LYSOSOMAL ACTIVITY IN BENIGN HYPERPLASTIC AND CANCEROUS HUMAN PROSTATE: AN ELECTRON CYTOCHEMICAL STUDY

by

Patricia Collins Temple, B.A.

A THESIS
Presented to the Department of Anatomy
and the
Graduate Division of the University of Oregon Medical School
in partial fulfillment of the requirements for the
degree of Master of Science.

June, 1969

APPROVED:

[redacted]

(Professor in Charge of Thesis)

[redacted]

(Chairman, Graduate Council)

ACKNOWLEDGEMENTS

I especially wish to thank Dr. Robert L. Bacon for his thoughtful guidance and encouragement during the planning and completion of this work. Mrs. Jacqueline Gellatly contributed a portion of the electron micrographs and much of the printing in the final manuscript; her contributions are gratefully appreciated. Dr. Dieter Kirchheim aided in the histochemical studies and in obtaining surgical material. The completion of the final manuscript was made possible by the editorial assistance of my husband, Gary Temple, and the secretarial help of Mrs. Cecilia Raddatz.

This investigation was supported by PHS Training Grant GM01659 and by contributions from the Sellings Fund.

CYTOCHEMISTRY

I. INTRODUCTION.

A. STATEMENT OF THE PROBLEM.

In 1955, de Duve discovered particles containing hydrolytic enzymes in liver cell homogenates.[1] They were called lysosomes because of their suspected function as lytic organelles. Subsequent biochemical, morphological and cytochemical studies have greatly elaborated on the function of the lysosome, proving de Duve's initial hypothesis to be correct.[2,3,4] Lysosomes have thus assumed an important role in normal and pathological cell physiology. They have been shown to function as intracellular digestive systems stimulated by various agents to digest foreign and autologous material.[2] Their role in carcinogenesis has been studied by Allison and associates[5,6,7,8,9] but little is known about how their activity differs in cancer tissue as compared to normal tissue.[9]

This study investigates the morphology and enzyme activity of lysosomes in hyperplastic tissue and compares it with activity in the cancerous human prostate using thiolacetic acid esterase activity to localize the lysosome with the electron microscope.

B. THE PROSTATE GLAND.

1. Normal anatomy and physiology.

The human prostate is a secondary male sexual organ. It functions primarily to secrete a transportation medium for sperm.[10] The main secretions are water, lipids, cholesterol and hydrolytic enzymes (especially acid phosphatase and fibrinolysin).[11]

The secretions are formed by tall epithelial cells arranged in glands around approximately 80 small ducts.[12] They empty into the urethra at the base of the bladder.

The ducts are arranged in three concentric circles around the urethra, as described by Adrion in 1922.[13] The central glands are called the mucosal group, the intermediate are the submucosal, and the peripheral are the true prostatic glands.[14]

The terminology for the anatomy of the gross specimen is confusing for several reasons. Lowsely described the embryology of the prostate and found the fetal gland had four distinct lobes; anterior, posterior, medial and lateral.[15] In the adult, the lobes are fused into a homogeneous spherical gland but investigators still apply the terminology used for the fetal organ.[16] The division into lobes has often been challenged and it has been more practical to look at the prostate as having an inner, mucosal group of glands (anterior prostate) and an outer, peripheral group (also called the posterior prostate).[16,17,18,19,20]

The glands throughout both zones are tubulo-alveolar;[20] their acini are lined by tall secretory cells. The ducts are formed by tall columnar cells, except near the urethra where they become transitional.[16] Small basal cells separate the epithelium from the basement membrane around each acinus.[21] The glands are supported by a stroma of smooth muscle, fibroblasts and collagen.[20] At the light microscope level, both inner and outer zones are the same,[16] but with the electron microscope more basal cells were found in the outer zone.[22]

There are important physiological differences between the two zones. Benign hyperplasia arises in the inner mucosal area,[16] while prostatic cancer is derived from glands in the outer zone.[17] Huggins noted that the two zones respond differently to estrogens.[23] The outer glands regress and the inner glands remain the same after estrogen administration.

2. Anatomy and physiology of benign hyperplasia.

Benign hyperplasia develops in men beginning in the fifth decade. Its occurrence increases with age and by the seventh decade, seventy percent of Caucasion males are affected.[24]

Morgagni first recognized benign prostatic hyperplasia as a clinical condition in 1760. He observed an Italian nobleman succumb soon after his urine stopped flowing; it was

due to a "preternatural swelling of the prostate gland."[16]

The pathological diagnosis of hyperplasia is made with gross inspection and confirmed with the light microscope. Nodules in an enlarged gland are characteristic of hyperplasia; most commonly the nodule is a fibromyoepithelialoma.[16] It has three constituents: fibroblasts, smooth muscle cells and epithelial cells. Eighty percent of the epithelial cells are tall, functioning cells that form large, papillated glands. About twenty percent of the glands in the nodule are formed by low columnar, nonpapillated glands with decreased secretory activity.[18] Other types of nodules may be formed of just stromal elements without epithelium, or by individual stromal elements such as smooth muscle myomas or fibroblast fibromas.[16]

The mass of the nodules added to the normal prostate tissue increases the weight from 15 grams to 100 grams or more.[24] The number of ducts draining the inner glands almost doubles in number,[12] but there is no evidence of an increased quantity of secretion or change in the chemical composition from the enlarged prostate.[25] Dogs secrete less from prostates with cystic hyperplasia than from the smaller normal prostates, but their cystic glands are formed by an inactive epithelium.[10]

3. Etiology of benign hyperplasia.

The hyperplastic cells have the same type of aerobic glycolysis as other neoplasms. For this reason, Huggins suggests they are a benign neoplasm similar in many respects to uterine fibroids.[26]

It was first thought that the hyperplastic nodules originated in the epithelial cells around the urethra, but Reischauer noted pure stromal nodules around the urethra where the mixed nodules occurred.[27] He showed one micrograph of a pure stromal nodule with glandular buds working their way towards the center of the nodule apparently coming from a normal gland nearby.[12] He concluded that stromal hypertrophy occurred first and induced the epithelial ingrowth into the nodule. Subsequent light microscope work supported Reischauer's findings,[12,18] but experimental proof of stromal induction of epithelial hyperplasia is still needed.[16] Benign hyperplasia has never been experimentally induced in any animal. Although it occurs spontaneously in elderly dogs, it is caused by cystic changes and is unlike the nodular hyperplasia seen in human tissue.[26]

The etiology of prostatic hyperplasia has been linked to a hormonal imbalance for a long time. John Hunter in 1786 first noted that the size of the prostate was affected by the endocrine system.[16] It was observed later that hyperplasia occurs only in the presence of androgens; men castrated in early life never

develop benign hyperplasia and the nodules in men with hyperplasia become smaller after castration.[26] Estrogens cause hyperplastic cells in the inner prostate to involute[23] — a response similar to androgen deprivation. However, estrogens delay the involution in castrated animals and can cause squamous metaplasia in prepubertal glands that results in an increase in the gland size.[28]

There has been very little evidence to support a hormonal imbalance as the cause of benign hyperplasia. The human male undergoes a natural change in his hormonal milieu; his urinary androgens sharply decrease in the third to fifth decade.[29] The total estrogens stay the same but estriol increases while its metabolic precursors estrone and estradiol decrease.[29] However, Moore compared urinary androgen levels in men with benign hyperplasia with normal males and found them statistically the same.[18]

Huggins suggested that the most likely cause of benign prostatic hyperplasia is the presence ". . . of a testicular stimulus presumably androgen acting over a long period of years on a tissue which at that time has a low threshold to androgens."[26] The evidence then for the etiology of hyperplasia favors a pathological change in the end organ tissue rather than a change in the hormonal stimulus.

4. Prostatic carcinoma.

Prostatic cancer has become an important clinical entity in the last thirty years. Before then it was considered a rare event.[17] In 1935, two studies announced a high frequency of incidental prostatic cancer in autopsy material.[30,31] Moore noted an incidence of 17% in the fifth decade and 29% by the ninth decade.[30] Rich found that 14% of the men over fifty years old had a small prostatic carcinoma at autopsy.[31] Edwards confirmed this in 1953.[17]

The same histological types of carcinoma are seen in occult and in clinical cases. The most common is called Class I by Auerbach[32] and Type I, "duct type" by Edwards.[17] In histological preparations it is recognized by the groups of small glands formed by cuboidal epithelial cells. The glands never have papillary projections into the lumen like normal glands and they lack the characteristic band of surrounding stroma. Occasionally, an epithelial cell is included in a smooth muscle bundle and this is considered diagnostic of cancer.[17]

The over-all architecture of the gland is the best indication of neoplasia.[17,32] This principle forms the basis of Auerbach's classification. Class II, III, and IV cancers are defined by the following:

Class II is the same as Edward's tubuloalveolar type; the epithelial cells are cuboidal but larger than in Class I.

They form glands with irregular lumens and very little intervening stroma. Class III has large, irregular sheets of cells with narrow slits for gland lumens. The cells are large with marked variation in size and prominent basophilic nucleoli. Class IV is the most anaplastic; large, sometimes multinucleated cells, form solid sheets and cords.[17,32]

Most clinically advanced cancers are still mature enough to secrete acid phosphatase.[33] Gutman and Gutman realized this fact in 1938 when they postulated that metastatic tumor cells would secrete their enzymes directly into the blood stream.[34] Their studies confirmed this; very high serum levels of acid phosphatase occurred in patients with known metastasis.

Huggins and Hodges later found that the levels of the acid phosphatase corresponded to the activity of the tumor.[35] High serum levels indicated active cells, low levels were found when the primary tumor regressed. They used the serum acid phosphatase level to monitor the response to different hormone therapies. Androgens increased the serum acid phosphatase; estrogens and castration decreased them and gave both objective and subjective relief from prostatic carcinoma. These principles of therapy were later applied extensively. In 1950, Nesbit and Baum reported the results on over 1000 cases.[36] Combined orchidectomy and estrogen therapy provided a 43% 5-year survival rate in patients with metastases. The survival rate was

only 20% without treatment.

5. Etiology of prostatic carcinoma.

The cancer arises in the outer prostate, independant of hyperplastic glands. Benign hyperplasia may occur in the same gland but there is no evidence that hyperplasia is the precursor of the cancer.[30]

In 1935, Moore observed cancerous glands in areas of senile atrophy.[30] He concluded that the atrophy predisposed to cancer formation. But McNeal recently studied the morphology of the cancer and found two types, "alveolar-medullary" and "tubulo scirrhous" cancers, both arising from active duct epithelium.[37] He demonstrated the cells progressing from active to hyperplastic, to carcinoma in-situ, and speculated that carcinoma arises in glands responsive to chronic androgen stimulation and therefore that androgens may be a carcinogen for prostatic carcinoma. Although prostatic cancer is often dependant upon androgens for growth, the concentration of neutral 17-ketosteroids is identical in men with cancer and in normal men.[38]

The etiology is still obscure. It probably will be answered when the etiology of other adenocarcinomas is solved.

C. HISTOCHEMISTRY OF THE PROSTATE.

Many enzyme systems have been studied in animal and human prostates. These studies have been reviewed recently in

two sources.[20,39]

1. The oxidative enzymes in the prostate.

The oxidative enzymes, such as glucose-6-phosphate dehydrogenase, succinic dehydrogenase and lactate dehydrogenase, were studied by the tetrazolium reductase system in malignant, hyperplastic and normal tissue.[40] The activity was the same in hyperplastic and normal tissue but it was increased in cancer cells. In areas of cancer responding to estrogen therapy, the activity was decreased; in the unresponsive tissue there was an increase in activity.

2. Aminopeptidase.

The results of aminopeptidase studies are especially interesting. It was first found that most hyperplastic nodules were devoid of activity.[40] Kirchheim and Scott later found sparse activity in the normal rat, but a marked increase in response after castration.[41] In a study on human material, activity was found to be "greatly decreased or absent" in cancer cells, while the surrounding normal tissue showed activity.[42,43]

3. Acid phosphatase.

Acid phosphatase is the most popular enzyme in the prostate. It was first localized in man by Kutscher and Wolbergs in 1935.[44] It is present in fetal glands in low amounts, probably because the gland is composed primarily of stroma with few active glands.[45] At puberty, the activity increases towards

normal values.[46] In the adult it is greater than in any other cells of the body.[47]

Several studies have compared the amount of enzyme reactivity in normal, hyperplastic and cancerous tissue. The inner and outer zones react the same in both hyperplastic and normal glands. The reaction product localizes in the tips of the epithelial cells in both and the same amount of activity occurs in both.[42,47,48]

Cancer cells in general have less activity than normal or benign hyperplastic cells,[42,43,47,48,49] but the staining intensity varies from cell to cell. Some may have more reaction than normal; others much less.[42,47] Anaplastic cells have less activity than mature cells.[43,47] Estrogens reduce the activity in hyperplastic tissue; castration and estrogens decrease it in carcinoma cells.[47,49]

Castration and ageing in laboratory rats both cause the accumulation of acid phosphatase positive granules in an area just beyond the nucleus, towards the lumen (the supranuclear area).[39] They correspond to electron opaque bodies seen with the electron microscope in the same area.[50,51] Hruban and Swift found them similar to areas of focal degeneration induced in the pancreas and liver after toxic stimuli and called them "autophagic vacuoles."[52] Brandes recently localized acid phosphatase

activity in the opaque bodies and concluded that they were lysosomes.[39]

Lysosomes probably accumulate in the same way after estrogen therapy but this probably cannot be stated with certainty without more work. Arcadi found that castrated and estrogen treated rats accumulated the same kind of "supranuclear globule".[53] They reacted positively to a periodic-acid-leukofuchsin stain and were thought to be Golgi apparatuses. However, Brandes suggested that they were probably lysosomes because Golgi apparatuses are not located in the supranuclear area in electron microscope studies of prostatic epithelium.[39] Groth and Brandes noted large bodies with complex inner structures scattered throughout the cytoplasm in estrogen treated rats.[54] These may have been lysosomes.

4. Esterases.

Huggins and Moulton used a spectrophotometric technique to survey nonspecific esterase activity in rat and dog tissue.[55] They were the first to report activity in the prostate. It had one-fifth to one-tenth the amount of activity of liver—the most reactive tissue.

Nachlas and Seligman developed a new histochemical technique to localize esterases.[56] In dog and human prostates,

they found much activity in some glands but not in others. A later study used frozen sections of dog prostates and found a "high order of esterase activity" in all acinar epithelium.[57] Incubation with an organophosphorus inhibitor, E-600, had no effect upon the reaction.

Brandes located esterases in granules similar to those in which acid phosphatase was located except that diffuse cytoplasmic staining in addition to the granules occurred with the esterase reaction.[39] The cytoplasmic staining disappeared after inhibition with E-600, but the granules remained. This was interpreted as an indication of their lysosomal nature. The granules disappeared after exposure to isotonic saline; saline is known to labilize the lysosomal membrane and release the enzymes from their particles.[58] This was added evidence that the acid phosphatase and esterase particles were lysosomes.[39]

Human prostates were first tested for esterases in 1956.[48] Acetone fixed paraffin sections were used with alpha-naphthal acetate as the substrate. The supranuclear area and the area just below the nucleus (basal part) of normal cells showed activity, but hyperplastic and cancerous tissue had greatly diminished activity. A later study from the same laboratory used formalin fixed, frozen tissue and produced different results.[42] They used the indoxyl acetate method of Holt[59,60] and the diazo dye technique of Seligman.[61] Both techniques

showed granular staining throughout the cytoplasm of hyperplastic and normal tissue but there was a preponderance of activity in the supranuclear region. Cancer cells showed the same cytoplasmic distribution but the results varied in intensity in different areas of involvement. However in most areas, the activity was similar in normal and benign hyperplasia.[42]

D. ULTRASTRUCTURE OF THE PROSTATE.

1. Mice and Rats.

There is much variability in the prostatic ultrastructure of mice and rats. They have four distinct prostatic lobes each with different ultrastructure.[39] Even in different species of mice, there is variation between homologous lobes. But all of the lobes have the distinctive feature of a cytoplasm full of dilated endoplasmic reticulum. There are only a few mitochondria, Golgi complexes and dense bodies scattered in the thin areas of cytoplasm between the dilated endoplasmic reticulum.[50,51,62,63,64,65]

The distinguishing characteristics of each lobe and species have been reviewed by Brandes.[39]

2. Dog and Rabbit.

The human prostate is much different than the rat and mouse. Dog and rabbit more closely approach the human type of ultrastructure.[39,66] The cytoplasm in the dog prostate is filled with dense secretory granules, similar to those seen

occasionally in the human. There is much less endoplasmic reticulum, a condition also similar to that in the human gland. Rabbit ultrastructure has only been briefly described and is said to be similar to the human, but containing a peculiar lamellar endoplasmic reticulum in the supranuclear area.[67]

Rats, mice, rabbits, dogs, and men share several basic features. They all have tall cuboidal or columnar secretory cells bordering the gland lumen with small basal cells[21] (Brandes calls them "reserve cells")[39] inserted between the secretory cells and the basal lamina. Fibroblasts and smooth muscle surround each acinus.

3. Normal and benign hyperplasia.

The ultrastructure of normal glands was examined from areas not involved in cancer and in the noncompressed outer zone of adenomatous prostates.[22] The tall epithelial cells have nuclei at their bases; the cytoplasm beyond contains a Golgi zone (called the supranuclear area) and secretory vacuoles especially concentrated in the luminal tip (called the apical pole).[22]

The epithelial cells in benign hyperplasia have the same cytoplasmic organelles as in the normal prostate.[22,67,68] One study noted an increase in the intercellular spaces at the base of the hyperplastic cells but this was the only reported deviation from normal.[22]

Mao's group found three types of columnar cells lining glands in hyperplastic nodules: tall active cells averaging 25 microns in height, low columnar cells with an average height of 15 microns, and cuboidal cells about 10 microns high.[68]

The secretions in the tall hyperplastic cells and in normal cells are of two types--merocrine and apocrine.[39,68] The merocrine secretions are either small vesicles enclosed in secretory vacuoles, or dense membrane bound bodies. Both types are released into the gland lumen. Apocrine secretions pinch off from the tips of cells and contain cell organelles, Golgi vesicles, glycogen, and free ribosomes.

