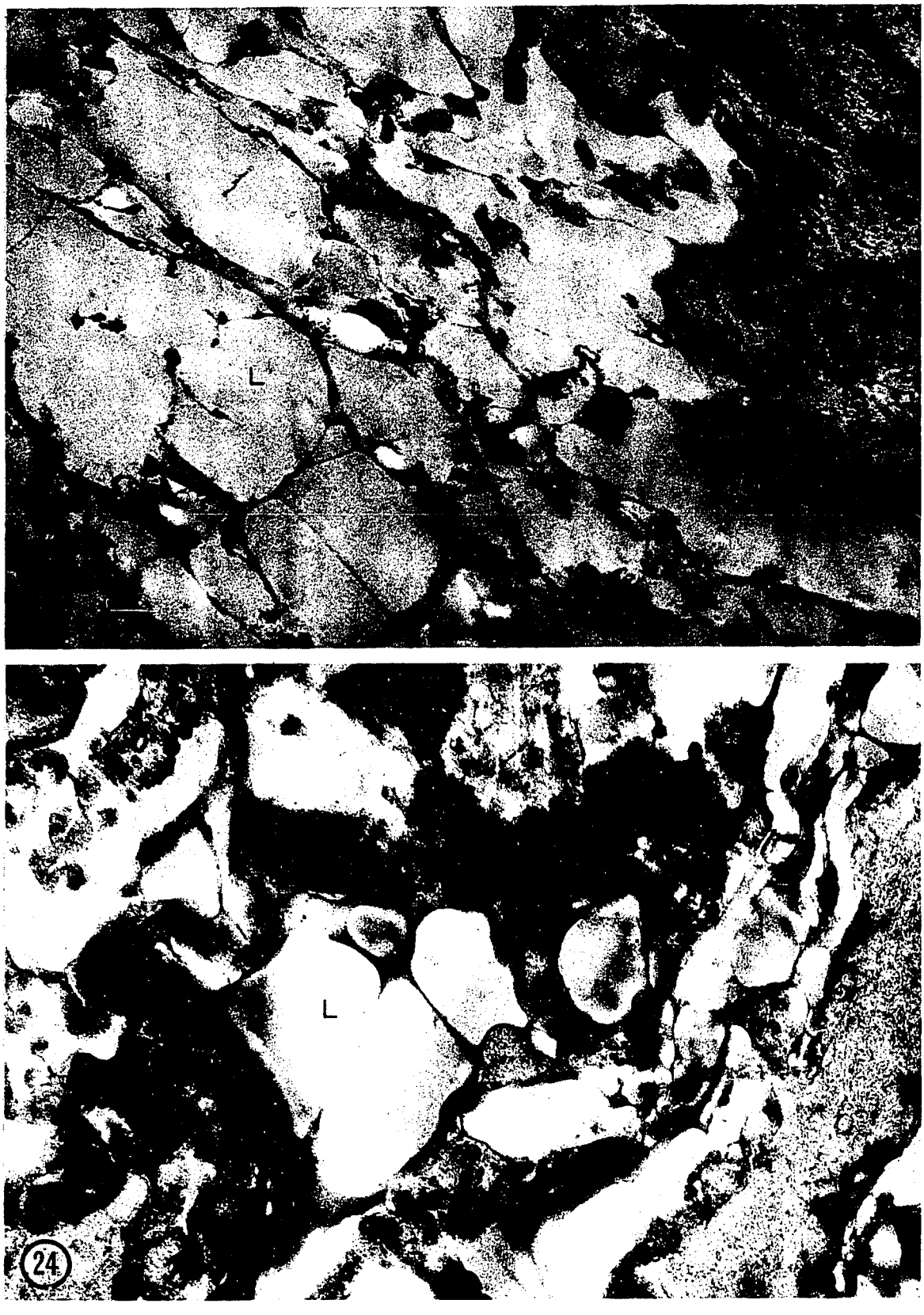
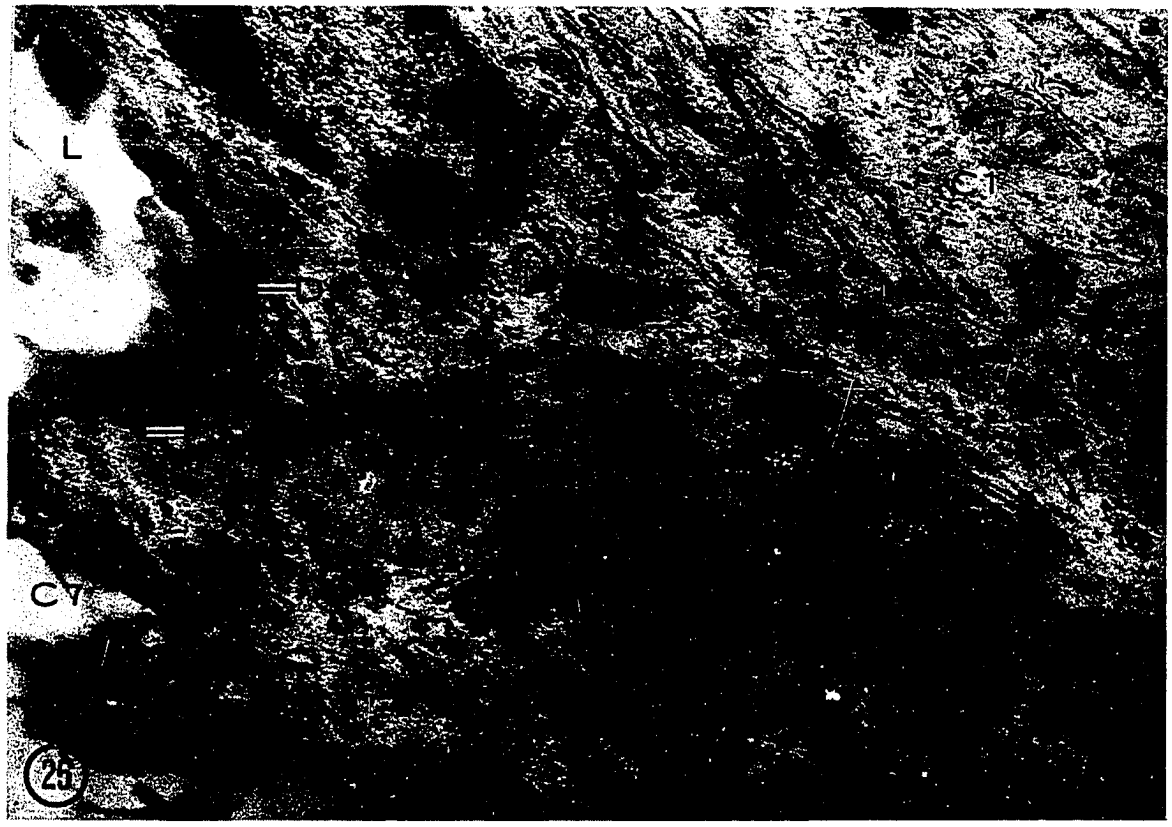

L
L
24

PLATE XV

Figure 25. Control preparation of terminal (Phase V) cell (C 7) and basal (Phase I) cell (C 1).

Figure 26. Terminal (Phase V) cell (C 7) and basal (Phase I) cell (C 1) showing no evidence of ATPase reaction product.

Note: lipid inclusions (L), plasma membrane (Pm) and desmosomes (D). (Stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X44,000).


L
C7
C7
25


C7
L

PLATE XVI

Figure 27. Control preparation of mid-secretory (Phase III) cell.

Figure 28. Mid-secretory (Phase III) cell showing positive ATPase reaction product (arrow) associated with external membrane system of mitochondria (M). Also finely granular ATPase-like reaction deposit (double arrows) associated with complex of Golgi membranes especially with the electron-dense cloudy area of lipid inclusion (L).

Note: Golgi complex (G), mitochondria (M) and lipid inclusion (L). (Stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X44,000).


M
G
L
27

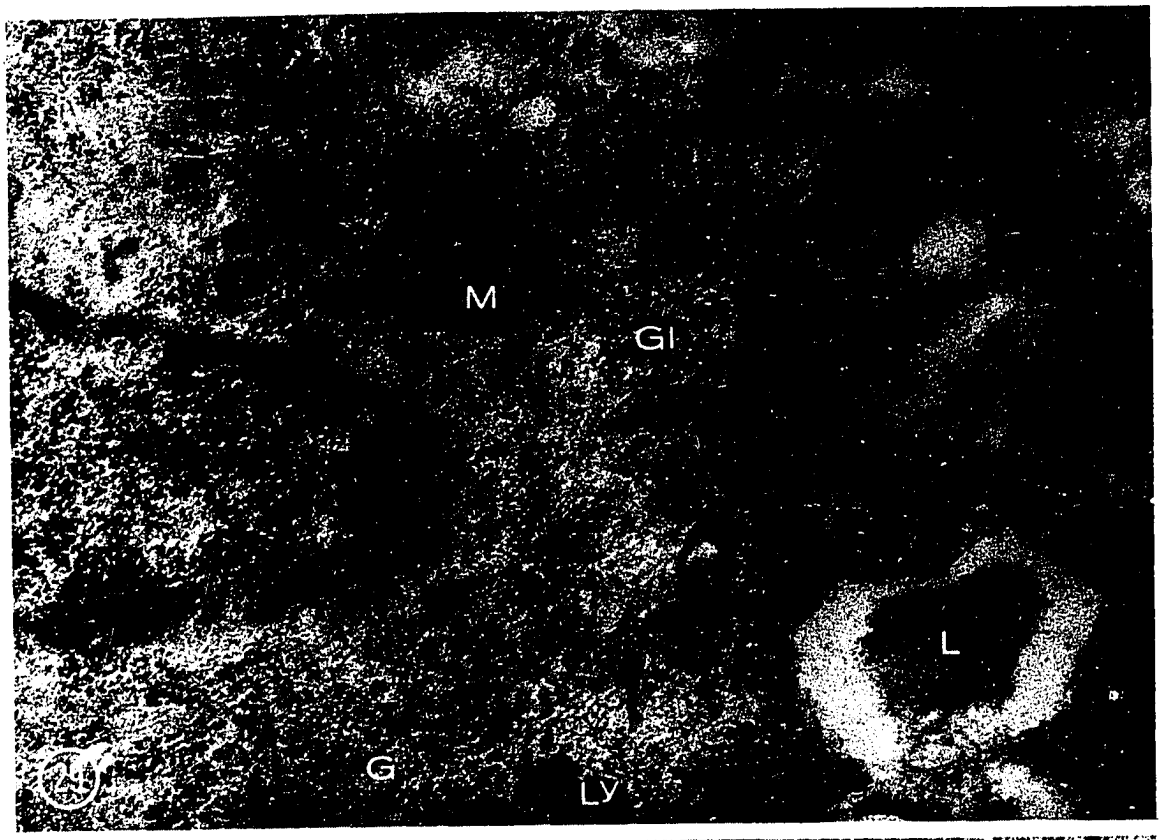

G
M
L
28

PLATE XVII

Figure 29. Control preparation of slightly early mid-secretory (Phase III) cell.

Figure 30. Slightly early mid-secretory (Phase III) cell showing positive ATPase reaction product (arrow) associated with external membrane system of mitochondria (M). Also finely granular ATPase-like reaction deposit (double arrows) associated with electron-dense area of the lipid inclusion (L).

Note: glycogen-like granules (Gl), Golgi complex (G), and lysosome-like (Ly) structures. (Stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X44,000).


M
GI
L
G
LY

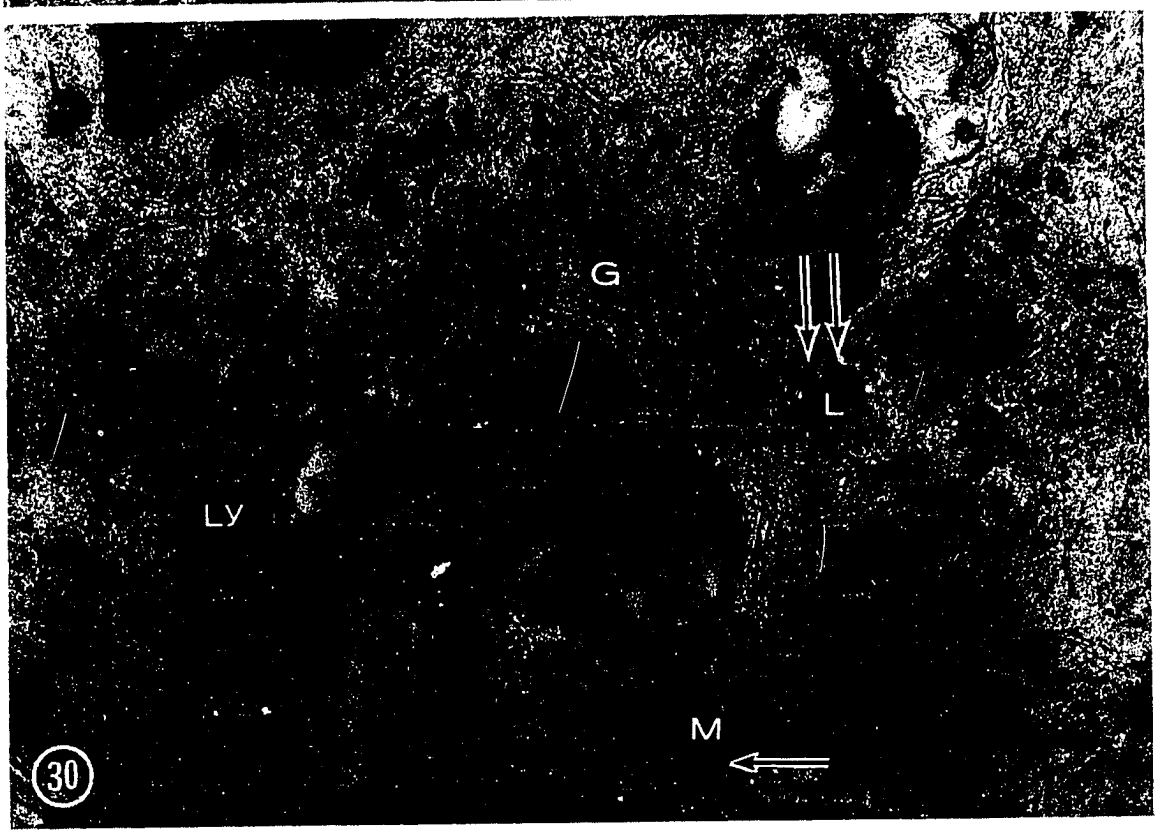

G
L
LY
M
30

PLATE XVIII

Figure 31. Control preparation of presecretory (Phase I) cell (C 3) and slightly advanced mid-secretory (Phase III) cell (C 5).

Figure 32. Slightly advanced mid-secretory (Phase III) cell showing no ATPase reaction product.

Note: mitochondria (M), rough endoplasmic reticulum (ER) and lysosome-like (Ly) body. (Stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X34,000).