Acid phosphatase and 5-nucleotidase activity have been located with the electron microscope in the Golgi vacuoles and apical secretory vacuoles of the merocrine secretions.[39,69] But the apocrine blebs were free of activity except when secretory vacuoles were located in them.[69] Low columnar and cuboidal cells lacked secretory activity of any type.

Lysosomes and autophagic vacuoles were found in all three cell types but especially in the inactive low columnar and cuboidal cells. Lysosomes were located in the Golgi zone in the tall cells; autophagic vacuoles were rarely seen in these active cells, but they were common in the inactive ones.[68]

Small triangular basal cells wedged between the epithelial cells and the basal lamina have an ultrastructure quite unlike the secretory cells. They are ". . . essentially poorly differentiated or undifferentiated cells."[21] The cytoplasm is filled with ribosomes and mitochondria giving it an electron dense appearance in sharp contrast to the lucency of the secretory cytoplasm nearby. Acid phosphatase activity was said to be essentially absent from basal cells unless a rare lysosome was present.[69]

The stroma closest to the acinus is made of collagen and fibroblasts. Smooth muscle cells usually lie beyond the fibroblasts; occasionally they touch the acinus. Acid phosphatase activity was absent in the stromal cells at both the light and electron microscope level.[69]

4. Human Cancer.

Well differentiated (Class I) cancer cells resembled benign hyperplastic cells except for a few differences,[22,39,67] but many changes were seen in less differentiated cells. In the well differentiated, the nucleoli were larger than normal. Dense bodies were frequently observed by one group[22] but another reported no difference in the number present compared to normal cells.[67]

Undifferentiated cells had cytoplasmic vacuoles considered to be pathological[22] and there were more lipid droplets than

normal.[67] The mitochondria were noticeably distorted; they were swollen and many had lost their cristae.[22,67,70] Lysosomes were rich in the Golgi region, but did not appear to be increased compared to hyperplastic or normal cells.[71] Isolated cells in the stroma lacked a surrounding basal lamina, but there were no reported changes in the stromal fibroblasts or muscle cells.[22]

E. THE LYSOSOME.

1. Introduction.

Christian de Duve wrote in 1955: ". . . there are strong grounds for the belief that the peculiar distribution of acid phosphatase reflects the existence of a distinct class of granules. ... For practical purposes, it is proposed to refer to these granules as lysosomes, thus calling attention to their richness in hydrolytic enzymes."[1] De Duve's concept originated from enzyme analysis of liver cell homogenates. In later biochemical studies, lysosomes were defined as membrane bound sacs of hydrolytic enzymes.[58] The enzymes emerged from their sacs under a wide variety of circumstances: a drop in pH, decrease in osmolarity, temperature increase,[72] ultraviolet irradiation, antigen-antibody reaction, shock, virus

infections and other conditions.[4] The enzymes released were primarily hydrolases such as acid phosphatase, esterases, DNA'ase, cathepsin and others.[4,58] They can digest liver cell homogenates into products similar to those resulting from liver autolysis.[73]

2. Terminology.

The morphologist defines the lysosome as a "membrane-delimited cytoplasmic granule" with acid phosphatase activity.[74] In morphological preparations they apparently function as intracellular digestive systems by breaking down a variety of foreign and endogenous materials.[4] The ultrastructural appearance of lysosomes depends upon the type of material digested and is therefore quite diverse. Probably because of this morphological heterogeneity, the terminology describing lysosomes is complicated. De Duve and Wattiaux have recently constructed a logical classification system based upon a division into primary and secondary lysosomes.[3] The primary lysosomes are considered to be "pure" enzymes that have never undergone digestive activity. Secondary lysosomes are divided into heterophagosomes, autophagosomes and residual bodies. Heterophagosomes result from the fusion of lysosomal enzymes with an ingested membrane bound particle called a phagosome.[3]

(These have also been called phagolysosomes.)

Autophagosomes contain cell organelles (such as mitochondria and endoplasmic reticulum) and lysosomal enzymes; they are surrounded by a limiting membrane. If the heterophagosome and the autophagosome exhaust their enzymatic activity but still retain undigested material, the structure is termed a residual body.[3]

3. Origin of lysosomes.

Novikoff and his colleagues proposed that lysosomal enzymes are formed in the rough endoplasmic reticulum and pass either to smooth endoplasmic reticulum or to Golgi saccules.[74] Buds then break off from the endoplasmic reticulum or the Golgi and coalesce with phagosomes to form phagolysosomes. Evidence for this theory comes largely from studies on acid phosphatase localization in rat hepatocytes. The acid phosphatase reaction product was located in the Golgi zone, the endoplasmic reticulum and the peribiliary dense bodies (these latter are considered to be lysosomes).[74] Brandes supported this theory later when he found acid phosphatase reaction product in the Golgi of the Euglena and in the dilated spaces of the rough endoplasmic reticulum in the rat prostate.[75] In human liver infected with viral hepatitis, the acid phosphatase

activity is located in the Golgi cisternae but this has not been observed in normal human liver.[76]

The granules of the polymorphonuclear leukocyte have been well established as pure primary lysosomes.[77,78] Bainton and Farquhar added impressive evidence that pure lysosomes are formed in the Golgi complex.[79] They studied their formation in rabbit bone marrow cells and demonstrated the electron dense material of the granules originating from small buds off the Golgi cisternae. They later united to form mature granules.[79]

4. Structure and function of lysosomes.

a. Primary lysosomes.

The leukocyte granules are about 500-800mμ in diameter. They have a homogeneous, electron dense matrix surrounded by a trilaminar membrane similar to other cell membranes.[79]

Cohn and Hirsch studied the function of pure lysosomes with the phase contrast microscope and recorded their findings with motion pictures.[77,78] They observed the degranulation of leukocytes after they ingested micro-organisms. The microorganisms fused with the "phase-dense granules, leaving in their place bright empty-appearing spaces which themselves soon vanished."[77] Zucker-Franklin

later studied the same phenomena with the electron microscope.[80] She demonstrated very nicely how an ingested foreign particle comes into the cell enveloped by the outer plasma membrane. An electron lucent vacuole forms between the particle and the surrounding membrane. The lysosome moves toward the particle, fuses with the surrounding membrane and injects its matrix into the vacuole, thus forming a phagolysosome.[80]

b. Secondary lysosomes.

The peribiliary bodies in the rat liver are considered to be secondary lysosomes.[3] They were first investigated with electron cytochemical techniques in 1961 and found to contain dense acid phosphatase reaction product.[81,82] Particles that were called lipofuschin granules with the light microscope were found to have an irregular electron dense matrix surrounded by reaction product.[81] They were also considered to be lysosomes.

Later studies on the morphology of secondary lysosomes traced the course of electron dense phagocytized material through the cell.[83,84] Miller and Palade studied hemoglobin particles phagocytized by the proximal convoluted tubule cells of the mouse kidney. Two hours after injection into the intraperitoneal cavity, hemoglobin appeared in phagosomes

in the tubule cells surrounded by a rim of acid phosphatase activity. Forty-eight hours later, the particles had disappeared but a large number of dense bodies with acid phosphatase activity remained.[84]

Autophagosomes are another type of secondary lysosomes. They appear occasionally in normal cells but more frequently after the cell has been stressed.[3] Starvation and toxic diets produce degenerative changes in the liver with sequestration of cytoplasmic organelles bounded by acid phosphatase activity.[52,85] Castration produced an increase in autophagic vacuoles in the rat prostate.[39] Other factors stimulating cellular autophagy are potassium deficient diets, cellular metamorphosis, hypoxia, irradiation and cold exposure. They have been discussed by de Duve and Wattiaux.[3]

5. Lysosomes in disease processes.

Lysosomes have been implicated in the pathogenesis of hemorrhagic, endotoxic and traumatic shock.[86,87,88] After experimental inducement of shock, lysosomal enzymes had decreased activity in liver samples, while plasma levels were increased.[86] If the animal was protected from shock by cortisone or by adaption to traumatic shock, the circulating and tissue lysosomal enzymes were normal.[86]

In reversible hemorrhagic shock, the lysosomal membrane was more fragile than normal (measured by a fragility test developed by Bitensky),[87] but in irreversible shock, the lysosomal membrane completely disintegrated, releasing enzymes into the cytoplasm.

Weissman has linked the pathogenesis of autoimmune diseases to derangements in lysosome function.[4] He found that the injection of lysosomal activating substances into joint spaces produces a disease similar to rheumatoid arthritis.[89] The intradermal activation of lysosomes in patients with systemic lupus erythematosis and their normal relatives produces a vasculitis identical to that seen in the original disease.[89] Treatment with chloroquin and cortisone, agents that stabilize lysosomal membranes, ameliorates the disease symptoms.[89,90]

Potassium deficiency induces osmiophilic, acid phosphatase-positive bodies in human and rat proximal convoluted tubule cells. The relationship between the appearance of the lysosomes and the decrease in kidney concentrating ability is obscure.[91,92]

Chediak-Higashi syndrome and Pompe's disease, are two hereditary diseases with abnormal lysosomes. Chediak-Higashi syndrome in humans and the Aleutian

mink disease in animals are associated with gigantic lysosomes in leukocytes and abnormal granulations in the bowel mucosa.[93] In Pompe's disease, the liver is deficient in acid alpha 1, 4-glucosidase which hydrolyzes the outer branch of glycogen to glucose.[94] This enzyme is localized in lysosomes in rat liver and thus considered to be a lysosomal enzyme. The liver of a patient with Pompe's disease had peribiliary bodies packed with abnormal accumulations of glycogen.

Allison and associates have developed the hypothesis that lysosomes play a major role in carcinogenesis.[9] Their evidence comes from experiments with carcinogens and lymphocyte chromosome studies. They found flouresecent polybenzenoid carcinogens localized in the hepatocyte in association with lysosomes.[5] Activated lysosomal enzymes caused a high incidence of gross chromosome abnormalities in lymphocyte cultures.[7] The defects resembled the deletions and triradial configurations seen after irradiation, viral infections and experimental chemical carcinogens. They proposed that carcinogens act upon the lysosome to stimulate the release of lysosomal enzymes which act upon the nucleus and cause chromosomal abnormalities leading to cancer.[9]

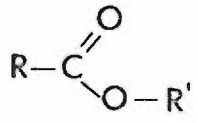
F. ESTERASES.

There is much evidence, as will be discussed later, that nonspecific esterases are lysosomal enzymes. They belong to the family of hydrolytic enzymes that split a variety of substrates and add water to the reaction products.[95, 96]

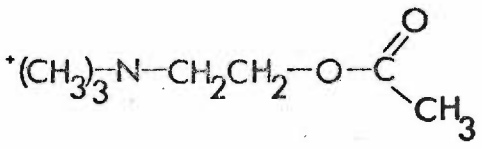
$$AB + HOH \xrightarrow{\text{hydrolase}} AOH + BH$$

1. Classification.

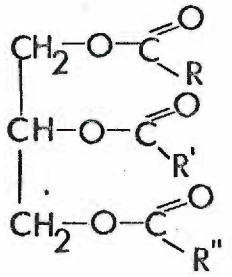
There are three general types of esterase substrates: carboxylic acid esters, sulfuric acid esters and phosphoric acid esters. The nonspecific esterases, acetylchoinesterase and lipases all belong to the class of carboxylic acid esterases. Their respective substrates are shown below.[96]



CARBOXYLIC ACID ESTER

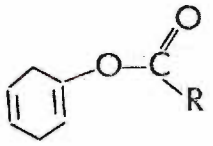


ACETYLCHOLINE

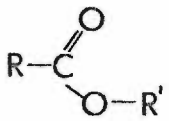


LIPID

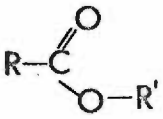
The nonspecific esterases are further divided into three groups, according to their substrate specificity.[95, 97]



Type A. Arom-esterases catalyze reactions where the alcohol group of the ester is an aromatic ring. It is not inhibited by concentrations of E-600 (diethyl-p-nitrophenyl phosphate) up to 10^{-3} M.[98,99,100]



Type B. Ali-esterases catalyze reactions in which the alcohol moiety is a short chain alcohol, inhibited by concentrations of E-600 as low as 10^{-8} M.[100,101]



R,R' = carbon chains

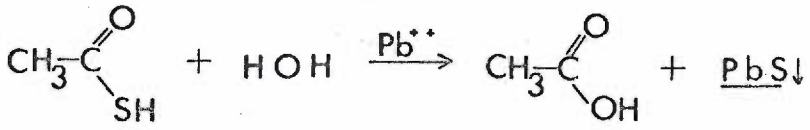
Type C. A lysosomal enzyme that acts like a cathepsin.[102,103] It is resistant to E-600.[102]

2. Localization methods.

The Azo dyes, Indigo dye and metal salts reactions are all used to identify esterase activity.[96] The Azo dye method utilizes napthyl acetate esters as the substrate; the acetate is released by the enzyme and combines with a diazonium salt in the incubating solution.[56] A colored Naphthol-diazonium salt is formed at the enzyme site. Indoxyl acetate is the substrate for the Indigo dye method; the acetate is hydrolyzed from the Indoxyl by an esterase.[59] Indoxyl is released and oxidized to blue indigo, an insoluable salt that marks the reaction site. The Azo dyes and indigo dye are ideal for

light microscopy but they are unsuitable for electron microscopy because they are electron lucent. (However, methods utilizing electron dense Azo dyes are being devised).[104,105] The methods involving metal salts give dense, electron opaque reaction products that are easily visualized with the electron microscope. Several methods for light histochemistry have been adapted to electron cytochemical work.[104]

Thiolacetic acid is the substrate used in the metal salts method for localizing nonspecific esterase activity. The SH group is hydrolyzed by esterases. Lead ions in the incubation medium combine with the sulfide ion to form a dense lead sulfide precipitate.[97]



Wilson in 1951 was the first to use the thiolacetic acid reaction, demonstrating that purefied acetylcholinesterase hydrolized thiolacetic acid, releasing H_2S.[106] This was the first evidence that esterases attack thiolacetic acid.[95] Crevier and Belanger later applied the principles of the reaction to tissue sections, and located prostigmine-sensitive and resistant activity in nervous tissue.[107] Barrnett in 1962 showed the fine structural localization of thiolacetic acid esterase in the terminal and junctional folds of myoneural

junctions, corresponding to acetylcholinesterase activity.[108]

3. Evidence that thiolacetic acid is hydrolyzed by nonspecific esterases.

Wachstien, Meisel and Falcon in 1961 compared thiolacetic acid esterase with nonspecific esterases (using naphthyl acetate and bromo indoxyl acetate as substrates) and found them both active in almost identical locations.[103] Barron, Bernsohn and Hess showed that electrophoresis of normal muscle homogenates produced two bands of esterase activity when naphtyol acetate was the substrate and the same two bands plus one other, when thiolacetic acid was the substrate.[109]

4. Evidence that esterases are located in lysosomes.

Initial cell fractionation studies found the microsome fraction to be the richest in esterase activity.[110,111] Histochemical evidence seemed to contradict this finding, especially with the indoxyl acetate method; it showed little cytoplasmic ("microsomal") staining.[111] The activity was confined to peribiliary cytoplasmic granules in the liver and in large droplets in the proximal kidney tubules. Later work by Shnitka and Seligman demonstrated that the indoxyl acetate method inhibited cytoplasmic esterases. They showed esterases in two locations: spread diffusely throughout the

cytoplasm and confined to specific granules.[112] The peribiliary granules and large proximal tubule droplets are thought to be lysosomes because acid phosphatase has been repeatedly located in them with the light and electron microscope.[82,95,113,114] The most convincing evidence for the presence of esterases in lysosomes comes from Holt: "If the Gomori acid phosphatase procedure is first applied to the fixed tissues, followed by the indoxyl method for esterases, it can easily be seen that the two enzymes are present in the same kidney droplets."[82] Wolman, Spicer and Burstone also found acid phosphatase and nonspecific esterase activity located together in the same cytoplasmic droplet in the rat thyroid.[115] In rat prostatic epithelium the organophosphorus resistant esterase corresponds to the location of acid phosphatase activity at the light microscope level.[116]

5. Evidence that thiolacetic acid esterase activity is found in the lysosome.

In 1960, Wachstien and Meisel reported localization of thiolacetic acid esterase activity in kidney and liver granules that they considered to be lysosomes.[117] Sabatini, Bensch and Barrnett in 1963 showed electron micrographs of esterases located with thiolacetic acid.[118] Dense bodies of muscle, kidney and liver cells reacted before and after

treatment with organophosphorus inhibitors. Similar results were reported by Bell.[97] Miller and Palade found both acid phosphatase and thiolacetic acid esterase located in dense bodies of the mouse proximal convoluted tubule cells.[84]

II. MATERIAL AND METHODS.

A. MATERIALS.

Twenty-one hyperplastic prostate specimens were obtained at surgery from patients of age 55 to 83 with urinary tract obstruction. Six cancer specimens came from patients of age 58 to 83; their prostates had been previously biopsied and found to have Class II or III malignancy.[32] The hyperplastic prostates were removed by open procedures using the suprapubic, retropubic, or perineal approach. Cancer samples were from whole prostates removed by an open procedure or from pieces of prostate resected transurethrally with a Thompson cold-punch resectoscope. Strips of tissue 0.2 cm x 1.0 cm were cut from hyperplastic nodules or from cancer tissue and immediately fixed in buffered glutaraldehyde or osmium tetroxide (concentrations and buffers will be described later). Larger pieces in the same area were taken for frozen and paraffin sections, and stained with hemotoxylin and eosin. Tissues for enzymatic studies were left as strips and chopped on the Smith-Farquhar chopper.[119,120] Tissues for morphological studies were cut into pieces less than 0.5 mm across with two razor blades.