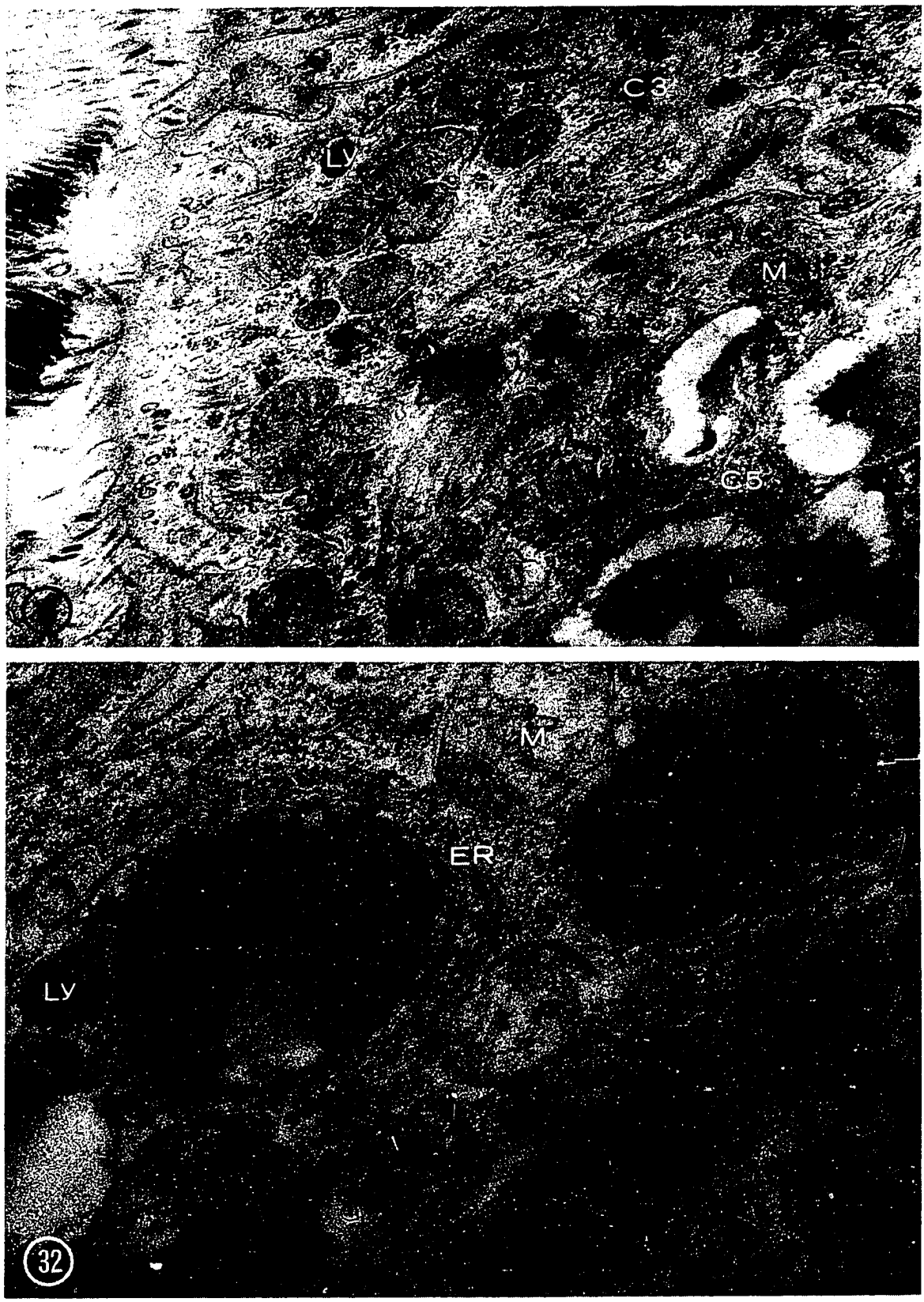
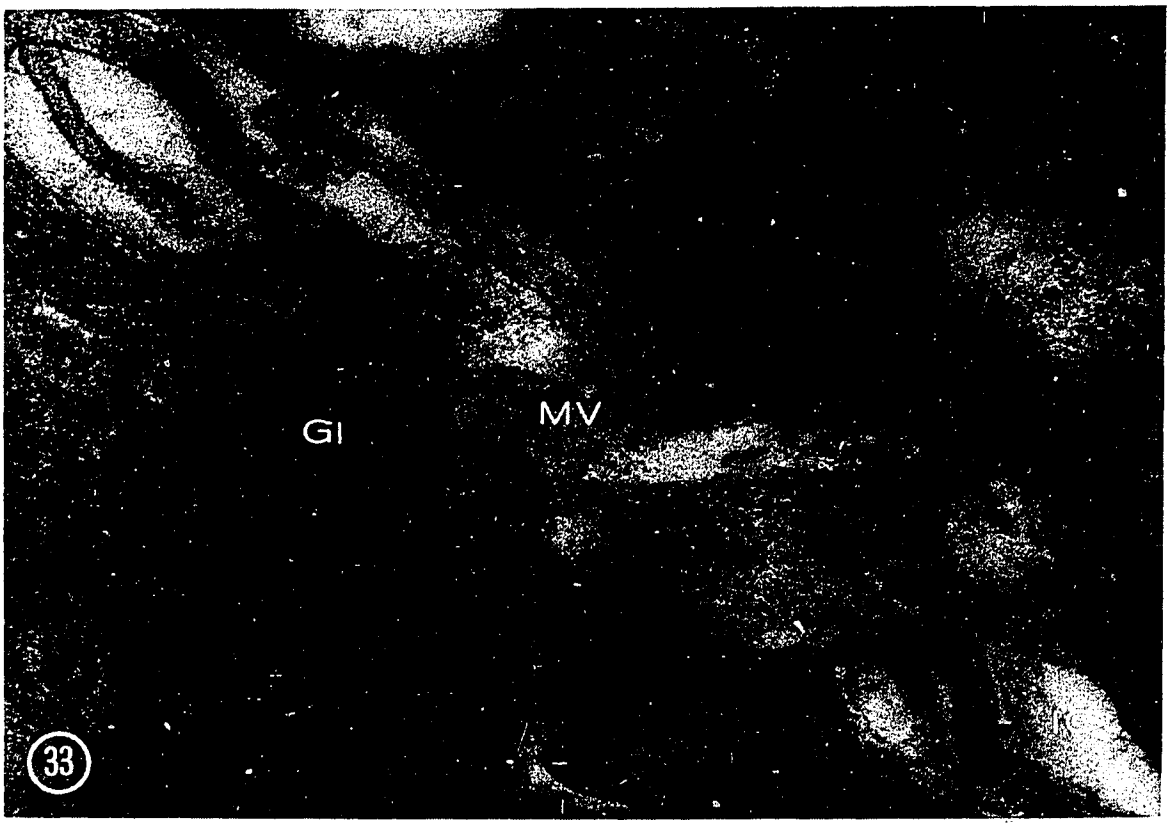

C3
LY
M
C5
M
ER
LY
32

PLATE XIX

Figure 33. Control preparation of mid-secretory (Phase III) cell.

Figure 34. Presecretory (Phase I) cell (C 3) and mid-secretory (Phase III) cell (C 5) showing no evidence of ATPase reaction product.

Note: microvilli (MV), intercellular channel (IC), glycogen-like granules (GI) and smooth endoplasmic reticulum (ER). (Stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X44,000).


GI
MV
33

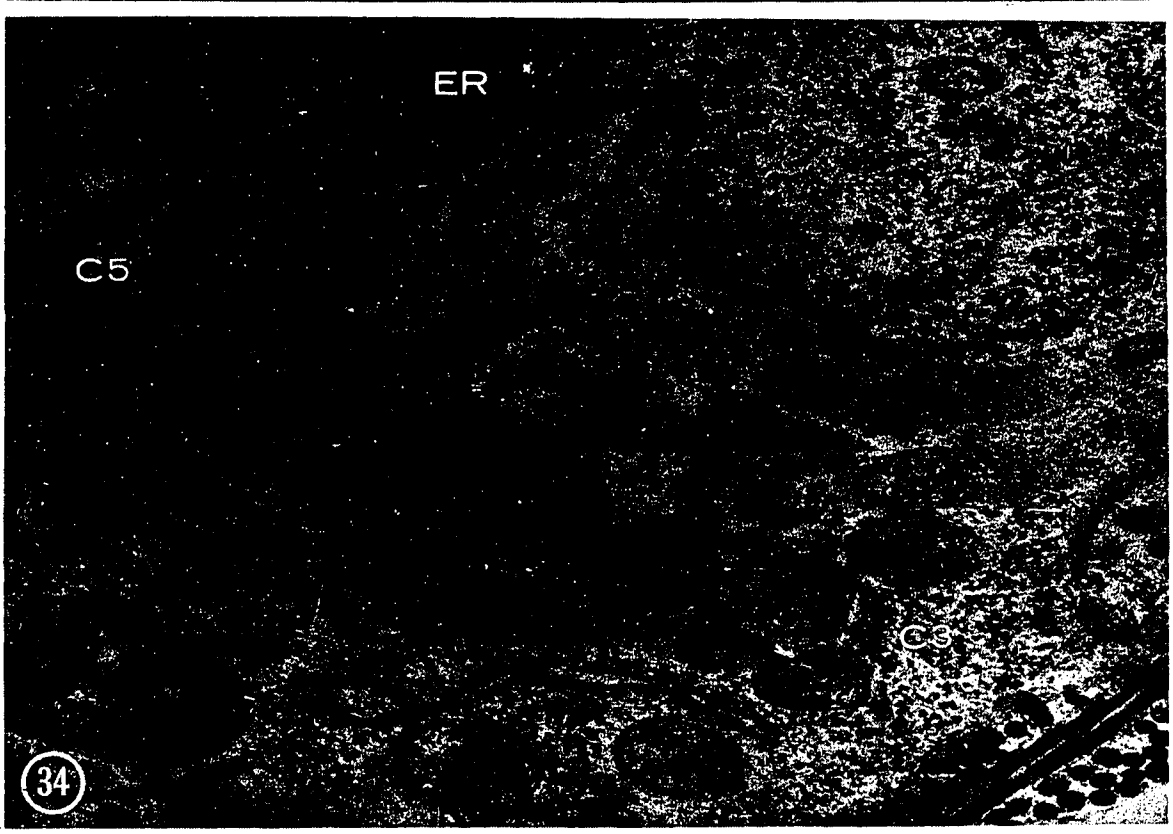
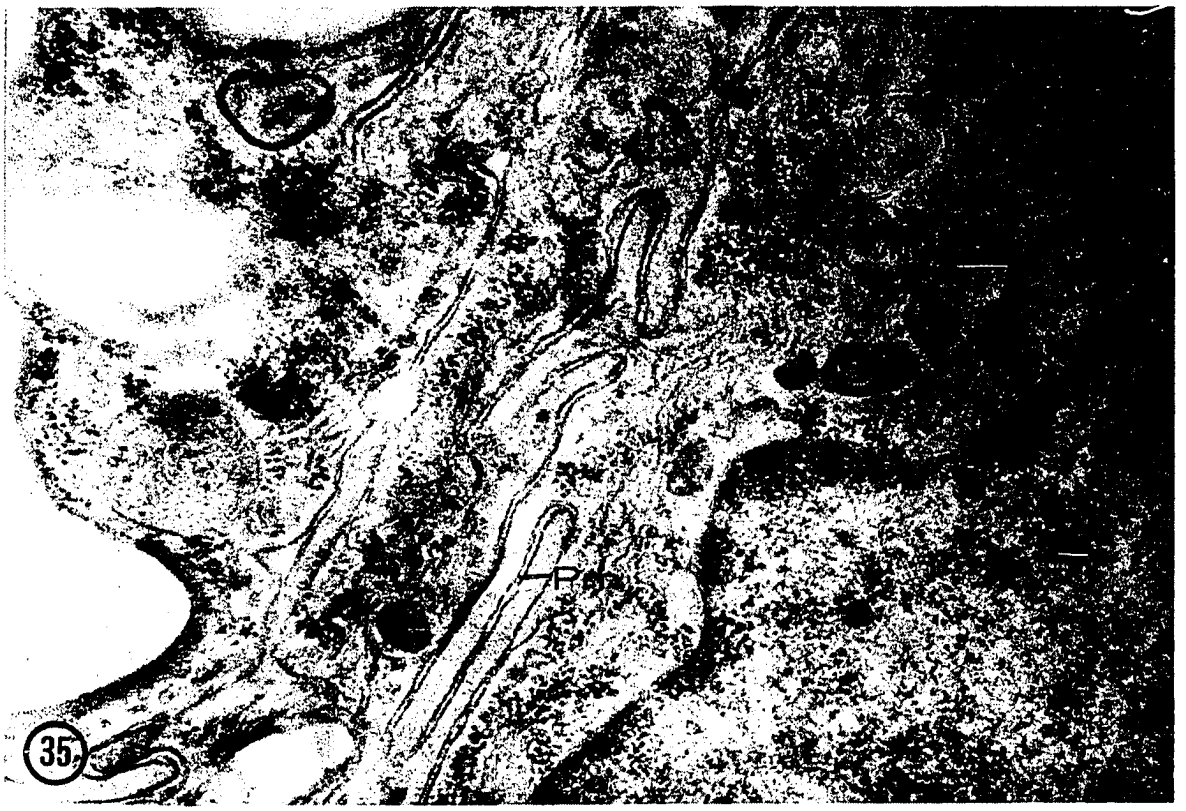

ER
C5
C3
34

PLATE XX

Figure 35. Control preparation of mid-secretory (Phase III) cell.

Figure 36. Mid-secretory (Phase III) cell showing no evidence of ATPase reaction product.

Note: mitochondria (M), plasma membrane (Pm) and lysosome-like (Ly) structures. (Stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X44,000).