B. METHODS.

1. Fixatives.

Several different concentrations of glutaraldehyde were used.[118] Tissue obtained at surgery was placed in 3.5%, 5%, 10%, 15%, or 20% glutaraldehyde prepared from a 25% stock solution (purchased from Fisher or Matheson, Coleman and Bell). The pH of the stock solution was adjusted with barium carbonate to about 4.0 by the method suggested in Kay.[121] The stock solution was then diluted to the desired concentration with 0.1M sodium cacodylate buffer adjusted to pH 7.4 with HCl.[122] The tissue was fixed for two hours at 4°C.

Osmium fixed tissue for morphological studies was placed for two hours in 1.0% Osmium tetroxide buffered in 1.0M S-Collidine adjusted with HCl to pH 7.4.[123]

2. Wash.

Tissue strips that were fixed in glutaraldehyde were then washed in 0.1M cacodylate buffer with .22M sucrose. Tissue stored longer than 48 hours in this solution showed poor preservation of morphology. The wash time for enzyme work was about 4 hours; for morphological studies the tissue was briefly rinsed and then post-fixed.

3. Post-fixation.

Tissue for morphological observation was transferred from the wash to 1.0% Osmium tetroxide buffered with 0.1 M S-Collidine and was prepared the same way as tissue fixed initially in Osmium tetroxide (page 33). The effects of 2.0% Osmium and several different buffer systems were studied. Two percent Osmium buffered in veronal-acetate and 1.0% Osmium buffered in veronal-acetate with 0.005M uranyl nitrate added were used. Dalton's, Caulfield's, Zetterquist's and Millonig's Osmium were also prepared according to methods in Kay[124] and Pease.[125] The results of these various procedures will be shown in a later section of this paper.

4. Enzyme reactions for light microscopy.

a. Thiolacetic acid esterase.

The esterase reaction gave the most consistent results for light microscopy when floating sections were used. These were prepared from tissue blocks (0.5 cm x 1.0 cm) fixed at surgery in 4% Ca-Formalin in 0.1M cacodylate buffer, pH 7.0 with 0.33M sucrose.[116] They were fixed at 4°C for 24-28 hours and then frozen by immersion in isopropyl alcohol cooled to -60°C with dry ice in acetone. The quenched tissue blocks were mounted on chucks for the Pearse-Slee cryostat. Thin sections 6 to 8 microns were cut at -15°C and brushed

from the knife blade into 0.1M cacodylate buffer. The floating sections were washed in buffer for 1 hour, and then lifted into the incubation medium with glass instruments.

The incubation medium for thiolacetic acid esterase activity was first used by Crevier and Belanger in 1955[107] and later by Bell and Barrnett in 1964.[126] The method used for this study was modified from Bell and Barrnett.

The medium was prepared over ice to prevent spontaneous hydrolysis of the thiolacetic acid. A 0.05M solution was prepared by adding 0.38 ml of thiolacetic acid (Sigma) to 50 ml of 0.1M cacodylate buffer at pH 6.0. The pH was readjusted to 6.0 with 1 N NaOH; 280 mg of $Pb(NO_3)_2$ was dissolved in 10 ml of 0.1M cacodylate buffer (pH 6.0) and added to the thiolacetic acid mixture. The total volume was adjusted to 100 ml with the cacodylate buffer (pH 6.0). The pH of the final solution was 6.0. The fine yellow precipitate that formed when the $Pb(NO_3)_2$ was added was removed by one passage through Whatman's No.2 filter paper. The tissue sections were incubated in the clear filtrate for 8 to 20 minutes at room temperature. They were washed in 2% acetic acid for 1 minute for light microscopy or put into cacodylate buffer (pH 7.4) for electron microscopy. The sections for light microscopy were counterstained with Harris' hematoxylin for 1 minute, rinsed in

buffer and mounted in glycerol gel.

b. Acid phosphatase.

Barka and Anderson's modification of the original Gomori lead phosphate method was used.[127] Sodium beta-glycerol phosphate (Sigma Grade I) was used as the substrate; 1.25 grams were added to a 100 ml volumetric flask and dissolved in 50 ml distilled water. The pH of this solution was adjusted to 5.2 with 1M HCl. The volume was adjusted to 100 ml with distilled water. Ten milliliters of the beta-glycerol phosphate solution were added to 10 ml of 0.2M Tris-maleate buffer pH 5.0 and 10 ml of distilled water.[122] Twenty milliliters of 0.2% $(Pb(NO_3)_2$ were slowly added to the mixture, always resulting in a clear final incubation solution. Floating sections and sections dried on coverslips in room air were incubated in the freshly prepared media for 1 to 5 minutes at room temperature. The reaction was stopped with a 2% acetic acid rinse for 2 minutes. The sections were counterstained with Harris' hematoxylin for 1 minute, then rinsed in buffer and mounted in glycerol gel.

5. The esterase reaction for the electron microscope.

The reaction was first tried on frozen tissue cut to a thickness of 25 to 30 microns on the Pearse-Slee

cryostat; but freezing disrupted the ultrastructure and was discontinued in favor of the Sorval, Smith-Farquhar fresh tissue chopper.[119,120] Sections 25 to 30 microns thick were cut by the procedure outlined by Smith and Farquhar.[120] Glutaraldehyde fixed tissue strips 0.2 cm x 1 cm long were stabilized with 7% agar on the platform of the chopper. The sections were cut with a Schick injectable razor blade and then placed in buffered 0.1M cacodylate (pH 7.4). They remained in the buffer while the substrate was prepared in the same procedure outlined for light microscopy. Following the removal of the buffer, the incubation was started by addition of the substrate medium. The incubation medium was removed after 6 to 15 minutes and 0.1M cacodylate buffer was added to stop the reaction. Four fresh changes of the buffer were used in the next hour. The tissue was then post-fixed in 1.0% Osmium 0.1M S-Collidine, for one hour at 4°C.

6. Controls.

Three types of controls were used; the first was the incubation medium prepared without the thiolacetic acid substrate added; the second, a cloudy control and the third preincubation with E-600 (diethyl nitrophenylphosphate). Medium without the substrate was prepared by dissolving

280 mg of $Pb(NO_3)_2$ in 100 ml of 0.1M cacodylate buffer (pH 6.0). Tissue segments were treated in the control solution for 6 to 15 minutes, then prepared for microscopy by the same method used for the experimental tissue. A cloudy control was prepared to test the nonspecific localization of a lead salt; 280 mg of $Pb(NO_3)_2$ was dissolved in 20 ml of cacodylate buffer at pH 6.0; 280 mg of potassium chloride was added and a white precipitate of lead chloride formed. The chopped tissue sections were then immersed in the solution for 10 minutes. Controls to specifically inhibit Esterase B were run at both the light and the electron microscope level. Tissue pieces were pre-incubated in 10^{-7} or 10^{-4} Molar E-600 for 10 minutes and were then incubated for 10 minutes in the complete thiolacetic acid incubation medium with the same concentration of E-600.

The tissue from each control experiment was rinsed in buffer for one hour and post-fixed the same as the incubated material.

7. Dehydration and embedding.

The osmium fixed tissue was dehydrated in graded dilutions of alcohol: 50% for 3 minutes, 70% for 3 minutes, 80% for 3 minutes, 95% for 3 minutes and 100% three changes of 5 minutes each. The tissue was then rinsed in propylene

oxide twice at 15 minutes each. Infiltration was started with a mixture of 1/2 propylene oxide and 1/2 epoxy resin with catalyst (Luft's method) for two hours.[128] The tissue was embedded in the 100% epoxy resin mixture and polymerized over night at 60°C.

8. Cutting and viewing the tissue.

Thin sections were cut on a Sorval MT1 with a glass or DuPont diamond knife or on an LKB Ultratome with a DuPont diamond knife. The ultra thin sections were placed on uncoated copper grids and stained with saturated uranyl acetate and Reynold's lead citrate stain.[129] Reacted tissues were viewed with and without the stains. Pictures were taken with an RCA EMU-3f electron microscope using glass projector plates from Kodak for negatives. The negatives were enlarged and printed on DuPont or Kodak polycontrast paper.

Sections one micron thick were stained with Methylene Blue-Azure II for orientation with the light microscope.[130]

III. RESULTS.

A. FIXATION.

The secretory products in the tall columnar cells were difficult to preserve; for example in light micrographs (Fig. 3, 5, and 6) the cytoplasm is full of clear vacuoles. In electron micrographs, the vacuoles correspond to empty or partially filled secretory vacuoles (Fig. 23, and 24). Efforts were made to keep the products in the vacuoles because it was thought that they would contain enzyme activity.

Experiments were done with both the prefixative and the postfixative. Glutaraldehyde was used as the prefixative since it is known to give good morphological results as well as to save enzyme activity.[118] It was followed by osmium tetroxide prepared with different buffer systems.

1. The prefixative.

The morphological effects of the osmolarity of the prefixative were tested. The osmolarity was changed by increasing the concentration of glutaraldehyde or by adding sodium chloride to the fixative. The results are tabulated below:

Glutaraldehyde concentration in ml%. Buffered in 0.1m Cacodylate at pH 7.0	Morphology
3.5%	Granular secretory products retained but many large vacuoles fill the cytoplasm. (Fig.3, 23, and 24).
5.0%	Dense and granular secretory products usually preserved. (Fig. 30, 49, and 53).
10.0%	Dense and granular secretions usually retained; very little difference between 5% and 10%. (Fig. 10, 25, and 51).
5.0% with .5MNaCl	Stroma and glands markedly distorted. (Fig. 9).
15.0%	Granular products well preserved but homogeneous and vesicular secretions are missing. (Fig. 12 and 26).
7.5% with .75MNaCl	Stroma and glands distorted and shriveled. (Fig. 11).
20%	Light microscope sections show many dense granules in the cytoplasm; there are few empty vacuoles. (Fig. 8). Homogeneous and granular secretions are well preserved in electron microscopy sections. (Fig. 27, 28, and 29).

Tissue prefixed in calcium-formalin had the same vacuolated cytoplasm as occurs with low concentrations of glutaraldehyde (Fig. 7).

2. The postfixative.

Five different osmium fixatives were tested; the results are as follows:

Fixative	Morphology
Dalton's 1% osmium (Dichromate buffer)	Empty secretory vacuoles remain in the cytoplasm. (Fig. 6).
Zetterquist's 1% osmium (Veronal-acetate buffer with Ringer's lactate)	Same as with Dalton's (Fig. 5).
1% osmium with 0.005 m uranyl nitrate in veronal-acetate buffer.	Very good fixation of low columnar cells, but tall secretory cells were not tested. (Fig. 31).
2% osmium in veronal-acetate buffer.	Cytoplasmic matrix denser than with 1% osmium, but the same organelles are preserved in low columnar cells. (Fig. 18 and 19).
1% osmium in 0.1m S-Collidine buffer.	Dense and vesicular secretions retained, general morphology is good. (Fig. 17,20, and 22).

Five and ten percent glutaraldehyde were the most useful fixatives, although this was a compromise between good morphology and enzyme activity. Only some of the secretory granules were preserved with 5% and 10% whereas 20% glutaraldehyde did keep the secretory granules in their vacuoles. But enzyme

activity was less than when glutaraldehyde was kept at lower concentrations. One percent osmium S-Collidine was used routinely as the postfixative and it gave satisfactory results.

B. MORPHOLOGY OF BENIGN HYPERPLASIA.

1. Histology.

Figures 5, 6, and 7 show the papillated glands typical of hyperplastic nodules. Secretory cells were crowded onto thin projections of stroma to give the papillated appearance. The secretory cells were about 25 microns tall; most of their cytoplasm was vacuolated, as is clearly shown in Fig. 3.

All of the glands had small basal cells between the secretory cells and the surrounding band of stroma. These cells had nuclei smaller and darker than in the secretory cell. Their cytoplasm was scant and contained no evidence of secretory activity. (Fig. 48).

Low columnar cells formed smaller, unpapillated glands as shown in Fig. 1, 2, and 13. The cells were about 15 microns tall; their cytoplasm was not vacuolated as in the tall epithelial cells, but was often attenuated at the lumenal tips as in Fig. 2. Small basal cells gave a double layered appearance in the Hematoxylin and Eosin section. (Fig. 1). They were similar to those found in papillated glands. Occasionally dense

secretions filled the lumens of low columnar cell glands (Fig. 13), but these were infrequent in the two other types.

A few benign nodules contained glands formed by inactive, cuboidal cells about 10 microns tall, shown in Fig. 4. The glands were small and without papillary projections. Their cells had a large nucleus with a small amount of cytoplasm. They had basal cells and surrounding stroma similar to that of other glands.

2. Ultrastructure of the secretory cells.

Tall columnar, low columnar and cuboidal cells were distinguished from each other with the light microscope primarily by their height. Their ultrastructural differences were primarily related to their secretory state.

a. Tall columnar cells.

There was evidence of much secretory activity in tall columnar cells. Three different types of secretions were prominent in their secretory vacuoles: one was filled with electron dense fine granules, another was electron dense but homogeneous, and the third consisted of small round vesicles floating in the vacuoles. Some cells had all three types of material (Fig. 23 and 24), while others had one or two types.

Osmium fixed tissue had both vesicular and homogeneous

types (Fig. 17 and 22). The type varied after glutaraldehyde fixation depending upon the strength used. Twenty percent glutaraldehyde preserved only granular or dense homogeneous secretions (Fig. 27 and 29). Less concentrated solutions fixed all three types (Fig. 23 and 24).

The dense homogeneous secretions often looked like the lysosomes found in other tissues.[131] They even contained myelin figures at times (Fig. 62) which are usually considered to be typical of lysosomes. It was not possible then to study the lysosome distribution in secretory cells without an enzyme reaction product to distinguish the lysosome from secretory granules. Other organelles found in all of the cell types were mitochondria and Golgi complexes. The mitochondria were round and contained only a few cristae. They were commonly surrounded by arcs of rough endoplasmic reticulum as described in other tissues.[131] They usually lay in an area around the nucleus but also appeared mixed with secretory granules at the cell apex. (Fig. 22 and 24).

Golgi complexes occurred also but they were obscured by the large amounts of secretions. They were found around the nucleus as in Fig. 22, but were more easily seen in less active low columnar and cuboidal cells. (Fig. 15 and 17).

The nuclei in all cell types were similar. They were

large, oval masses with smooth borders. The chromatin was finely dispersed in osmium fixed cells, but was clumped around the periphery after glutaraldehyde fixation. Nucleoli were prominent in most cells after both kinds of fixation.

b. Low columnar cells.

Low columnar cells lacked the abundant secretory vacuoles found in the taller cells. Mao[68] described apocrine and merocrine secretory mechanisms in tall columnar cells exclusively, but they were also seen in low columnar cells in the preparations being described here.

Apocrine secretions formed at the lumenal pole of the low columnar cells in Fig. 14 and 16. The apical cytoplasm was filled with electron lucent material devoid of most organelles. There often was a constricted zone between the sparsely populated cytoplasm and the area richer in organelles near the nucleus. Round blebs of cytoplasm filled with the same electron lucent material appeared in the gland lumen, apparently pinched off from the cell tip (Fig. 16).

Other low columnar cells had small dense secretory granules in the cell apex (Fig. 17) which were apparently released into the lumen as merocrine secretions. Figure 33 shows a duct with merocrine type secretory granules filling the lumen.

The rest of the cytoplasm in low columnar cells was filled with mitochondria and free ribosomes. Golgi complexes which have already been mentioned, occurred near the nucleus, either above it or below it. (Fig. 14 and 17). Lysosomes were difficult to tell from secretory granules in cells with merocrine secretions, but cells with apocrine secretory mechanisms had two to three small dense bodies usually around the nucleus that were probably lysosomes (Fig. 14 and 16).

c. Cuboidal cells.

The nucleus consumed most of the space in cuboidal cells (Fig. 30 and 31). If present, the secretions were concentrated in small dense granules similar to those found in low columnar cells. But many cells lacked evidence of secretory ability. The rest of the cytoplasm was rather bleak. It was filled with free ribosomes, a few small mitochondria and an electron lucent material with fine, short filaments in it.

Several small dense bodies with limiting membranes were found in each cell; but they were difficult to distinguish from secretory granules. (Fig. 30 and 32).

3. Ultrastructure of basal cells.

The ultrastructure of basal cells was much

different than that of secretory cells. The cytoplasm contained many free ribosomes and was more electron dense than the surrounding secretory cells. The nucleus had an irregular outline and was smaller and more dense than in secretory cells. The chromatin was packed closer together, often in large clumps around the periphery. Each cell had several slender mitochondria and one or two small dense bodies.

There appeared to be a maturation sequence from basal cell to secretory cell. Cells shown in Figures 17, 19, 20 and 50 had features of young, undifferentiated cells. Their cytoplasm was dense with ribosomes, but they contained only a few other organelles. More mature basal cells contained many mitochondria and several small Golgi complexes in addition to the ribosomes. The nucleus was dense and irregular but similar to that found in younger cells. (Fig. 20).

4. Dark cells.

After 20% glutaraldehyde fixation, there was a sharp contrast between two cell types (Fig. 8 and 29). Darkly staining cells appeared close to light colored cells in light and electron micrographs. The dark cells had an

electron dense cytoplasm filled with ribosomes similar to that found in basal cells. But they also had secretory granules like mature cells. The light cells had an ultrastructure identical to mature cells fixed in lower concentrations of glutaraldehyde (Fig. 29 and 62). The two cell types were found in hyperplastic tissue only after 20% glutaraldehyde fixation, but they were also seen in cancer cells after routine osmium fixation, as will be shown later.

C. CANCER MORPHOLOGY.

1. Histology.

The tissue used conformed to Auerbach's criteria for Class II, (Fig. 70) and Class III, (Fig. 66 and 69).[32] The classification was established from hematoxylin and eosin sections of large pieces of the gland. Although each specimen contained one predominant class of cells, more than one class existed in each case. Therefore, it was necessary to study the small pieces of plastic embedded material used for electron microscopy in order to show the histology representative of a given electron micrograph. Light micrographs accompany most of the electron micrographs for that purpose.

2. Ultrastructure of cancer cells.

Cancer cells differed from hyperplastic cells

in many respects. The most prominent difference was the two cell types seen in five of the six specimens examined. One type was electron opaque and was called a dark cell; the other was electron lucent and was called a light cell.

Dark cells were usually filled with mitochondria and with small vacuoles containing a moderately electron dense material (Fig. 38 and 39). The cytoplasm between the vacuoles and mitochondria was filled with ribosomes similar to the cytoplasm in dark cells of hyperplastic tissue fixed in 20% glutaraldehyde. The nucleus was very electron dense but otherwise similar to the basal cell nucleus in hyperplastic tissue (compare Fig. 20 with 40).

The cytoplasm of light cells contained fewer organelles than in the dark cells. There were vacuoles with electron lucent material, mitochondria similar to those in dark cells, and free ribosomes which were less concentrated than those of the dark cells. The nucleus was much more electron lucent than that in dark cells. It resembled the nucleus of secretory cells seen in glutaraldehyde fixed tissue, being large and oval with a smooth border. The chromatin was finely clumped and there was usually a prominent nucleolus (Fig. 17, 20 and 39).

The mitochondria in both cell types were similar. In

osmium fixed material, they were altered from normal mitochondria in two respects. Some were swollen and had short irregular cristae as shown in Fig. 39, while others contained a dense, fibrous material shown in Fig. 36.

Dark cells had two to three small dense bodies inconspicuously mixed with the mitochondria and vacuoles (Fig. 38). Light cells sometimes had the same kind of small dense bodies (Fig. 36 and 39), but they also were seen with many very small dense bodies at their cell peripheries. (Fig. 36 and 75).

An incidental finding was noted in a Class II cancer specimen: Several glands lined with dark epithelial cells were invaded with white blood cells (Fig. 83 and 84). The dark cells had advanced towards the stroma but were still surrounded by a basal lamina. (Fig. 85). White blood cells were mixed in with the dark cells forming the gland. The dark cells had two to three small lysosomes on each cell tip; the white blood cells had a few small lysosomes and occasionally very large ones (Fig. 86 and 87).

D. CYTOCHEMISTRY.

Acid phosphatase and thiolacetic acid esterase activity were localized in benign hyperplastic cells with the light microscope. Thiolacetic acid esterase activity was

studied in benign hyperplastic and cancer tissue with the electron microscope.

1. Light microscopy.

Acid phosphatase reaction product was localized in granules around the nucleus (Fig. 43 and 45). This corresponded to the areas designated as supranuclear and basal by Brandes.[22] Granules from the thiolacetic acid esterase reaction also appeared in the same basal and supranuclear areas (Fig. 44).

Incubation with the organophosphorous inhibitor E-600 seemed to reduce the number of positive esterase granules at the light microscope level (Fig. 46). But sections viewed with the electron microscope showed the same kind of activity with or without inhibition.

Control sections incubated without the thiolacetic acid or β-glycerol-phosphate substrates had a clear cytoplasm, free of dense granules (Fig. 47). Cloudy control sections were also free of reaction products, as shown in Fig. 89.

Epon embedded sections stained for light microscopy had small dark granules in the supranuclear and basal areas. They occurred both in tissue reacted for esterases or prepared just for morphology (Fig. 3 and 48).

2. Ultrastructure.

a. Localization in tall columnar cells.

Homogeneous and granular secretions usually contained reaction products but vesicular secretions were not encountered in the incubated material. It was therefore not possible to determine their reactivity.

All of the homogeneous secretions had activity. The granules around the nuclei (Fig. 51 and 53) were exceptionally dense. Individual reaction products were obscured by the denseness, but at high magnification a fine haze of reaction product was observed over the dense background. This was not found in control sections (compare Fig. 52 and 61). Control sections also contained secretions that were almost as dense as the reacted secretions (Fig. 60). The underlying density therefore was due to the nature of the secretory product but it was intensified by enzyme activity. The more electron lucent homogeneous secretions have a moderate amount of reaction product clearly visible (Fig. 56).

Granular secretions had activity similar to the homogeneous products. They were more concentrated towards the apical pole but also appeared around the nucleus with the dense secretions (Fig. 53).

When lipid droplets were present with the secretions, they had a narrow band of precipitate around their outer rim. (Fig. 53).

b. Localization in low columnar cells.

Enzyme activity was confined to several small dense granules in each cell (Fig. 59). They were located in the supranuclear region and in the apical pole. The apocrine secretions did not show activity.

c. Basal cells.

Basal cells had several small lysosomes with enzyme activity in glands with tall columnar and low columnar cells. Figure 50 shows a cluster of small lysosomes in two basal cells, but this was not typical of other basal cells. They were usually more randomly distributed throughout the cytoplasm. All dense bodies present had a slight amount of activity.

d. Cancer cells.

Enzyme activity in dark cells was located in one to two small dense bodies found in each cell (Fig. 75). In light cells, lysosomes had a size and activity similar to those in dark cells but were more abundant. The many small lysosomes at the cell tips in some cells lacked significant activity. (Fig. 75 and 76).

The stroma near cancer cells contained pockets of cytoplasm with large lysosomes in them (Fig. 67, 68, and 71). They were consistently positive for enzyme activity. The cytoplasm surrounding the lysosomes was electron lucent, similar to that

present in light cells. But the plasma membranes of the pockets rarely contained desmosomal attachments to nearby membranes characteristic of epithelial cells. The lymphocytes and macrophages in the area of carcinoma cells had moderate sized lysosomes occasionally as large as those in the cytoplasmic pockets. They always displayed vigorous enzyme activity (Fig. 73, 86 and 88).

e. Controls.

Tissue incubated without the thiolacetic acid substrate had ultrastructure similar to other glutaraldehyde-fixed tissue. There were occasional dense secretory products as shown in Fig. 60 and discussed under the section on the results of benign hyperplastic cytochemistry. All of the secretory products were always free of reaction deposits (Fig. 62, 63 and 64).

Cloudy control specimens were likewise always free of dense reaction products (Fig. 89).

f. Nonspecific reaction.

A fine scattering of lead sulphide granules was present in all tissues. It was found over the nucleus and in the cytoplasm unassociated with specific organelles. It was assumed to be an artefact not indicative of true enzymatic activity. The amount and size of the individual granules seemed to increase as the incubation time lengthened. In initial

experiments incubation times of fifteen minutes were used, but this was later cut to less than ten minutes and gave much cleaner results.

E. SUMMARY.

1. Fixation.

Five and ten percent glutaraldehyde followed by 1% Osmium-S-Collidine gave acceptable results. But the ideal fixative that would preserve enzyme reactivity and give excellent morphology was not discovered.

2. Size of lysosomes.

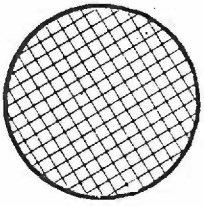
Terminology	Size magnified 10,000 x's	Approximate actual size in tissue.
Very small		0.1u
Small		0.4u
Moderate		0.8u
Large		3.0u

Table 1. Definition of the terms used to describe the lysosomes found in hyperplastic and cancerous tissue.

Cells	Location of lysosome activity.	Average number of lysosomes in each cell.	Size of lysosomes as defined in Table 1.	Intensity of reaction.
Benign Hyperplastic:				
Tall columnar	Granular and homogeneous secretory products	20	moderate	++ and +++
Low columnar	Throughout the cytoplasm	2-3	small	++
Basal	Throughout the cytoplasm	1-2	small	++
Cancer:				
Light	Cell tips and throughout the cytoplasm	10-20	very small	+
		2-3	small	++
Dark	Throughout the cytoplasm	1-2	small	++
Pockets of cytoplasm	Mixed in stroma and between cancer cells	2-4	moderate and large	++ and +++

Table 2. Summary of lysosome morphology and activity in benign hyperplastic and cancerous prostates.

IV. DISCUSSION.

A. FIXATIVES.

The use of glutaraldehyde concentrations of 10% and above seemed radical at first because the usual concentrations employed are from 1.5% to 6.5%.[118,124] The two published accounts of prostatic tissue fixed with glutaraldehyde used 5% and 6.25%. Miller and Palade, however, reported a trial of fixation with 18% glutaraldehyde but did not give pictures of their results.[84] The increased concentrations of glutaraldehyde probably serve two functions; i.e. to increase osmotic pressure and to increase fixative ability.

In light microscope work, it has been found that the osmolarity of the fixative has little effect upon the final morphology, apparently because the tissue is fixed before it has a chance to react to osmotic pressure differences.[132] Changes that do occur, such as shrinking or swelling are often too subtle to be recognized with the light microscope. But in electron microscope studies, fixatives give the best results if the osmolarity is kept isotonic with the tissue.[133] The tonicity of the specimen is usually assumed to be that of serum.[125] Human serum has an ionic concentration of

282-300 meq/liter[139] which is close to that calculated for prostatic secretions (245-305 meq/liter).[10] It could be predicted that fixatives with an osmolarity similar to that of serum would be satisfactory for preserving prostatic secretions.

No attempt was made to match the osmolarity of the fixative with the prostatic secretions because of the difficulty in accurately calculating the osmolarity of glutaraldehyde. This is probably due to impurities in the stock solution.[137] In this study, the concentration of the prefixative was empirically varied and 20% was found to conserve most of the secretory products. When the fixative concentration was low but the osmolarity was increased with sodium chloride, the fixation was very poor, indicating that the secretions were probably retained due to the fixative action of glutaraldehyde rather than to a correction of an osmotic imbalance.

The finding of decreased enzymatic activity in the material fixed with 20% glutaraldehyde, even though more secretions were present, agrees with the concept that strong fixatives compromise enzyme activity, probably by binding with the active site or covering it up with protein folds of fixed material.[132,135]

The empty secretory vacuoles in the material fixed with 3.5%, 5% and 10% glutaraldehyde probably occurred for two reasons: 1) the small amount of protein (2.46-2.64 gm/100ml) in the secretions was not exposed to enough fixative to form cross links to bind them in the cytoplasm; and 2) there are more lipids in benign hyperplastic tissue than in normal glands.[10] Aldehydes fix proteins by forming cross linkages between amino groups, but they are unable to form bridges with lipids; therefore, the lipids were probably not secured in the vacuoles and washed out during the wash or incubation period.[136, 137] For example, Osmium tetroxide is a very good fixative for lipids and tissue fixed first in Osmium had much better preservation of the secretory products, probably because the lipids were retained.[132]

Different buffering systems for Osmium tetroxide are known to affect the fine structure of cells.[138] Some of the influence of the buffer had been attributed to a "specific ion effect," whereby the chemistry of the tissue is changed slightly by the presence of a special ion. Such a change is registered as a different staining property at the light microscopic level. The post-fixatives in this study may have had subtle effects on cytoplasmic organelles, such as an increase in the microfilaments after fixation with Osmium buffered

with veronal acetate with uranyl acetate added. However, the significance of this finding is difficult to evaluate, since, in light of the known variability among prostate specimens it could have been due to a normal variation in the tissue. The different buffer systems did not increase the retention of the secretory products, probably because they did not enhance the cross-linking ability of the fixative.

The ideal fixative for retention of the secretory products would have a high degree of cross-linking ability to enmesh the lipid and protein secretions in the cytoplasm, and yet still not obscure the active enzyme sites. The secretions and their enzymes would then be intact. Even with an ideal fixative, the proper fixation for human surgical material might not be achieved. Tissue at surgery may be cut off from major blood supplies for one to two hours before it is received; it is manipulated and subjected to a general anesthesia before it is fixed. All of these factors may compromise fixation and are drawbacks associated with using human surgical material.

B. MORPHOLOGY.

1. Secretory cells.

The most striking result obtained in morphological preparations was the variety of cell types

found between specimens and even within the same specimen. The three cell types corresponded to those defined by Mao as low columnar, tall columnar and cuboidal.[68] He did not mention whether or not all three types were found in the same organ. In nine of the 21 specimens used in this study, the predominant cell type was low columnar; the rest had tall columnar cells. When cuboidal cells were found, they probably represented the 20% that were found by Moore in hyperplastic specimens at the light microscope level, and were not indicative of the cell type found in the entire specimen.[18]

Mao described apocrine and merocrine secretory activity in tall columnar cells, but found the other two types of cells to be without activity.[68] Apocrine secretions were found associated with the low columnar cells in this study, and merocrine type secretions were found in all three cell types.

The finding of apical blebs forming apocrine secretory bodies agrees with earlier light microscope studies of the secretory mechanism of prostate cells.[11] "Opalescent bodies" have been described in the prostatic fluid situated in the gland lumen which probably are the tips of the columnar cells that have been pinched off for secretion (Fig. 16).[11] This process has been described in the dog prostate and is probably the same mechanism that occurs in humans. In dogs, the apical pole separates from the

rest of the cell and "migrates" into the lumen where the "package" disintegrates and releases the prostatic secretions in amorphous form.[139]

Three types of merocrine secretory bodies were found in this study and described as dense homogeneous, granular and vesicular secretions. Vesicular secretions have been described in other studies but dense homogeneous and granular secretions were not reported.[22,67,68] If dense bodies were present in other studies, they were termed lysosomes and considered to be separate from the secretions. In this study, the dense bodies appeared separate from the secretions in Osmium fixed material of tall columnar cells (Fig. 22) and thus agreed with the other studies. But in low columnar cells or tall cells fixed with glutaraldehyde, the dense bodies were mixed in with the secretory products or were located at the cell periphery (Fig. 17) where it was difficult to make the distinction between secretions and lysosomes without further characterization of their enzymatic activity. Part of the differences between this study and others can be attributed to the use of glutaraldehyde fixation which was not used by other investigators. There is no way to tell which fixative represents the most accurate picture of prostatic secretions, but it seems likely that both contribute to the understanding of the secretory products manufactured by the prostate.

2. The relationship between basal cells and dark and light cells.

The relationship between the basal cells and the secretory cells has been the focus of speculation and debate of several investigators.[21,68] It was once believed that they represented myoepithelial cells similar to those found in breast tissue, and presumed that they served the same purpose, that is, to contract and expel the secretions.[21] But this concept was challenged because the same kinds of myofilaments were not present in the basal cells as in other myoepithelial cells, and because the abundant smooth muscle in the prostate adequately explained the expulsion of the secretions. In their study on the basal cell, Mao and Angrist showed a transition of maturation from the primitive basal cell morphology to that of the specialized secretory cell.[21] Their contention that the basal cell was the precursor cell agreed with the similar conclusion of Brandes.[39] However, there is often a sharp demarcation between secretory cells and basal cells, with no obvious transitional stages. This could be explained by postulating different ages for the glands; those glands with transitional cells may be younger than those with only mature and young cells--the young cells having stayed behind after mitosis, and their older sister cells having migrated to the lumen and become mature secreting cells.

The finding of dark and light cells in material fixed with 20% glutaraldehyde may provide a useful test to discriminate between young and old cells; the dark cells have cytoplasmic features characteristic of basal cells and therefore may be younger than the light cells. Occasional dark cells, similar to those seen in the present study with 20% glutaraldehyde fixation, were reported by Mao and Angrist in their morphological studies with Osmium fixation,[21] but have not been mentioned before by other investigators. The other possibility is that the light and dark cells represent two mature cell types. The prostate has been considered to function as a "dual" organ in response to estrogen stimulation.[23] It is possible that there are two predominant cell types, one in the inner and the other in the outer prostate and that these might be distinguished by certain fixatives, such as 20% glutaraldehyde. A study of the response of cells sampled from the inner and outer prostate to fixation in 20% glutaraldehyde would be one means of testing this hypothesis.

If the basal cells do function as the prostatic "stem" cells, then carcinoma cells would most likely originate from these cells. However, in the five existing studies on the morphology of cancer cells, there has been no evidence to suggest a close association between basal cells and cancer cells.[22,67,70,71,140] Two of the studies investigated less differentiated cancers and found the

cells quite pleomorphic, sometimes with dark and light cells side by side.[71,140] The dark cells resemble the basal cell architecture, with abundant cytoplasmic free ribosomes giving it the electron dense appearance. In this study, dark and light cells often appeared in marked contrast to one another as in Fig. 38 and 39. At other times, dark cells were alone as in Fig. 40. Cells that appeared to represent a transition between the dark and light cells also occurred with about the same frequency as the dark and light cells. (Fig. 35 and 36).

The significance of the two cell types in the cancer tissue is possibly related to the two cell types found in hyperplastic tissue. Although their origin is obscure, if cancer cells are derived from basal cells, it seems likely there might be two different types of basal cells to account for the two cancer cell types, but this has never been reported. The explanation offered by Mao and Angrist for the appearance of a dark secreting cell next to a light secreting cell is probably the most logical, that is, the dark cells represent younger cells, the light cells more mature cells--with both probably derived from the same stem cell.[21]

3. Cancer cells.

It is difficult to reconcile the reports that cancer cells from well-differentiated carcinoma have an

ultrastructure similar to normal cells.[22,67,70] This probably results from a comparison of the cancer cells with normal cuboidal or low columnar cells which appear similar to Grade I cancer cells at the light microscope level rather than with the tall secretory cells which they do not resemble at either the light or electron microscopic level.

Grade II and Grade III cancer cells were used in this study and tended to resemble cuboidal cells in size and nuclear-cytoplasmic ratio. The nucleus was large and filled most of the cytoplasm as in cuboidal cells. But here the similarity ended. Cancer cells had an active cytoplasm with evidence of some kind of secretory activity, but the secretions present were different than those seen in hyperplastic cells. There was a large amount of lipid in both cells, often not as prominent as in Fig. 38, but nevertheless, obviously more abundant than observed in hyperplastic cells. Mao, et.al. and Braunstein also noted the increased amounts of lipid present in cancer cells.[71,141] Mao suggested that it represented androgenic metabolytes or androgens themselves "acting directly on the prostatic cancer cells."[71] Braunstein suggested that the increased lipid represented deposits unable to reach an excretory duct for release.[141] However, because they are seen in acini that have open lumens, it seems unlikely that an inability to leave the cell fully explains the

increased amount of cytoplasmic lipid.