Pm
35

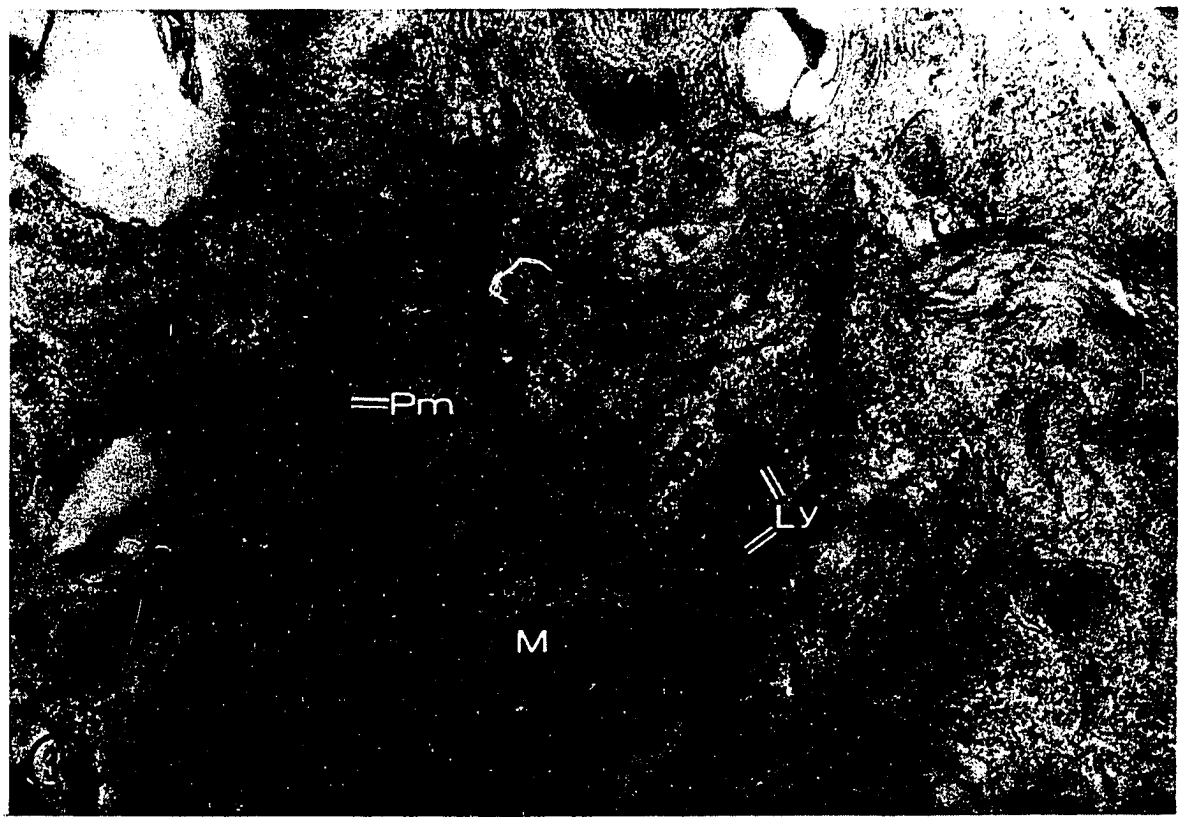

Pm
Ly
M

PLATE XXI

Figure 37. Control preparation of slightly advanced mid-secretory (Phase III) cell.

Figure 38. Slightly advanced mid-secretory (Phase III) cell showing no evidence of ATPase reaction product.

Note: mitochondria (M), large lipid inclusions (L) have extensive flattening of membrane system (MS) in their compressed surfaces, and desmosomes (D). (Stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X35,000).


MS
L
37

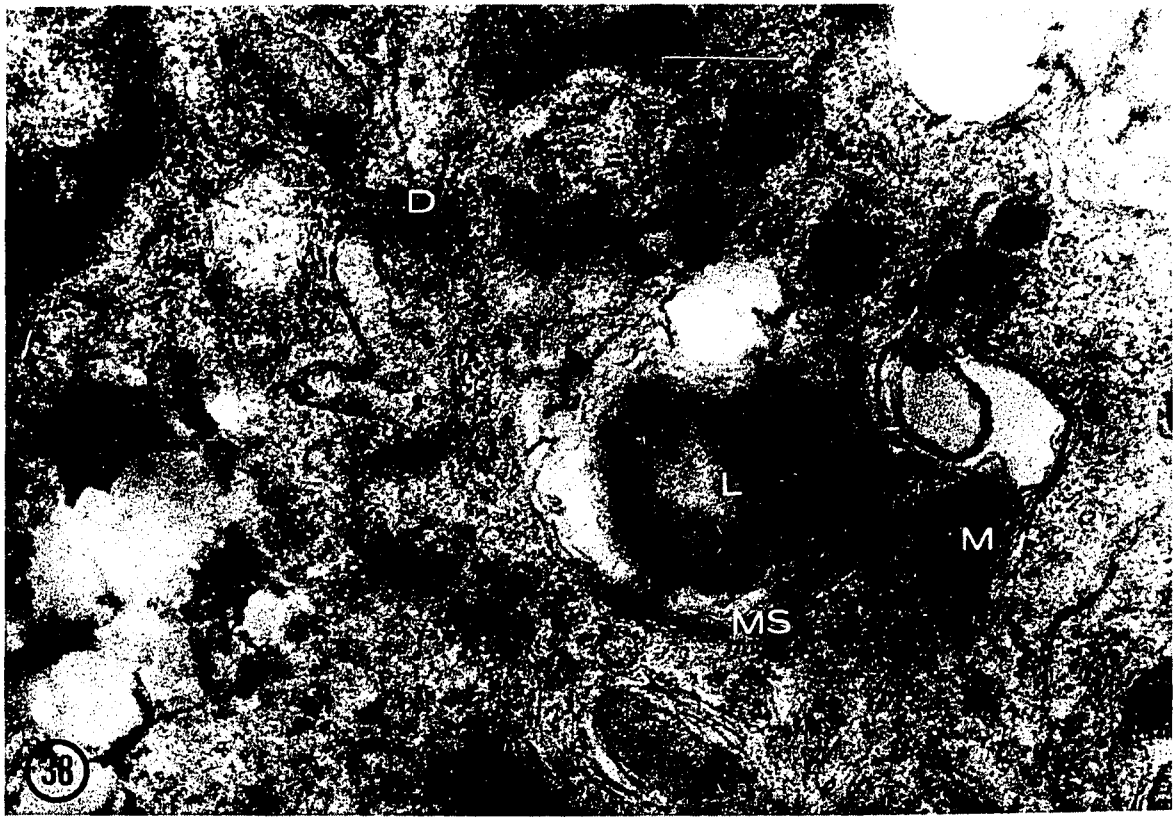

D
L
M
MS
38

PLATE XXII

Figure 39. Control preparation of basal (Phase I) cell nucleus (N).

Figure 40. Basal cell (Phase I) nucleus (N) showing no ATPase reaction product within the nuclear structure.

Note: a typical double nuclear membrane, outer nuclear membrane (ONm) and inner nuclear membrane (INm). Also note the size of the nucleus.

Figure 41. Control preparation of presecretory (Phase I) cell nucleus (N).

Figure 42. Presecretory (Phase I) cell nucleus (N) showing no ATPase reaction product within the nuclear structure.

Note: karyoplasmic granularity and beginning nuclear polymorphism. (Figures 39, 40, 41 and 42 were stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X35,000).


ONM
N
39


N
41


ONM
N
INM
40


N
42

PLATE XXIII

Figure 43. Control preparation of an early secretory (Phase II) cell nucleus (N).

Figure 44. Early secretory (Phase II) cell nucleus (N) showing no ATPase reaction product within the nuclear structure.

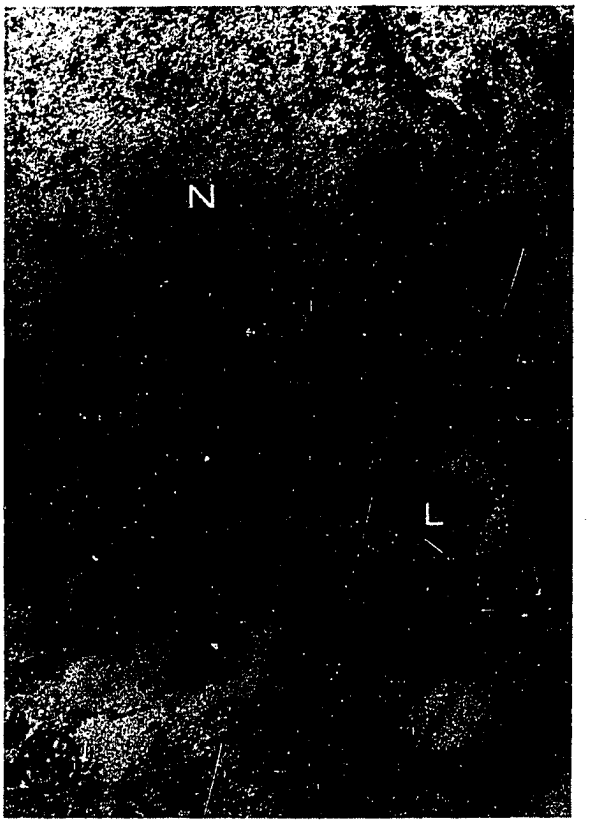
Note: further increased karyoplasmic granularity.