The vacuolated cytoplasm frequently seen in dark cancer cells may represent the "pathological vacuolation" referred to by Brandes.[22] In some cases, the vacuoles associated with Golgi complexes probably are Golgi vesicles (Fig. 42). In the dark cells, the vacuoles often are the size of mitochondria and contain small remnants of cristae around the edges--they are possibly formed by degenerating mitochondria (Fig. 39). Vacuolated cytoplasm similar to that seen in the dark cells of the present study was also reported by Mao who was also unsure of their origin.[71]

Secretory products and processes noted in hyperplastic cells were not found in cancer cells, although cancer cells are known to secrete many of the same enzymes found in hyperplastic cells.[42] The secretions might originate from dilated Golgi complexes, smooth endoplasmic reticulum, or unattached ribosomes. Although the secretions are proteins and classically should come from rough endoplasmic reticulum, very little rough endoplasmic reticulum is present. Nevertheless, in keeping with the evidence that proteins are synthesized on ribosomes, the secretions most likely were formed in the dark cells with the abundant ribosomes. The heterogenous reactivity of prostatic cancer cells for acid phosphatase has been well established.[42,47]

It is possible that the difference in intensity of staining represents a real difference in cell physiology which is reflected at the electron microscope level by the structural differences between dark cells with many ribosomes and light cells with few cytoplasmic organelles.

The relationships between stromal cells and cancer cells were similar to those predicted from light micrographs and similar to those found by Mao, et.al.[71] Acini usually retained a basal lamina but lacked the characteristic bundles of collagen and fibroblasts; this could have been due to a rapid proliferation of epithelium into the stroma without allowing time for the stroma to respond by building the normal elements around the cells, or it could have been due to a decrease in the collagen fibers and fibroblasts which allowed the glands to grow into the stroma. It is generally thought, however, that cancer cells possess the ability to invade the stroma, possibly due to the presence of proteolytic enzymes at their advancing edge,[71] and that instead, the initial change in carcinogenesis occurs in the epithelial cells rather than the stroma. That no changes were reported in stromal cells in the prostatic cancer studies is consistent with this last interpretation.[22,71,140]

C. CYTOCHEMICAL RESULTS.

1. Type of esterase localized and its possible functions.

It is most likely that the thiolacetic acid esterase activity located in the present study was a nonspecific type C esterase. Although acetylcholinesterase and lipases will also hydrolyze thiolacetic acid,[103] both are unlikely to occur in the prostate gland.[20] Acetylcholinesterase activity has been reported to be very weak in seminal fluid (which contains portions of epithelial cells) and therefore it appears unlikely that this enzyme was present in amounts sufficient to explain the esterase activity observed in the epithelium.[20] Lipases have been found by others to act on thiolacetic acid in pancreatic cells only after stimulation by taurocholate, a substance not known to be present in the prostate; therefore, it seems unlikely that the substrate was hydrolyzed by a lipase.[103]

Nonspecific esterase type A and type B can presumably hydrolyze thiolacetic acid and may have contributed to some of the enzyme activity observed. However, type A reacts most readily with a substrate containing an aromatic ring rather than with the short carbon chain of thiolacetic acid.[97] Type A is also inhibited by lead ions; the lead ions in the incubation medium would probably prevent type A from reacting.[95] Type B acts upon thiolacetic acid to give a diffuse cytoplasmic reaction at the light microscope level. This is inhibited by E-600.[103] After incubation with E-600, cytoplasmic granules

with thiolacetic acid activity remain intact; this has been considered characteristic of esterase type C and since it is associated with granules, it is considered to be a lysosomal enzyme by most authors.[102,103,118,126] In the present study, the granules were not inhibited by E-600 at 10^{-7} M concentration, supporting the conclusion that the thiolacetic acid positive granules contain esterase type C activity.

The role of cathepsin C in prostatic function is presently speculative. Cathepsins are thought to act as intracellular proteinases splitting peptide bonds;[142] however, they can stimulate the polymerization of amino acid chains and catalyze trans-amidation reactions besides acting as hydrolases.[142] DeDuve has explored the function of the class of hydrolytic enzymes and concluded that they probably function only as hydrolases but that they have roles other than in intracellular digestion.[143] For example, they may be important in the regulation of activating and deactivating systems as appears to be true for acetylcholinesterase at myoneural junctions. The hydrolytic enzymes in the lysosome of the anterior pituitary have been suggested to have a regulating role in the secretion of mammotrophic hormone from the pituitaries of nursing mice.[144] Their role in the mediation of androgenic stimulation on the cell has not been investigated and although there is evidence that androgens act upon rate-limiting enzyme systems to cause

biochemical or structural changes in responsive cells, it is most likely that the enzymes are not hydrolytic in nature.[145]

The purpose of hydrolytic enzymes in prostatic secretions has been an enigma for many years. Although acid phosphatase is found in abundance, its function is obscure. The only known substrate available for acid phosphatase in semen is phosphoryl choline.[146] It has been suggested that the choline released after hydrolysis serves as a methyl donor for sperm metabolism, but this is still unproven.[69] Similarily, the role of esterases is also unclear.

2. The differences in the esterase localized in hyperplastic and cancerous tissue.

In benign hyperplastic cells the reaction product lies in dense homogeneous and granular secretions. The dense homogeneous secretions correspond to the organelles called "dense bodies" (which is a descriptive term for lysosomes) in other studies and therefore they represent lysosomes. The granular secretions also fit the criteria established for defining lysosomes: they are membrane bound organelles containing hydrolytic enzymes. One possible conclusion is that the prostate secretes the contents of lysosomes into the glandular lumen. This concept of excretion of lysosomes has been invoked by Morgan to explain how ingested neutral red

particles escape from pancreatic cells.[147] De Duve has also discussed a cycle in the function of lysosomes which includes the secretion of lysosomal enzymes from bone fragments and cartilage cells into the extracellular environment where they act as hydrolytic agents.[3] Mao, et.al. suggested that the prostate is "unique in that its mature secretory granules contain acid phosphatase, 5-nucleotidase, and presumably other enzymes, and are discharged from the cell to constitute part of the prostatic secretion, possibly to function in the extracellular digestion of material in the genital tract."[69] The results of the present study suggest that one more enzyme be added to the previously described enzymes which are secreted by the prostate. Presumably this enzyme functions in an extracellular digestive capacity. The prostate, then appears to be a unique organ in that it may function to secrete lysosomal enzymes. The evidence from this study suggests the enzymes are secreted either as small droplets(Fig. 33) or as part of the cell apex (which eventually disintegrates,)[139] and therefore are not secreted as the whole lysosome with intact membrane.

The basal cells in this study have small lysosomes which sometimes have irregular contours and appear much smaller than the secretory products seen in the mature cells. The contents of these lysosomes probably represent non-secretory

hydrolytic enzymes which might function as classical intracellular digestive organelles.

The lysosomes in the cancer cells appear to be derived from two sources. The first is clearly from the cancer cells themselves, either as small lysosomes at the cell periphery with sparse reaction product in them, or as moderate sized lysosomes located throughout the cell containing more reaction product granules. The second source of lysosomes seen with cancer cells is unclear. The lysosomes appear in cytoplasmic pockets in the stroma, they are often very large, up to 3 microns in diameter, and contain abundant sprinklings of reaction product. The pockets rarely contain desmosomal attachments to nearby membranes, suggesting they are not of epithelial origin, and unlikely to be prostatic cancer cells. However, Mao, et.al. noted that anaplastic invasive prostatic cells almost altogether lack desmosomal attachments[71] and therefore this finding is probably not helpful in determining their origin. The presence of other organelles such as mitochondria and endoplasmic reticulum in the pockets of cytoplasm are also of little help in determining their cell of origin. For instance, the mitochondria associated with them are normal slim mitochondria which are usually not seen in cancer cells, but the endoplasmic reticulum and the nucleus is similar to that seen

in the electron lucent (light) cancer cells (Fig. 68, 73, and 80). Cancer cells around the cytoplasmic pockets have cell extensions characteristic of pseudopodia. The cell organelles are located around the middle of the cell and the extensions have an empty, electron lucent cytoplasm, occasionally containing small lysosomes. These characteristics agree in general with the description given by Mao of invasive tumor cells seen in his study,[71] but large lysosomes were not noted in his study.

The large lysosomes are similar to the granules shown by Bernhard and Leplus in macrophages of the normal lymph node.[148] These authors believed that the granules were ingested pieces of erythrocytes because they had the same electron density as hemoglobin. The large lysosomes also have a superficial resemblance to the early granules seen in the eosinophil,[149] but this is an unlikely source of the great number of them seen in the stroma and around malignant cells.

Mao found no characteristics which would distinguish the membranes of invasive cells from non-invasive ones.[71] He found no suggestion of dense bodies contributing to the invasiveness of cancer cells. Similar findings were reported by Brandes, et. al. in a study of leukemic cells invading muscle.[150] The invading cells did not evidence lysosomes or

acid phosphatase activity, but the normal macrophages which were present with the invading cells did have strong acid phosphatase activity. Pearse and Hess also investigated the possibility that tissue cathepsins and other hydrolytic enzymes were instrumental in causing tumor cell advances into normal tissue but found high acid phosphatase activity only in stromal macrophages.[151] It has also been found that lysosomes lack the ability to break down collagen, a property that seems vital for invading stromal cells.[152]

It is possible that because the prostate has a large amount of acid phosphatase in the normal cells, it would be present in the cancer cells and possibly contribute to their invasiveness; but the fact that most prostatic cancers are slower growing than many adenocarcinomas without acid phosphatase activity, suggests that the invasiveness of cancer cells is little influenced by the presence or absence of acid phosphatase. The source of the large lysosomes still remains in contention; they may be associated with invasive cancer cells or they may belong to stromal macrophages as was reported in other cancer studies.[150,151]

The finding of several glands surrounded and invaded by white blood cells, primarily small and large lymphocytes, may represent an immune response to the cancer cells and suggests that prostatic carcinoma may be an antigenic tumor. Responses

similar to graft rejections, with lymphocyte and plasma cell infiltrates, have been reported for other antigenic tumors; the immune reaction at the edge of other tumors apparently reduces their invasiveness and subdues their growth rate.[153] The immune response to prostatic cancer may be one of the factors contributing to its well recognized slow rate of growth.[24]

IV. SUMMARY AND CONCLUSIONS.

Tissue from hyperplastic and Grade II and III human prostatic cancers were prepared for study of lysosomal activity at the ultrastructural level. A nonspecific esterase reaction with thiolacetic acid as the substrate was used for the enzyme localization procedure. The reaction was carried out at pH 6.0 in order to assure the optimal reactivity of the lysosomal acid hydrolases.

Several different fixatives were used in order to achieve good morphological preparations and maintain adequate enzymatic activity. Five and ten percent glutaraldehyde followed by postfixation with 1% osmium-S-Collidine gave the best results.

Fixation with twenty percent glutaraldehyde resulted in glands with two distinctive cell types, called dark and light cells. The dark and light cells were also seen in cancer tissue after routine fixations. The origin and significance of the two cell types was discussed.

Enzyme activity in hyperplastic tissue was found in the secretory products of the tall secretory cells; small lysosomes in basal cells also contained activity. It was

suggested that the secretory cells in the prostate contribute lysosomal enzymes to the prostatic secretions, the esterase located with thiolacetic acid being one of the enzymes.

In cancer cells, the enzyme activity was located in very small lysosomes 0.1 micron in diameter and in large lysosomes up to 3.0 microns in diameter. The large lysosomes were found in cytoplasmic pockets, often in the stroma. The cell type associated with the large lysosomes was discussed; they were thought to arise from either invading cancer cells or macrophages.

In conclusion, it is proposed that there are differences in sites of localization and in sizes of lysosomes between cells of cancerous and hyperplastic prostate. These differences may be due to changes in the function of the cells. The lysosomes of well differentiated secretory cells in the hyperplastic tissue may principally function in external secretions, whereas the lysosomes of the cancer cells probably function primarily in intracellular digestive processes. The large lysosomes seen in the prostatic cancer tissue and not in the hyperplastic tissue, are probably associated with tissue macrophages and related to the immune response to the prostatic tumor.

Bibliography

1. De Duve, C., Pressman, B.C., Gianetto, R., Wattiaux, R., and Appelmans, F.: Tissue fractionation studies. Intracellular distribution patterns of enzymes in rat-liver tissue. Biochem. J. 60:604-617, 1955.

2. De Reuck, A.V.S., and Cameron, M.P.: (Ed.) Lysosomes. Ciba Foundation Symposium. Boston, Mass., Little, Brown, and Co., 427 pages, 1963.

3. De Duve, C., and Wattiaux, R.: Functions of lysosomes. In: V. E. Hall (Ed.) Annual Review of Physiology. Palo Alto, Calif., Annual Reviews, Inc. pp.435-492, 1966.

4. Weissman, G.: Lysosomes. New Eng. J. Med. 273:1084-1089, 1143-1149, 1965.

5. Allison, A.C., and Mallucci, L.: Uptake of hydrocarbon carcinogens by lysosomes. Nature (London) 203:1024-1027, 1964.

6. Allison, A.C., and Mallucci, L.: Lysosomes in dividing cells, with special reference to lymphocytes. Lancet ii, 1371-1373, 1964.

7. Allison, A.C., and Paton, G.R.: Chromosome damage in human diploid cells following activation of lysosomal enzymes. Nature (London) 207:1170-1173, 1965.

8. Allison, A.: Lysosomes and disease. Sci. Amer. 217:62-72, 1967.

9. Allison, A.: Lysosomes in relation to cancer inducation and treatment. European J. Cancer 3:481-490, 1968.

10. Huggins, C.: The physiology of the prostate gland. Physiol. Rev. 25:281-295, 1945.

11. Mann, T.: The Biochemistry of Semen. New York: Wiley, 493 p., 1964.

12. Le Duc, I.E.: The anatomy of the prostate and the pathology of early benign hypertrophy. J. Urol. 42:1217-1241, 1939.

13. Adrion, W.: Ein Beitragzur Atiologie der Prostatahypertrophie., Beitr. Z. Path. Anat. u. Z. Allg. Path. 70 (suppl. 9) 179-202, 1922. Cited by Grant, J.C.B.: A Method of Anatomy. Baltimore: The Williams & Wilkins Co., 350-351, 1958.

14. Grant, J.C.B.: A Method of Anatomy. Baltimore: The Williams & Wilkins Co., 350-351, 1958.

15. Lowsley, O.S.: Embryology, anatomy, and surgery of the prostate gland. Am. J. Surg. 8:526-541, 1930.

16. Franks, L.M.: Benign nodular hyperplasia of the prostate: A review. Ann. Roy. Coll. Surg. 14:92-106, 1954.

17. Edwards, C.N., Steinthorsson, E., and Nicholson, D.: An autopsy study of latent prostatic cancer. Cancer 6:531-554, 1953.

18. Moore, R.A.: Benign hypertrophy of the prostate. A Morphological Study. J. Urol. 50:680-710, 1943.

19. Andrews, G.S.: The histology of the human foetal and prepubertal prostates. J. Anat. 85:44-54, 1951.

20. Price, D., and Williams-Ashman, H.G.: The accessory reproductive glands of mammals. In: Young, W.C. (Ed.) Sex and Internal Secretions. Baltimore, The Williams & Wilkins Co., pp.366-448, 1961.

21. Mao, P., and Angrist, A.: The fine structure of the basal cell of human prostate. Lab. Invest. 15:1768-1782, 1966.

22. Brandes, D., Kirchheim, D., and Scott, W.W.: Ultrastructure of the human prostate: Normal and neoplastic. Lab. Invest. 13:1541-1560, 1964.

23. Huggins, C., and Webster, W.O.: Duality of human prostate in response to estrogen. J. Urol. 59:258-266, 1948.

24. Fallis, B.D.: Textbook of Pathology. New York, McGraw-Hill Book Co., 515-521, 1964.

25. Moore, R.A., Miller, M.L., and McLellan, A.: Chemical composition of prostatic secretion in relation to benign hypertrophy of prostate. J. Urol. 46:132-137, 1941.

26. Huggins, C.: The etiology of benign prostatic hypertrophy. Bull. N.Y. Acad. Med. 23:696-704, 1947.

27. Reischauer, F., Virchows Archiv. Path. Anat. 256:357. Cited by Franks, L.M.: Benign nodular hyperplasia of the prostate: A Review. Ann. Roy. Coll. Surg. 14:95, 1954.

28. Vidgoff, B.: The hormonal control of the prostate and its relation to clinical prostatic hypertrophy. J. Urol. 42:359-371, 1939.

29. Pincus, G, Romanoff, L.P., and Carlo, J.: The excretion of urinary steroids by men and women of various ages. J. Gerontol. 9:113-132, 1954.

30. Moore, R.A.: The morphology of small prostatic carcinoma. J. Urol. 33:224-234, 1935.

31. Rich, A.R.: On the frequency of occurrence of occult carcinoma of the prostate. J. Urol. 33:215-223, 1935.

32. Shelley, H.S., Auerbach, S.H., Classen, K.L, Marks, C.H., and Wiederanders, R.E.: Carcinoma of the prostate: A new system of classification. Arch. Surg. 77:751-756, 1958.

33. Huggins, C., Stevens, R.E., and Hodges, C.V.: Studies on prostatic cancer. II. The effect of castration on clinical patients with carcinoma of the prostat e. Arch. Surg. 43: 209-223, 1941.

34. Gutman, A.B., and Gutman, E.B.: An "acid" phosphatase occurring in the serum of patients with metastasizing carcinoma of the prostate gland. J. Clin. Invest. 17:473-478, 1938.

35. Huggins, C., and Hodges, C.V.: Studies on prostatic cancer. I. The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. Cancer Res. 1:293-297, 1941.

36. Nesbit, R.M., and Baum, W.C.: Endocrine therapy of prostatic carcinoma; clinical and statistical survey of 1818 cases. JAMA 143:1317-1320, 1950.

37. McNeal, J.E.: Morphogenesis of prostatic carcinoma. Cancer 18:1659-1666, 1965.

38. Birke, G., Franksson, C., and Plantin, L.O.: On the excretion of androgens in carcinoma of the prostate. Acta Endocrin. Suppl. 17:3-35, 1954.

39. Brandes, D.: The fine structure and histochemistry of prostatic glands in relation to sex hormones. Int. Rev. Cyt. 20:207-276, 1966.

40. Neimi, M., Harkonen, M., Larmi, T.K.I.: Enzymic histochemistry of human prostate. Localization of oxidative enzymes, esterases and aminopeptidases in the normal and hyperplastic human prostate. Arch. Path. 75:528-537, 1963.

41. Kirchheim, D., and Scott, W.W.: The effects of castration and sex hormones upon aminopeptidases and phosphatases of the rat prostate. Invest. Urol. 2:393-404, 1965.

42. Kirchheim, D., Gyorkey, F., Brandes, D., and Scott, W.W.: Histochemistry of the normal, hyperplastic, and neoplastic human prostate gland. Invest. Urol. 1:403-421, 1964.

43. Kirchheim, D., Niles, N.R., Frankus, E., and Hodges, C.V.: Correlative histochemical and histological studies on thirty radical prostatectomy specimens. Cancer 19:1683-1696, 1966.

44. Kirscher, W., and Wolbergs, H.: Prostatophosphatase. Ztschr. Physiol. Chem. 236:237-240, 1935. Cited by Gutman, A.B., and Gutman, E.B., An "acid" phosphatase occurring in the serum of patients with metastasizing carcinoma of the prostate gland. J. Clin. Invest. 17:473, 1938.

45. Gyorkey, F.: The appearance of acid phosphatase in human prostate gland. Lab. Invest. 13:105-111, 1964.

46. Gutman, A.B., and Gutman, E.B.: "Acid" phosphatase and functional activity of the prostate (man) and preputial glands (rat). Proc. Soc. Exper. Biol. & Med. 39:529-532, 1938.

47. Goetsch, J.B.: A clinical and histochemical study of acid and alkaline phosphatase in normal and abnormal prostatic tissue. J. Urol. 84:636-642, 1960.

48. Brandes, D., and Bourne, G.H.: Histochemistry of the human prostate, normal and neoplastic. J. Pathol. Bacteriol. 71:33-36, 1956.

49. Parkin, L., Bylsma, G., Torre, A.V., Drew, D., and Madden, R.J.: Acid phosphatase in carcinoma of the prostate in man. J. Histochem. Cytochem. 12:288-292, 1964.

50. Harkin, J.C.: An electron microscope study of the castration changes in the rat prostate. Endocrinology 60: 185-198, 1957.

51. Brandes, D., Gyorkey, F., and Groth, D.P.: Occurrance of lysosomes in the prostatic epithelium of castrate rats. Exper. Cell Res. 28:61-68, 1962.

52. Swift, H., and Hruban, Z.: Focal degradation as a biological process. Fed. Proc. 23:1026-1037, 1964.

53. Arcadi, J.A.: Hormonally induced changes in the glycoprotein component of the Golgi apparatus in the prostate of the rat. J. Endocrinology 10:311-315, 1954.

54. Groth, D.P., and Brandes, D.: Correlative electron microscope and biochemical studies on the effect of estradiol on the rat ventral prostate. J. Ultrastruct. Res. 4:166-181, 1960.

55. Huggins, C., and Moulton, S.H.: Esterases of testis and other tissues. J. Exp. Med. 88:169-179, 1948.

56. Nachlas, M.M., and Seligman, A.M.: The histochemical demonstration of esterase. J. Nat. Cancer Inst. 9:415-425, 1949.

57. Seaman, A., and Winell, M.: A histochemical study of the esterases of the prostate gland of the dog. Acta Histochem. 8:381-392, 1959.

58. De Duve, C.: Lysosome concent. In: De Reuck, A.V.S., and Cameron, M.P. (Ed.) Lysosomes. Ciba Foundation Symposium. Boston, Mass., Little, Brown, and Co., 1-31, 1963.

59. Holt, S.J., and Withers, R.F.J.: Cytochemical localization of esterases using indoxyl derivatives. Nature (London) 170: 1012-1014, 1952.

60. Shnitka, T.K., and Seligman, A.M.: Role of esteratic inhibition on localization of esterase and the simultaneous cytochemical demonstration of inhibitor sensitive and resistant enzyme species. J. Histochem. Cytochem. 9:504-527, 1961.

61. Pearse, A.G.E.: Histochemistry. Ed. 2, p. 886. Boston; Little, Brown, and Co., 1961.

62. Harkin, J.C.: Ultrastructural alterations with age in prostatic epithelial cells of the rat. Lab. Invest. 10:696-706, 1961.

63. Brandes, D.: Histochemical and ultrastructural observations on prostatic epithelium of older rats. Lab. Invest. 12:290-305, 1963.

64. Brandes, D., and Groth, D.P.: The fine structure of the rat prostatic complex. Exper. Cell. Res. 23:159-175, 1961.

65. Brandes, D., and Portela, A.: The fine structure of the epithelial cells of the mouse prostate. II. Ventral lobe epithelium. J. Biophys. Biochem. Cytol. 7:511-514, 1960.

66. Seaman, A., and Winell, M.: An electron microscope study of the dog prostate gland. Anat. Record 139:272-273, 1961. (Abstract).

67. Fisher, E.R., and Jeffrey, W.: Ultrastructure of human normal and neoplastic prostate. With comments relative to prostatic effects of hormonal stimulation in the rabbit. Am. J. Clin. Path. 44:119-134, 1965.

68. Mao, P., Nakao, K., Bora, R., and Geller, J.: Human benign prostatic hyperplasia. Arch. Path. 79:270-283, 1965.

69. Mao, P., Nakao, K., and Angrist, A.: Acid phosphatase and 5-nucleotidase activities of human nodular prostatic hyperplasia as revealed by electron microscopy. Lab. Invest. 15:422-434, 1966.

70. Takayasu, H., and Yamaguchi, Y.: An electron microscopic study of the prostatic cancer cell. J. Urol. 87:935-940, 1962.

71. Mao, P., Nakao, K., and Angrist, A.: Human prostatic carcinoma: An electron microscope study. Cancer Res. 26: 955-973, 1966.

72. Sawant, P.L., Desai, I.D., and Tappel, A.L.: Factors affecting the lysosomal membrane and availability of enzymes. Arch. Biochem. 105:247-253, 1964.

73. Tappel, A.L., Sawant, P.L., and Shibko, S.: Lysosomes: Distribution in animals, hydrolytic capacity and other properties. In: de Reuck, A.V.S., and Cameron, M.P. (Ed.) Lysosomes. Ciba Foundation Symposium. Boston, Mass.; Little, Brown, and Co., 78-106, 1963.

74. Novikoff, A.B., Essner, E., and Quintana, N.: Golgi apparatus and lysosomes. Fed. Proc. 23:1010-1022, 1964.

75. Brandes, D.: Observation on the apparent mode of formation of "pure" lysosomes. J. Ultrastruct. Res. 12:63-80, 1965.

76. Bartolini, B., and Hassan, G.: Acid phosphatase associated with the Golgi apparatus in human liver cells. J. Cell. Biol. 32:216-218, 1967.

77. Cohn, Z.A., and Hirsch, J.G.: The isolation and properties of the specific cytoplasmic granules of rabbit polymorphonuclear leukocytes. J. Exp. Med. 112:983-1004, 1960.

78. Hirsch, J.G., and Cohn, Z.A.: Digestive and autolytic functions of lysosomes in phagocytic cells. Fed. Proc. 23:1023-1025, 1964.

79. Bainton, D.F., and Farquhar, M.G.: Origins of granules in polymorphonuclear leukocytes. J. Cell. Biol. 28:277-301, 1966.

80. Zucker-Franklin, D.: Electron microscope studies on the degranulation of rabbit peritoneal leukocytes during phagocytosis. J. Exp. Med. 120:569-576, 1964.

81. Essner, E., and Novikoff, A.B.: Localization of acid phosphatase activity in hepatic lysosomes by means of electron microscopy. J. Biophys. Biochem. Cytol. 9:773-784, 1961.

82. Holt, S.J., and Hicks, M., The localization of acid phosphatase in rat liver cells as revealed by combined cytochemical staining and electron microscopy. J. Biophys. Biochem. Cytol. 11:47-66, 1961.

83. Daems, W.T., van der Ploeg, M., Persijn, J.P., and van Duijn, P.: Demonstration with the electron microscope of injected peroxidase in rat liver cells. Histochemie 3: 561-564, 1964.

84. Miller, F., and Palade, G.E.: Lytic activities in renal protein absorption droplets. An electron microscopical cytochemical study. J. Cell Biol. 23:519-552, 1964.

85. Novikoff, A.B., and Essner, E.: Cytolysosomes and mitochondrial degeneration. J. Cell Biol. 15:140-146, 1962.

86. Janoff, A., Wessman, G., Zweifach, B., and Thomas, L.: Pathogenesis of experimental shock. IV. Studies of lysosomes in normal and tolerant animals subjected to lethal trauma and endotoxemia. J. Exp. Med. 116:451-466, 1962.

87. Bitensky, L., Chayer, J., Cunningham, G.J., and Fine, J.: Behaviour of lysosomes in haemorrhagic shock. Nature (London) 199:493-494, 1963

88. Bitensky, L.: The reversible activation of lysosomes in normal cells and the effects of pathological conditions. In: de Reuck, A.V.S., and Cameron, M.P. (Ed.) Lysosomes. Ciba Foundation Symposium. Boston, Mass.; Little, Brown, and Co., 362-383, 1963.

89. Weissman, G.: Lysosomes, autoimmune phenomena, and diseases of connective tissue. Lancet ii, 1373-1375, 1964.

90. Weissman, G., and Thomas, L.: Steroids, lysosomes, and systemic lupus erythematosus. Bull. N.Y. Acad. Med. 38:779-787, 1962.

91. Muehrcke, R.C., and Rosen, S.: Hypokalemic nephropathy in rat and man: Light and electron microscopic study. Lab. Invest. 13:1359-1373, 1964.

92. Morrison, A.B., Panner, B., and Gasic, G.: Lysosomes in renal papillae of rats: formation induced by potassium-deficient diet. Science 142:1066-1067, 1963.

93. Dent, P.B., Fish, L.A., White, J.G., and Good, R.A.: Chediak-Higashi syndrome. Observations on the nature of the associated malignancy. Lab. Invest. 15:1634-1642, 1966.

94. Baudhuin, P., Hers, H.G., and Loeb, H.: Electron microscopic and biochemical study of type II Glycogenosis. Lab. Invest. 13:1139-1152, 1964.

95. Pearse, A.G.E.: Histochemistry. Ed. 2, p. 456-490; Little, Brown, and Co., Boston, 1961.

96. Deane, H.W., Barrnett, R.J., and Seligman, A.M., 1960. Enzymes (Part One); Histochemical methods for the demonstration of enzymatic activity. In: Handbuch der Histochemie, Graumann, W., and Neumann, K., Eds. Vol. VII, pp. 67-93, Gustav Fischer, Stuttgardt.

97. Bell, M.: Enzyme activity related to fine structure: Histochemical and electron microscope observations on enzymes that catalyze the hydrolysis of thiol-substituted carboxylic acids. Unpublished doctoral dissertation. Yale University School of Medicine, 1965.

98. Aldridge, W.N., 1951. The enzymatic hydrolysis of diethyl-p-nitrophenyl phosphate (E-600). Biochem. J. 49: i (Proc. Biochem. Soc.).

99. Aldridge, W.N., 1953. Serum esterases. II. An enzyme hydrolyzing diethyl-p-nitrophenyl phosphate (E-600) and its identity with the A-esterase of mammalian sera. Biochem. J. 53:117-124.

100. Aldridge, W.N., 1954. Some esterases of the rat. Biochem. J. 57:692-702.

101. Aldridge, W.N., 1953. Serum esterases. I. Two types of esterase (A and B) hydrolyzing p-nitrophenyl acetate, proprionate, and butyrate, and a method for their determination. Biochem. J. 53:110-117.

102. Hess, R., and Pearse, A.G.E.: The histochemistry of indoxylesterase of rat kidney with special reference to its cathepsin-like activity. Brit. J. Exp. Path. 39:292-299, 1958.

103. Wachstein, M., Meisel, E., and Falcon, C.: Histochemistry of thiolacetic acid esterase: A comparison with non-specific esterase with special regard to the effect of fixatives and inhibitors on intracellular localization. J. Histochem. Cytochem. 9:325-339, 1961.

104. Barrnett, R.J., and Tice, L.W.: The use of the electron microscope as a tool for the investigation of problems in cytochemistry. First Int. Congress of Histochem. and Cytochem. Pergamon Press, 139-180, 1963.

105. Hanker, J.S., Katzoff, L., Rosen, H.R., Seligman, M.U., Ueno, H., Seligman, A.M.: Design and synthesis of thiol-esters for the histochemical demonstration of esterase and lipase via the formation of osmiophilic diazo thioethers. J. Med. Chem. 9:288-291, 1966.

106. Wilson, I.B.; Mechanism of hydrolysis. II. New evidence for an acylated enzyme as intermediate. Biochem. et Biophys. Acta, 7:520-525, 1951.

107. Crevier, M., and Belanger, L.F.: Simple method for histochemical detection of esterase activity. Science 122:556, 1955.

108. Barrnett, R.J.: The fine structural localization of acetylcholinesterase at the myoneural junction. J. Cell. Biol. 12:247-262,

109. Barron, K.D., Bernsohn, J., and Hess, A.R.: Esterases and proteins of normal and atrophic feline muscle. J. Histochem. and Cytochem. 14:1-24,

110. Omachi, A., Barnum, C.P., and Glick, D.: Quantitative distribution of an esterase among cytoplasmic components of mouse liver cells. Proc. Soc. Exp. Biol. (N.Y.) 67:133-137, 1948.

111. Holt, S.J.: Some observations on the occurrence and nature of esterases in lysosomes. In: de Reuck, A.V.S., and Cameron, M.P., Lysosomes. Ciba Foundation Symposium. Boston, Mass.; Little, Brown, and Co. 114-125, 1963.

112. Shnitka, T.K., and Seligman, A.M.: Role of esteratic inhibition on localization of esterases and the simultaneous cytochemical demonstration of inhibitor sensitive and resistant enzyme species. J. Histochem. & Cytochem. 9:504-527, 1961.

113. Ericsson, J.L., and Trump, B.F.: Observations on the application to electron microscopy of the lead phosphatase technique for the demonstration of acid phosphatase. Histochemie 4:470-487, 1965.

114. Goldfischer, S., Essner, E., and Novikoff, A.B.: The localization of phosphatase activities at the level of ultrastructure. J. Histochem. & Cytochem. 12:72-95, 1964.

115. Wollman, S.H., Spicer, S.S., and Burstone, M.S.: Localization of esterase and acid phosphatase in granules and colloid droplets in rat thyroid epithelium. J. Cell Biol. 21:191-201, 1964.

116. Frost, J.L., and Brandes, D.: Nonspecific esterases in rat prostatic epithelial cells. J. Histochem. & Cytochem. 15:589-595, 1967.

117. Wachstein, M., and Meisel, E.: Histochemistry of "thiolacetic acid esterase" in relation to De Duve's lysosomes. J. Histochem. & Cytochem. 8:317-318, 1960 (Abstract).

118. Sabatini, D.D., Bensch, K.G., and Barrnett, R.J.: Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. J. Cell Biol. 17:19-58, 1963.

119. Smith, R.E., and Farquhar, M.G.: Preparation of thick sections for cytochemistry and electron microscopy by a non-freezing technique. Nature (London) 200:691, 1963.

120. Smith, R.E., and Farquhar, M.G.: Preparation of nonfrozen sections for electron microscope cytochemistry. Scient. Instr. News (RCA) 10:13, 1965.

121. Kay, D.H. (Ed.): Techniques for electron microscopy. 2nd Edition. Philadelphia; F.A. Davis Co. p. 181, 1965.

122. Gomori, G. : Microscopic Histochemistry. Chicago: University of Chicago Press, 1952.

123. Bennett, H.S., and Luft, J.H.: S-Collidine as a basis for buffering fixatives. J. Biophys. Biochem. Cytol. 6:113-114, 1959.

124. Kay, D.H. (Ed.): Techniques for electron microscopy. 2nd Edition. Philadelphia: F. A. Davis Co., 1965.

125. Pease, D.C.: Histological techniques for electron microscopy. New York:Academic Press. 274 p., 1960.

126. Bell, M., and Barrnett, R.J.: The use of thiol-substituted carboxylic acids as histochemical substrates. J. Histochem. & Cytochem. 13:611-628, 1965.

127. Barka, T., and Anderson, P.: Histochemical methods for acid phosphatase using hexazonium pararosanilin as coupler. J. Histochem. & Cytochem. 10:741-753, 1962.

128. Luft, J.H.: Improvements in epoxy resin embedding methods. J. Biophys. & Biochem. Cytol. 9:409-414, 1961.

129. Reynolds, E.S.: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17:208-213, 1963.

130. Richardson, K.C., Jarrett, L., and Finke, E.: Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Techn. 35:313-323, 1960.

131. Fawcett, D.: An Atlas of Fine Structure. The cell. Philadelphia: W. B. Saunders, Co., 1966.

132. Baker, J.R.: Principles of biological microtechnique; A study of fixation and dyeing. New York: Wiley. 357 p., 1958.

133. Maunsbach, A.B., Madden, S.C., and Latta, H.: Variations in fine structure of renal tubular epithelium under different conditions of fixation. J. Ultrastruct. Res. 6:511-530, 1962.

134. West, E.S., Todd, W.R., Mason, H.S., and Van Bruggen, J.T.: Textbook of Biochemistry, 4th Edition, MacMillan Co., New York.

135. Janigan, D.T.: The effects of aldehyde fixation on acid phosphatase activity in tissue blocks. J. Histochem. & Cytochem. 13:476-483, 1965.

136. Trump, B.F., and Bulger, R.E.: New ultrastructural characteristics of cells fixed in a glutaraldehyde-osmium tetroxide mixture. Lab. Invest. 15:368-379, 1966.