Figure 45. Control preparation of a mid-secretory (Phase III) cell nucleus (N).

Figure 46. Mid-secretory (Phase III) cell nucleus (N) showing no evidence of ATPase reaction product within the nuclear structure.

Note: start to show some pyknotic appearance and relationship with lipid inclusions (L). (Figures 43, 44, 45 and 46 were stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X35,000).


N
43


N
L


L
N
44

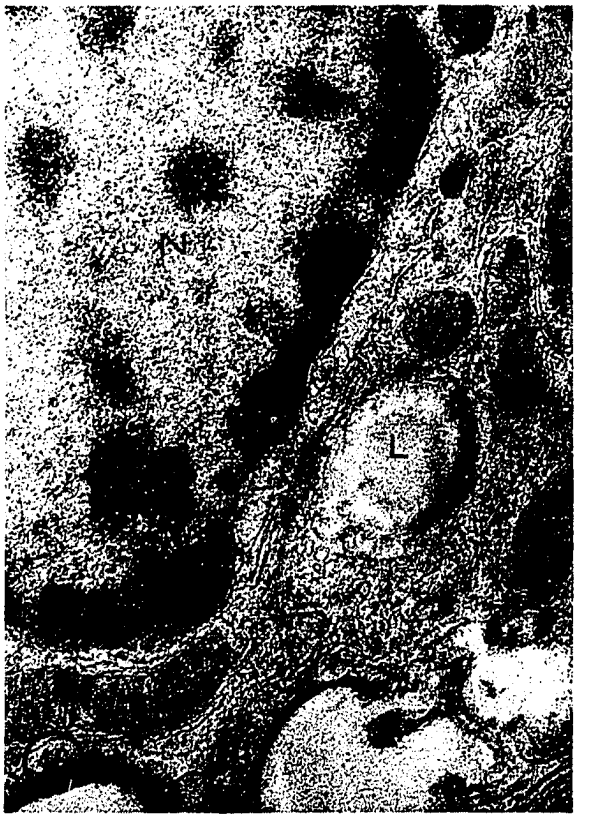

N
L

PLATE XXIV

Figure 47. Control preparation of a late secretory (Phase IV) cell nucleus (N).

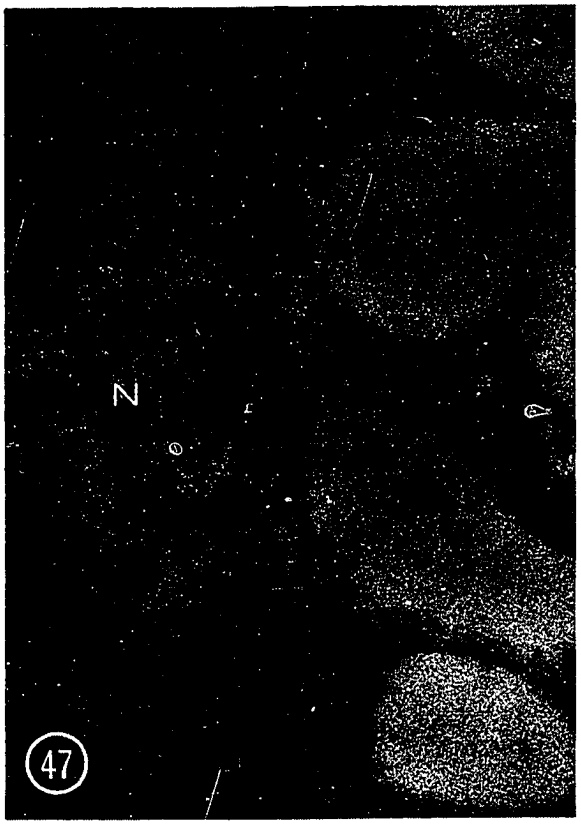
Figure 48. Late secretory (Phase IV) cell nucleus (N) showing no ATPase reaction product within the nuclear structure.

Note: large lipid inclusions (L) compressed against the nucleus.

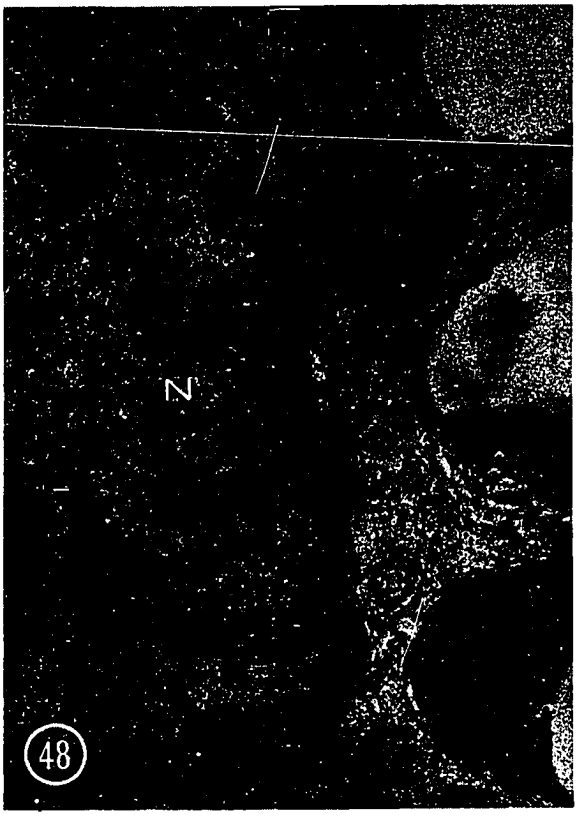
Figure 49. Control preparation of a terminal secretory (Phase V) cell nucleus (N).

Figure 50. Terminal secretory (Phase V) cell nucleus (N) showing no ATPase reaction product within the nuclear structure.

Note: showing karyorrexis and nuclear remnants dispersed with general cytoplasmic debris among coalesced lipid material. (Figures 47, 48, 49 and 50 were stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X35,000).