137. Anderson, P.J.: Purification and quantitation of glutaraldehyde and its effect on several enzyme activities in skeletal muscle. J. Histochem. & Cytochem. 15:652-661, 1967.

138. Wood, R.L., and Luft, J.H.: The influence of buffer systems on fixation with osmium tetroxide. J. Ultrastruct. Res. 12: 22-45, 1965.

139. Maser, M.: Fine structure of prostatic secretion in the dog. J. Cell. Biol. 39:85a, 1968 (Abstract).

140. Kirchheim, D., Bacon, R.L.: Elektronenmikroskopische Untersuchungen der normalen Prostata, der Prostatahypertrophie und des Prostatacarcinoms. Sonderdruck Aus, Der Urologe. Jahrgang, Heft 5. 5:292-305, 1968.

141. Braunstein, H.: Staining lipid in carcinoma of the prostate gland. Am. J. Clin. Path. 41:44-48, 1964.

142. Fruton, J.S.: Cathepsins. In: Boyer, P.D., Lardy, H., and Myrback, K., (Eds.) The Enzymes. 2nd Edition. Academic Press, Inc., New York, Vol. IV, pp. 223-241.

143. De Duve, C.: The function of intracellular hydrolases. Exp. Cell Res. Suppl. 7:169-182, 1959.

144. Smith, R.E., and Farquhar, M.G.: Lysosome function in the regulation of the secretory process in cells of the anterior pituitary gland. J. Cell Biol. 31:319-347, 1966.

145. Williams-Ashman, A.G., Shutsung, L., Hancock, R.L., Jurkowitz, L., and Silverman, D.A.: Testicular hormones and the synthesis of ribonucleic acids and proteins in the prostate gland. Recent. Progr. Hormone Res. 20:247-301, 1964.

146. Lundquist, F.: Function of prostatic phosphatase. Nature (London) 158:710, 1946.

147. Morgan, W.S.: Ductal excretion of neutral red lysosomes in the mouse pancreas. J. Cell Biol. 36:261-265, 1968.

148. Bernhard, W., and Leplus, R.: Fine Structure of the Normal and Malignant Human Lymph Node. New York: MacMillan Co., 1964.

149. De Harven, E.:
Human leukemic cells in tissue culture: An electron microscope survey. Cancer Res. 27:2447-2467, 1967.

150. Brandes, D., Anton, E., and Schofield, B.: Invasion of skeletal and smooth muscle by L1210 leukemia. Cancer Res. 27:2159-2178, 1967.

151. Pearse, A.G.E., Hess, R., and Snyder, R.: Stromal and tumor cathepsins and their relationships to metabolic activity and invasiveness. In: M. J. Brennan and W.L. Simpson (Eds.), Biological Interactions in Normal and Neoplastic Growth. Henry Ford Hospital International Symposium, pp. 657-670. Boston: Little, Brown, and Co., 1962.

152. Uriuhara, T., and Movat, H.Z.: The role of polymorphonuclear leukocyte lysosomes in tissue injury, inflammation and hypersensitivity. IV. The inability of leukocyte lysosomes to alter native collagen fibrils. Am. J. Path. 50:245-257, 1967.

153. Smith, R.T.: Tumor-specific immune mechanisms. New Eng. J. Med. 278:1207-1214, 1268-1274, 1326-1331; 1968.

ABBREVIATIONS USED IN FIGURES 1-89.

A.	
APICAL REGION	AR
APICAL SECRETORY BODY	ASB
B.	
BASAL CELL	BC
BASAL LAMINA	bl
BASAL REGION	BR
BASEMENT MEMBRANE	Bm
BLOOD VESSEL	Bv
C.	
CANCER CELL	CC
CELLULAR INTERDIGITATIONS	ci
CENTRIOLE	C
CHROMATIN	ch
COLLAGEN	Co
CYTOPLASMIC FILAMENTS	cf
CYTOPLASMIC POCKETS	CP
D.	
DARK CELL	DC
DENSE BODIES	db
DESMOSOMES	d

E.		
	ENDOPLASMIC RETICULUM	er
	ENDOTHELIAL CELL	END
F.		
	FIBROBLASTS	FB
G.		
	GOLGI COMPLEX	GC
	GRANULAR ENDOPLASMIC RETICULUM	GER
I.		
	INTERCELLULAR LACUNAE	il
J.		
	JUNCTIONAL COMPLEX	JC
L.		
	LARGE LYSOSOME	LLy
	LIGHT CELL	LC
	LIPID	L
	LYSOSOME	Ly
M.		
	MICROVILLI	mv
	MITOCHONDRIA	m
	MULTIVESCICULAR BODY	mb
	MYELIN FIGURES	mf

N.

NUCLEAR MEMBRANE	nm
NUCLEOLUS	nu
NUCLEUS	n

P.

PLASMA MEMBRANE	PM

R.

REACTION PRODUCT	rp
RED BLOOD CELL	rbc
RIBOSOMES	r
ROUGH ENDOPLASMIC RETICULUM	RER

S.

SECRETORY CELL	SC
SECRETORY GRANULES	
HOMOGENEOUS	Sg_1
GRANULAR	Sg_2
VESICULAR	Sg_3
SECRETORY PRODUCTS	SP
SECRETORY VACUOLES	SV
SMOOTH MUSCLE	SM
STROMA	S
SUPRANUCLEAR REGION	SR

T.

TONOFILAMENTS	tf

V.

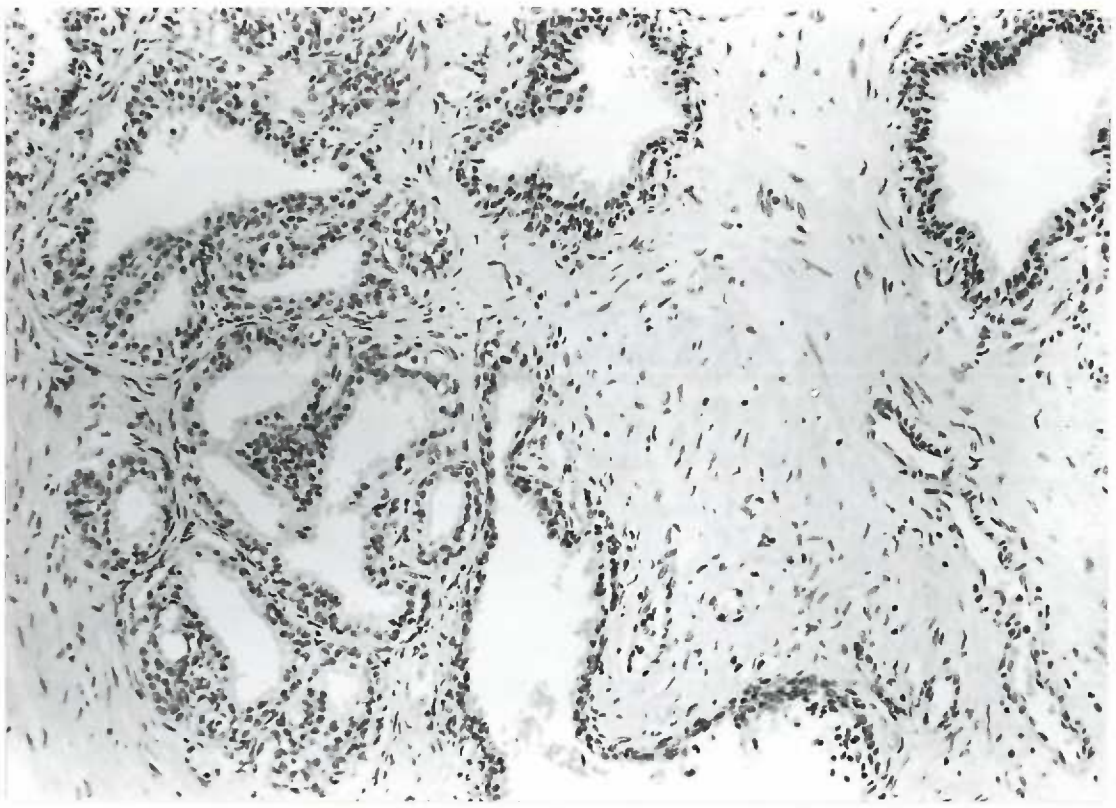
VACUOLES	V

W.

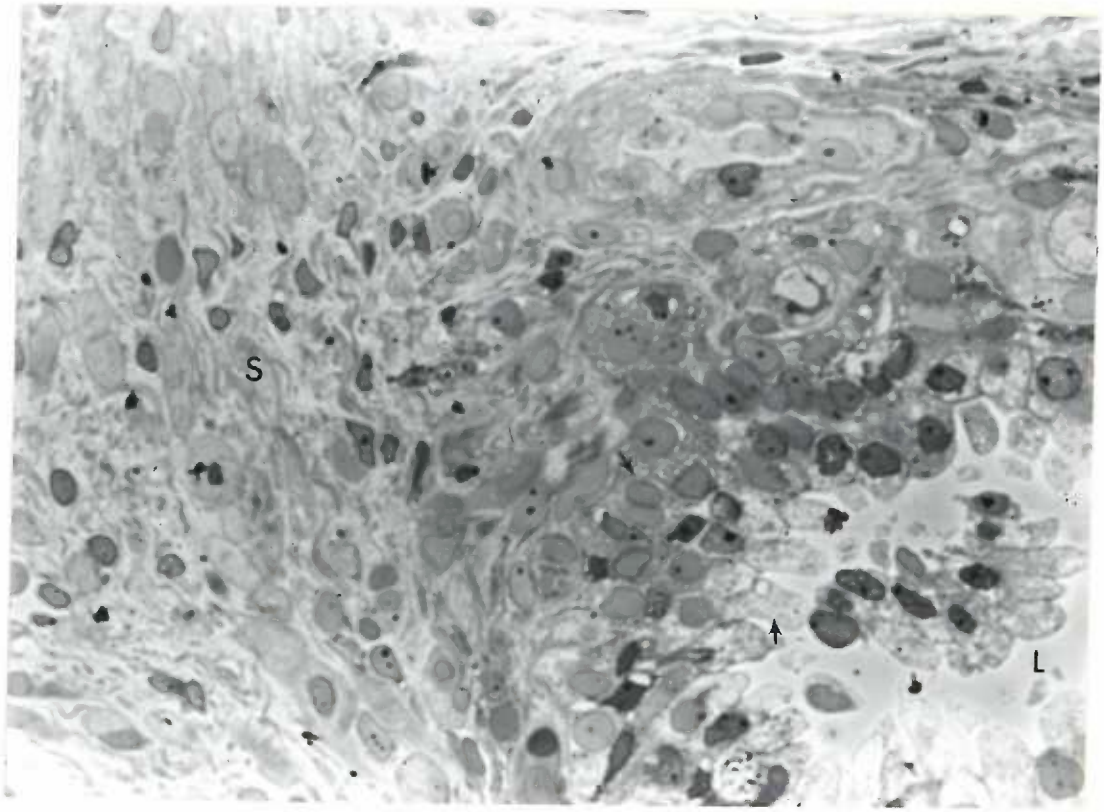
WHITE BLOOD CELLS	wbc

Figure 1. A frozen section of benign hyperplastic tissue stained with hematoxylin and eosin for light microscopy. The glands are formed by low columnar cells, and appear similar to glands found in normal prostates, but since they came from a nodule, they are considered to be hyperplastic glands. They lack the characteristic papillated projections found in many hyperplastic specimens, which are demonstrated in later figures. 180 X.

Figure 2. A micron thin section from hyperplastic tissue prepared for electron microscopy by fixing in 1% oxmium-S-Collidine for 2 hours. Low columnar cells form a small gland without papillary projections. The small arrow points to the tip of a secretory cell that probably corresponds to the attenuated apical pole seen in the electron microscopy preparation in Fig. 16. 180 X.



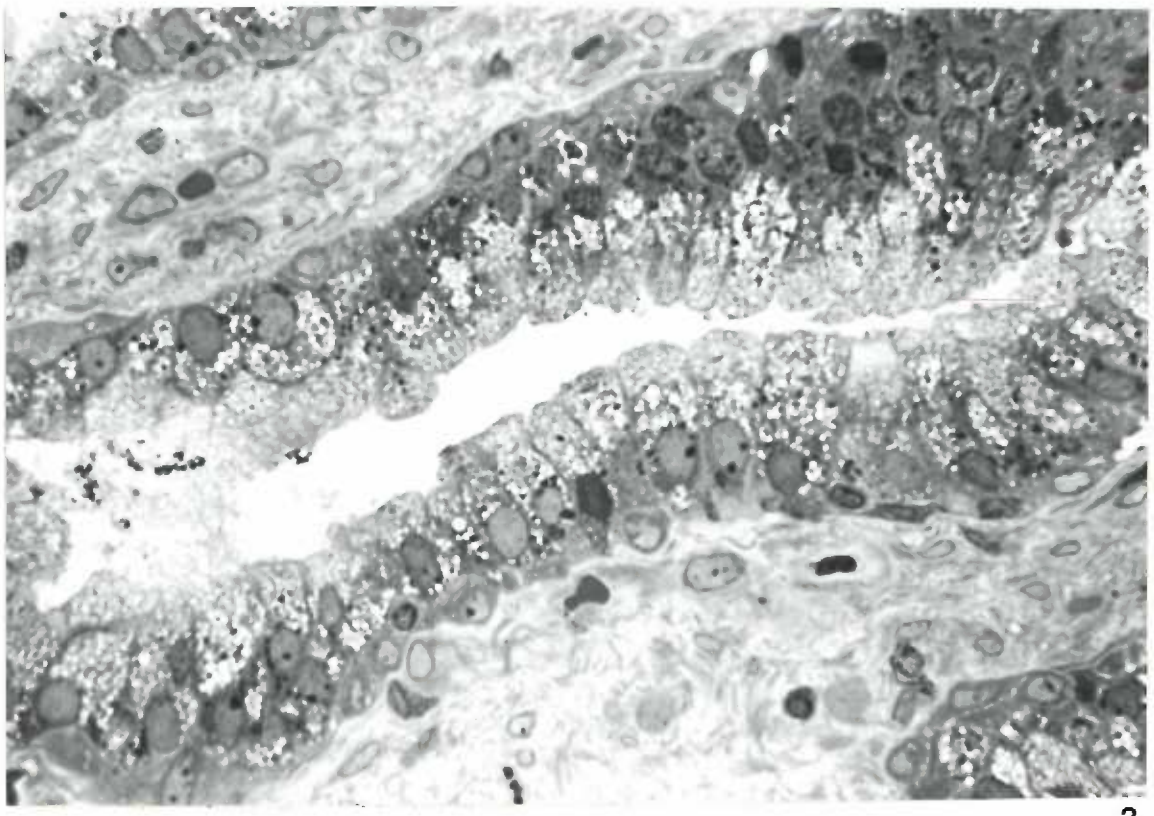
1



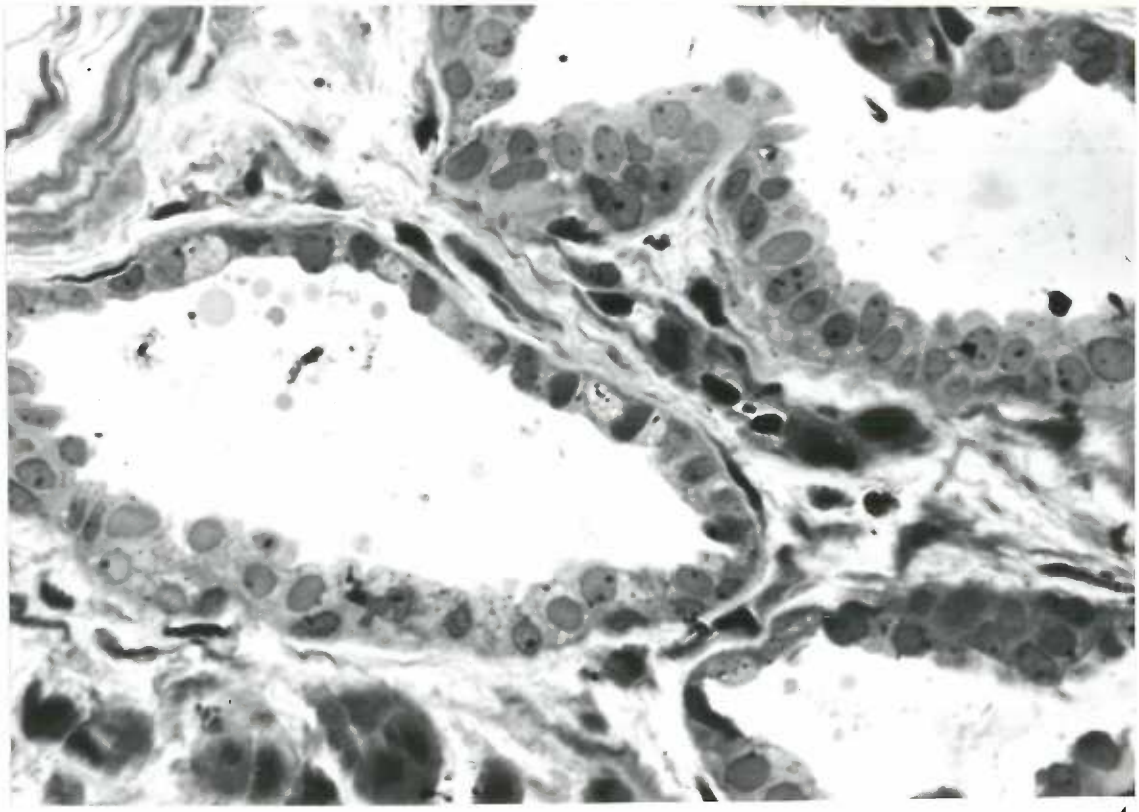
2

Figure 3. One micron sections of hyperplastic tissue prepared with 3.5% glutaraldehyde for 2 hours, rinsed briefly and post-fixed in 1% osmium-S-Collidine for morphological study. The secretory cells are tall, with highly vacuolated cytoplasm. The small arrow points to a clear vacuole that corresponds to those present in electron micrographs in Fig. 23 and 24. 720 X.