N
47


N
49


N
48


N

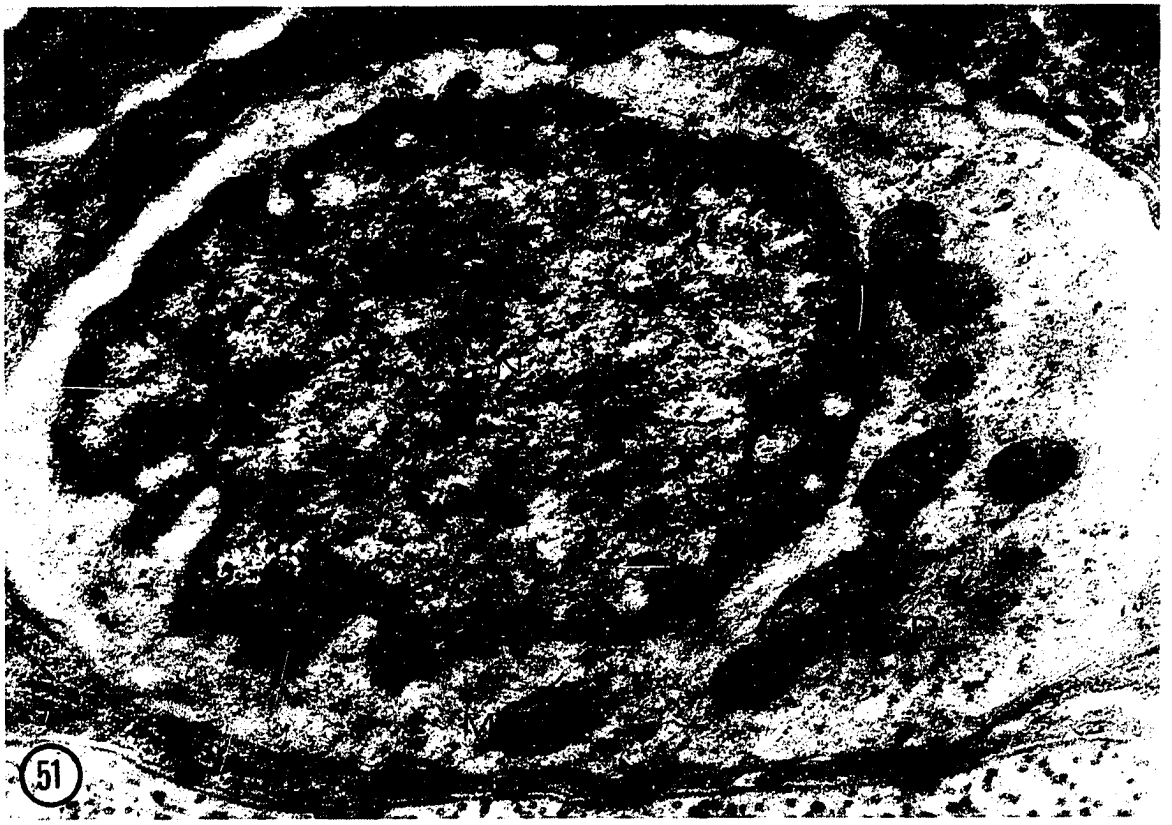
PLATE XXV

Figure 51. Control preparation of "Clear Cell" which shows osmiophobic cytoplasm and is comparable to the "Langerhans' cell" in the general epidermis.

Note: slight nuclear indentation (N), dense mitochondria (M) and abundant cytoskeleton reticulum (SR). (Stained with aqueous uranyl acetate, approximately X19,000).

Figure 52. Control preparation similar to Figure 51 except its position is slightly more toward the center.

Note: the "Clear Cell" (C 2) is completely free from tonofibrils and desmosomes attaching to the surrounding sebaceous cell (C 1) of Malpighi-origin. Long foot-like processes (F) protrude from the cell surface but they do not interdigitate with comparable folds of adjacent cells of Malpighi-origin. The nucleus (N) is more indented and lobulated than Figure 51. (Stained with aqueous uranyl acetate, approximately X19,000).


51

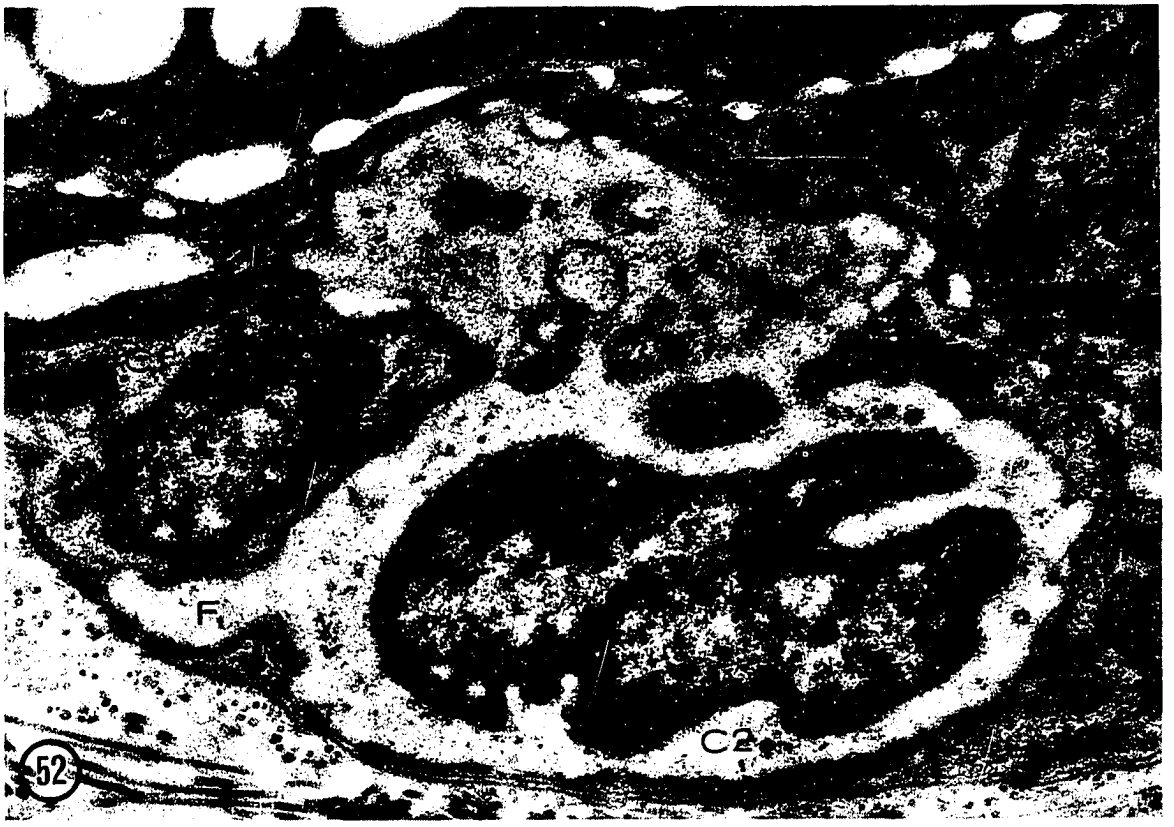

F1
C2
52

PLATE XXVI

Figure 53. Similar to Figures 51 and 52 except the section was treated for ATPase localization. The cell shows very high metabolic activity among the sebaceous secretory cells (C 3 and C 5).

Note: lobulated nucleus (N), Golgi complex (G), centriolar apparatus (C), lysosome-like (Ly) body, mitochondria (M), abundant fine cytoskeleton reticulum (SR) and Birbeck's granules (BG). Although no clear-cut evidence of ATPase activity in the aforementioned structures is shown, there is some possible reaction product (arrow) associated with the external membrane system of some, but not all, mitochondria. (Stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X21,000).

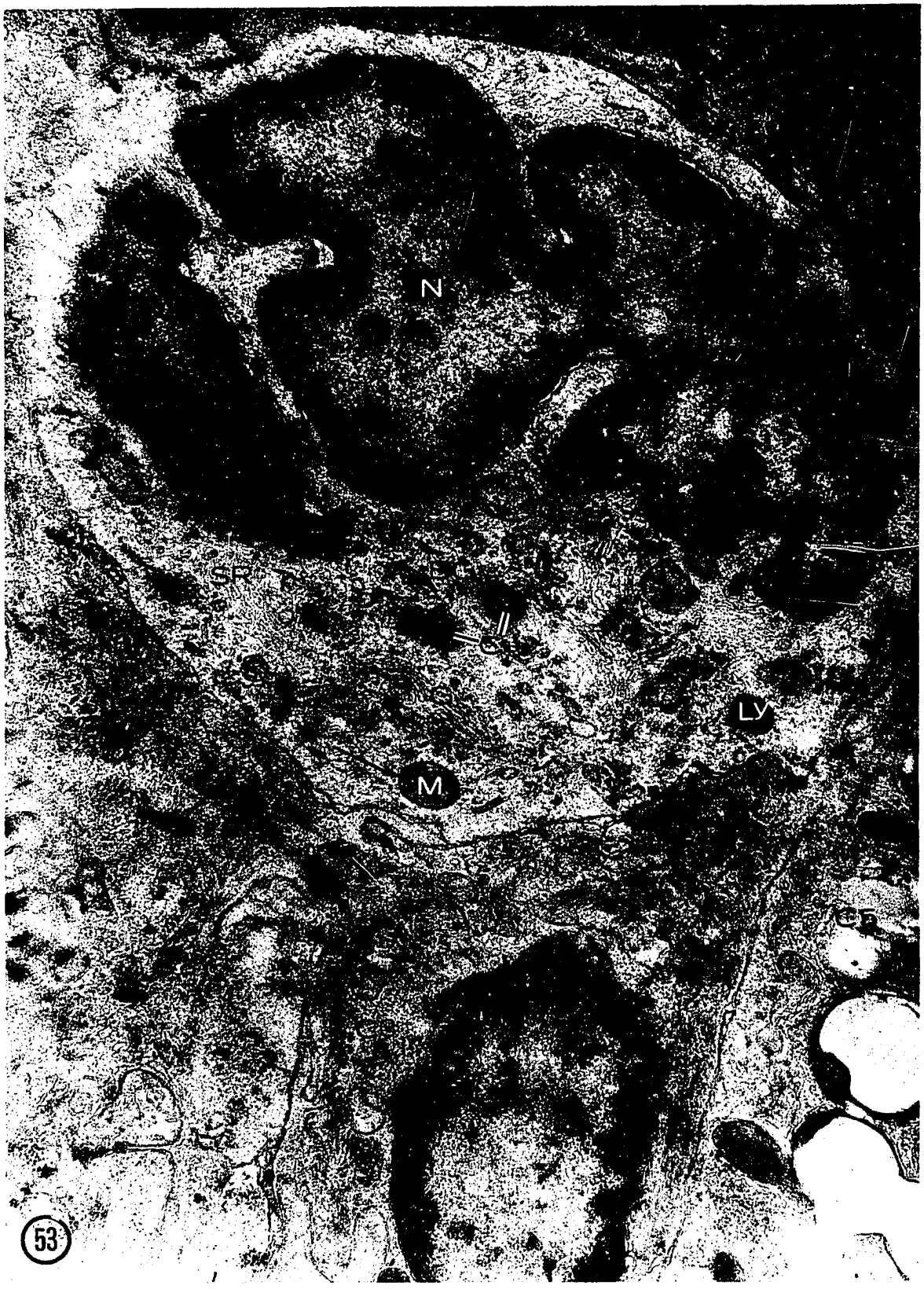
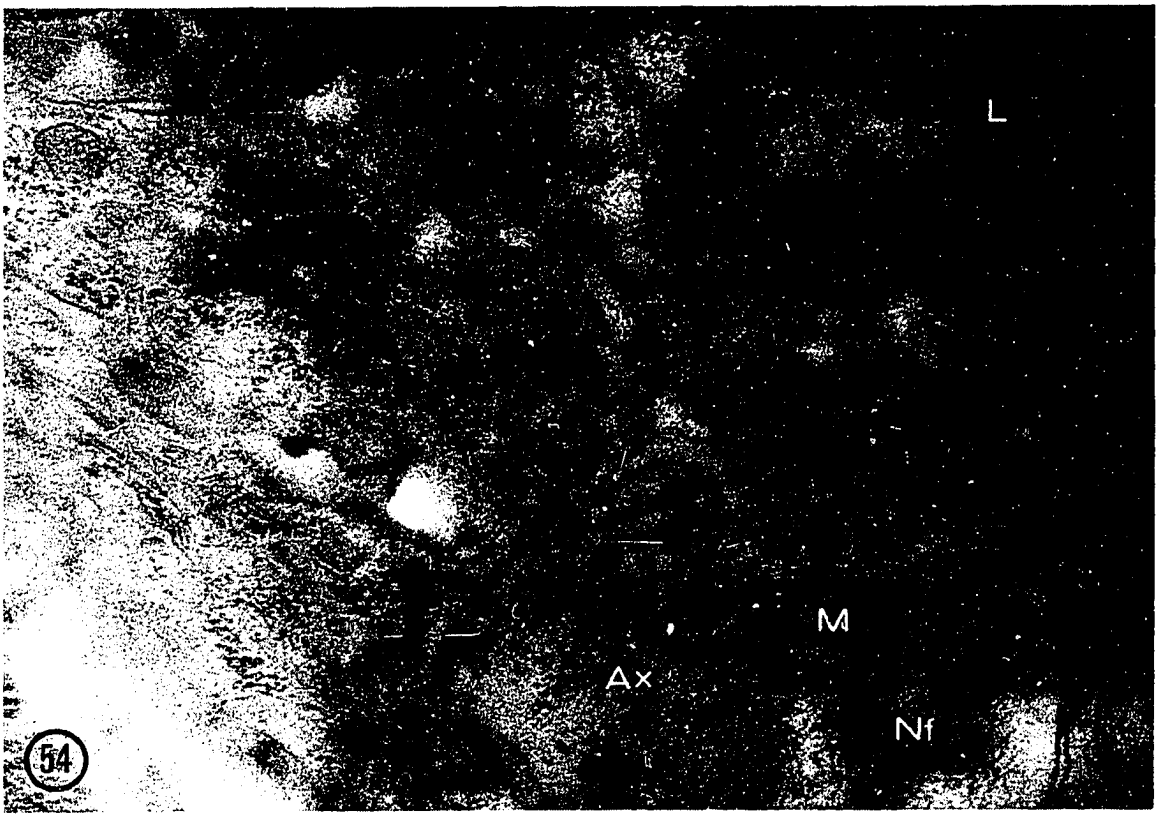

N
SR
LY
M
53

PLATE XXVII

Figure 54. Control preparation of an axon-like (Ax) structure occurring intercellularly among sebaceous cells with lipid inclusions (L) on the supra-basal cell level.

Figure 55. An axon-like (Ax) structure showing a diffuse distribution of ATPase reaction product (arrow) in the general cortical axoplasmic zone.

Note: mitochondria (M) and neurofilament-like fine fibers (Nf). (Stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X44,000).


L
M
Ax
Nf
54


L
Ax
Nf

PLATE XXVIII

Figure 56. Tangental section through a portion of a sebaceous gland showing ATPase localization in a basal (Phase I) cell (C 1), early (Phase II) cell (C 4), and late (Phase IV) cell (C 6).

In the basal (Phase I) cell (C 1) the reaction product (arrow) is associated with mitochondria and in limited areas in the cortical cytoplasm and near the plasma membrane.

In early secretory (Phase II) cell (C 4), the product (arrow) appears as finely granular aggregates which are characteristically associated with mitochondria of near or normal structure. Also, small irregularly fine granular deposits (double arrows) occur near the surface of lipid inclusion (L) droplet.

The late secretory (Phase IV) cell (C 6) also shows a finely granular reaction product (arrow) associated with mitochondria.

(Stained with aqueous uranyl acetate and aqueous lead hydroxide, approximately X19,200).


C6
C4
C1
56