Figure 4. Cuboidal cells from hyperplastic tissue. Prepared for morphological study by fixing for 1 hour in 1% osmium buffered with veronal acetate. The nuclei consume most of the cell cytoplasm, the glands are small without papillary projections into the lumen. 720 X.



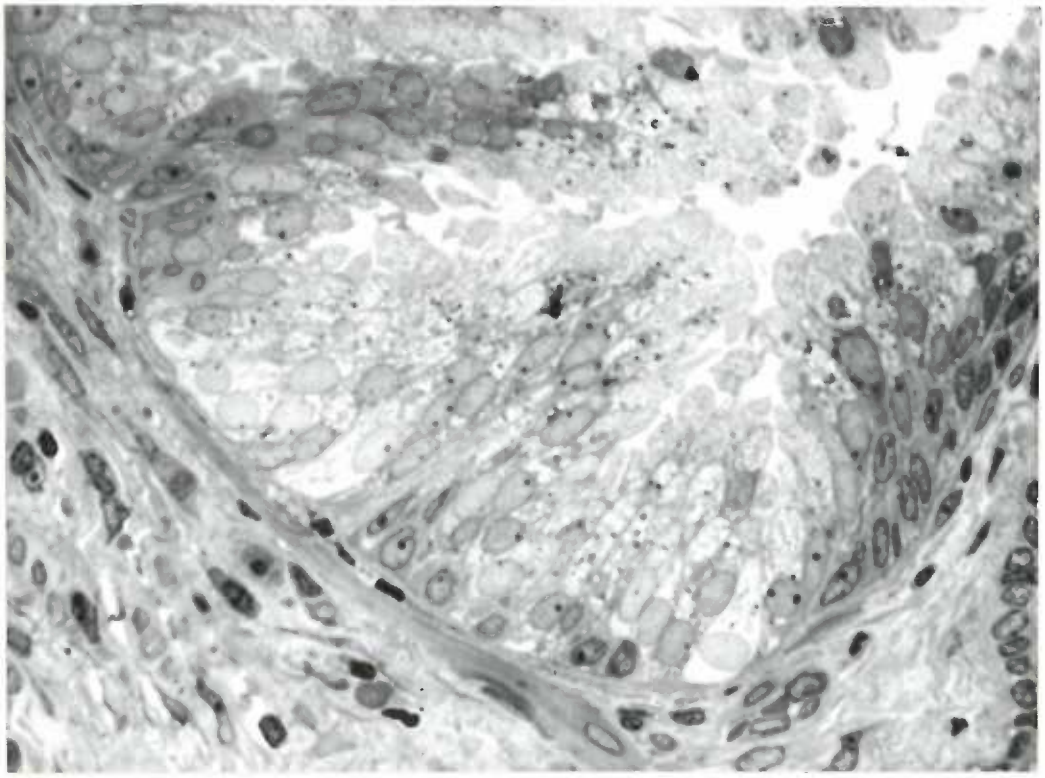
3



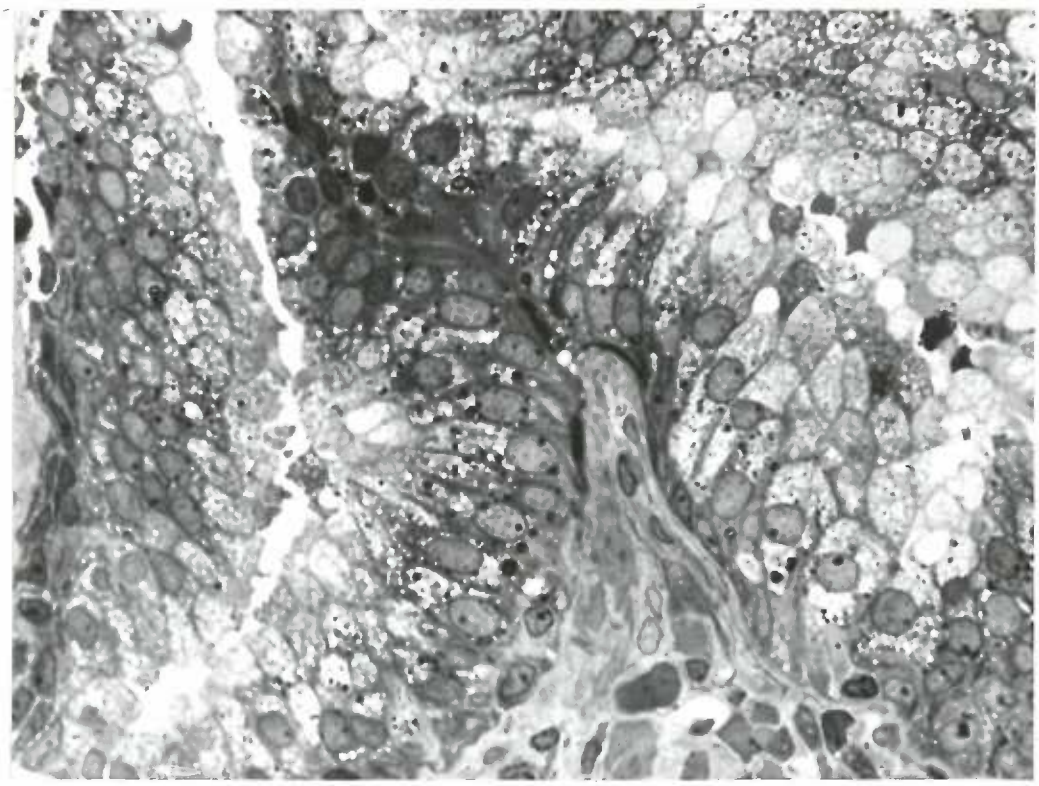
4

Figure 5. Tall secretory cells in papillated glands prepared for morphological study with fixation for 2 hours in 3.6% glutaraldehyde, washed for 24 hours in buffer and post-fixed for 1 hour in Zetterquist's osmium solution. The tall cells are crowded together and project into the gland lumen; the cytoplasm is foamy, with occasional densely staining bodies around the nucleus. Two small basal cells (BC) with a scant amount of dark cytoplasm lie between the stroma and the tall cells. 615 X.

Figure 6. Hyperplastic tissue prepared the same as the tissue in Fig. 5, except Dalton's osmium was used for the postfixative. The tall secretory cells still have prominent cytoplasmic vacuoles as pointed out by the arrows. 615 X.



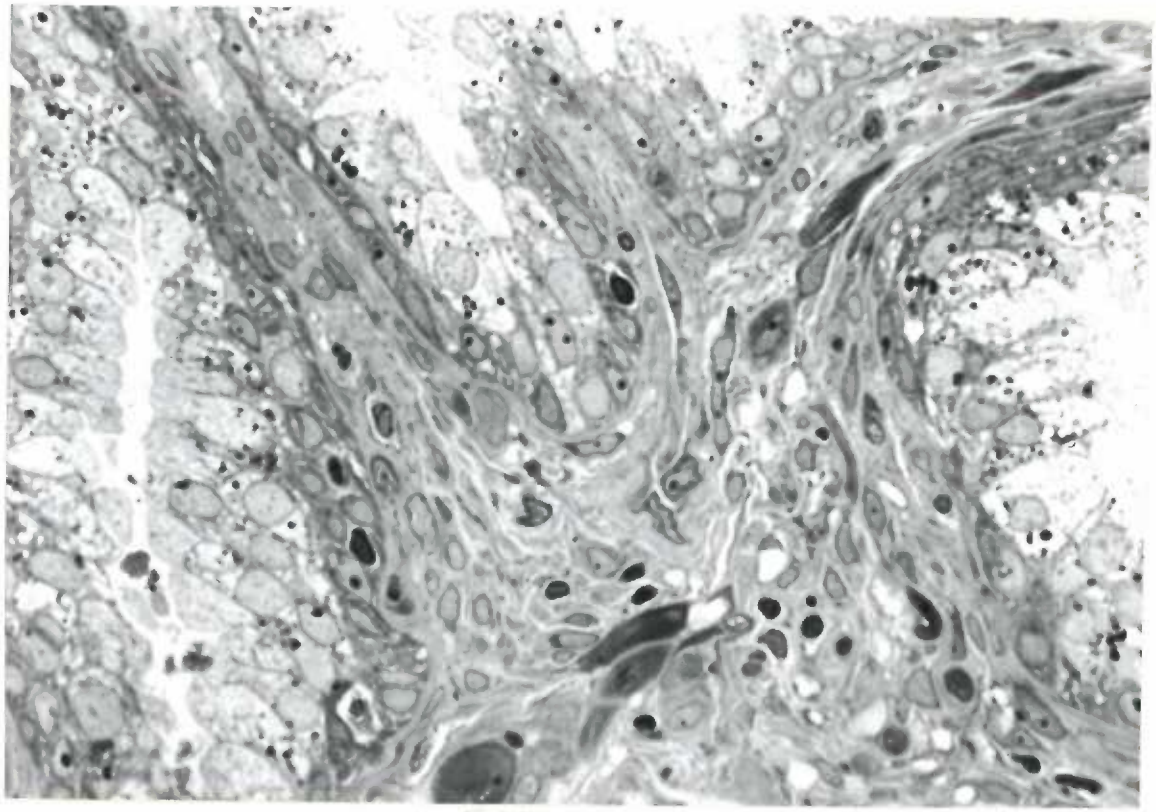
5



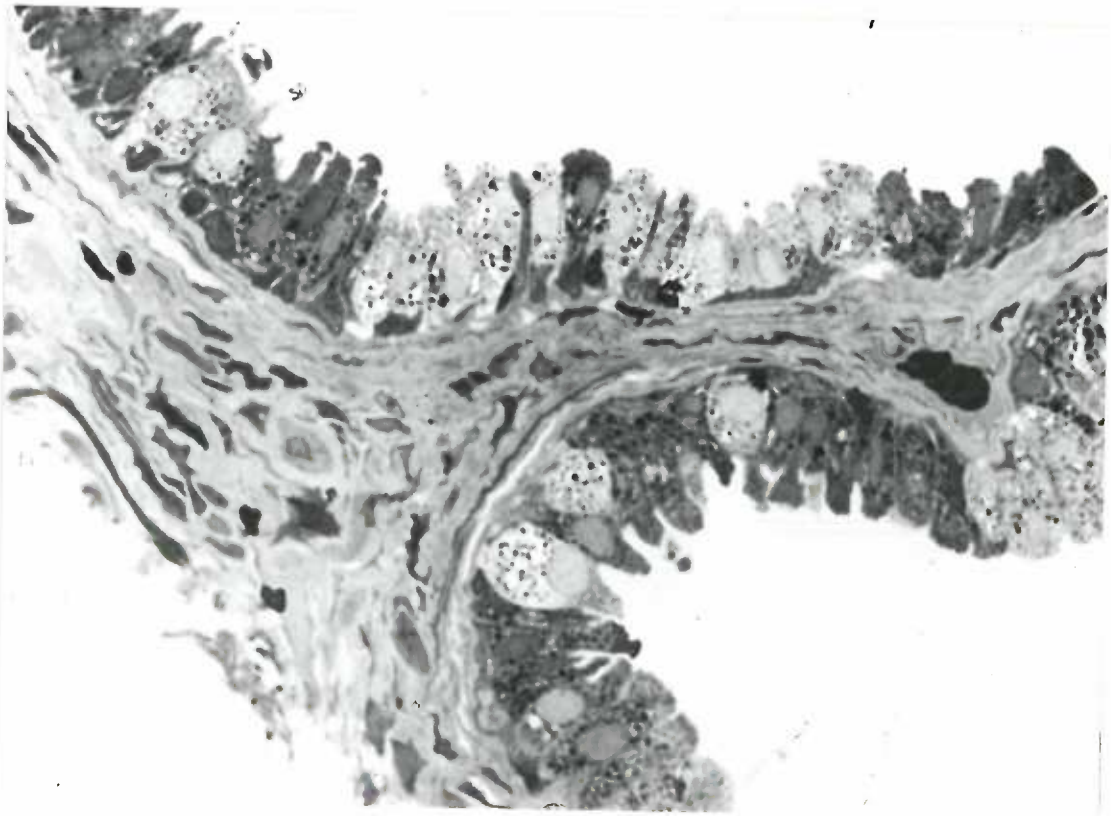
6

Figure 7. A one micron thick section of hyperplastic tissue prefixed in 4% Ca-formol for 24 hours, and post-fixed for 1 hour in 1% osmium-S-Collidine. The morphology of the tall secreting cells and of the stroma is similar to that found after fixation with 3.6% glutaraldehyde, in Fig. 5. The vacuolated cytoplasm still remains, as pointed out by the small arrow. 720 X.

Figure 8. A light micrograph from hyperplastic tissue prepared for electron microscopy by fixing in 20% glutaraldehyde for 2 hours and postfixing for 1 hour in Dalton's osmium. The tall epithelial cells are divided into dark (DC) and light (LC) cells. The stromal cells are shriveled and distorted as compared to the stroma in Fig. 7. The light and dark cells are shown in an electron micrograph in Fig. 29. 615 X.



7 7



8 8

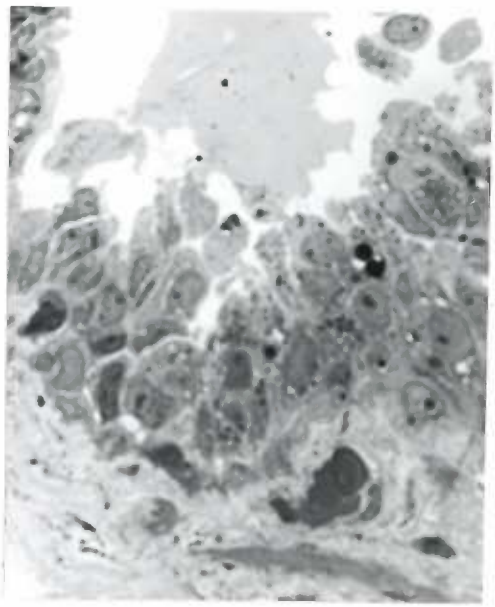
Figure 9. Hyperplastic tissue fixed for electron microscopy in a mixture of 5% glutaraldehyde containing 0.5 M NaCl, washed for 24 hours in buffer and postfixed 1 hour in 1% osmium-S-Collidine. The architecture of the glands and stroma is completely distorted. 750 X.

Figure 10. Tissue prepared in the same manner as that in Fig. 9, except 10% glutaraldehyde was substituted for the 5% glutaraldehyde and 0.5M NaCl. The low columnar cells have an appearance similar to the low columnar cells in Fig. 2.

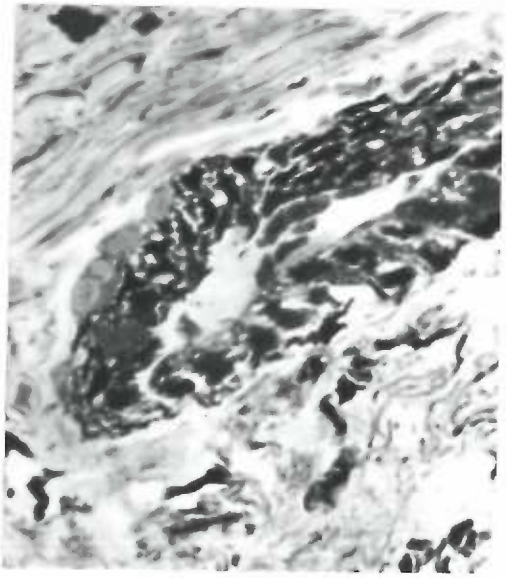
Figure 11. Hyperplastic cells fixed initially with 7.5% glutaraldehyde containing 0.75M NaCl, and postfixed the same as the tissue in Fig. 9. Again, the morphology of the tissue is quite distorted as it is in Fig. 9.

Figure 12. Tissue fixed the same way as that in Fig. 11, except 15% glutaraldehyde was used in place of the combination of glutaraldehyde and NaCl. The morphology at the light microscope level is comparable to that in Fig. 10. 750 X.

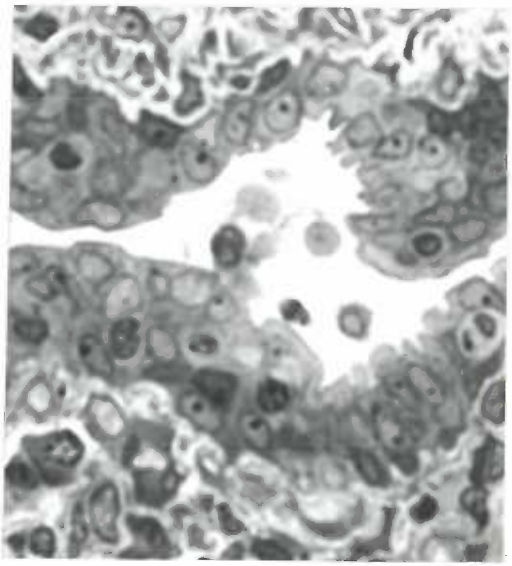
9



10



11



12

Figure 13. Low columnar cells fixed by the procedure outlined for Fig. 3. The small gland contains dense secretory material with large vacuoles and evidence suggesting that crystals of material have been removed. The retained secretory products as demonstrated here, were only occasionally observed in the lumen of glands. They were noticed a few times in ducts (Fig. 33) but the glands and ducts were usually empty. 720 X.

Figure 14. Low columnar cells fixed in Caulfield's osmium for 1 hour and embedded for morphological study. The cell is divided into three zones as suggested by Brandes, et.al.,[22] the basal region (BR) contains mitochondria and unattached ribosomes. The supranuclear region (SR) contains the Golgi complex as shown in more detail in Fig. 15. The apical pole is free of most organelles; it contains a diffuse electron lucent material similar to that found in apical secretory bodies as shown in Fig. 16. The cells that lie between the secretory cell (SC) and the basal lamina (BL) have a small amount of cytoplasm filled with ribosomes, mitochondria and occasional dense bodies similar to the basal cells described by Mao.[21] 8,300 X.

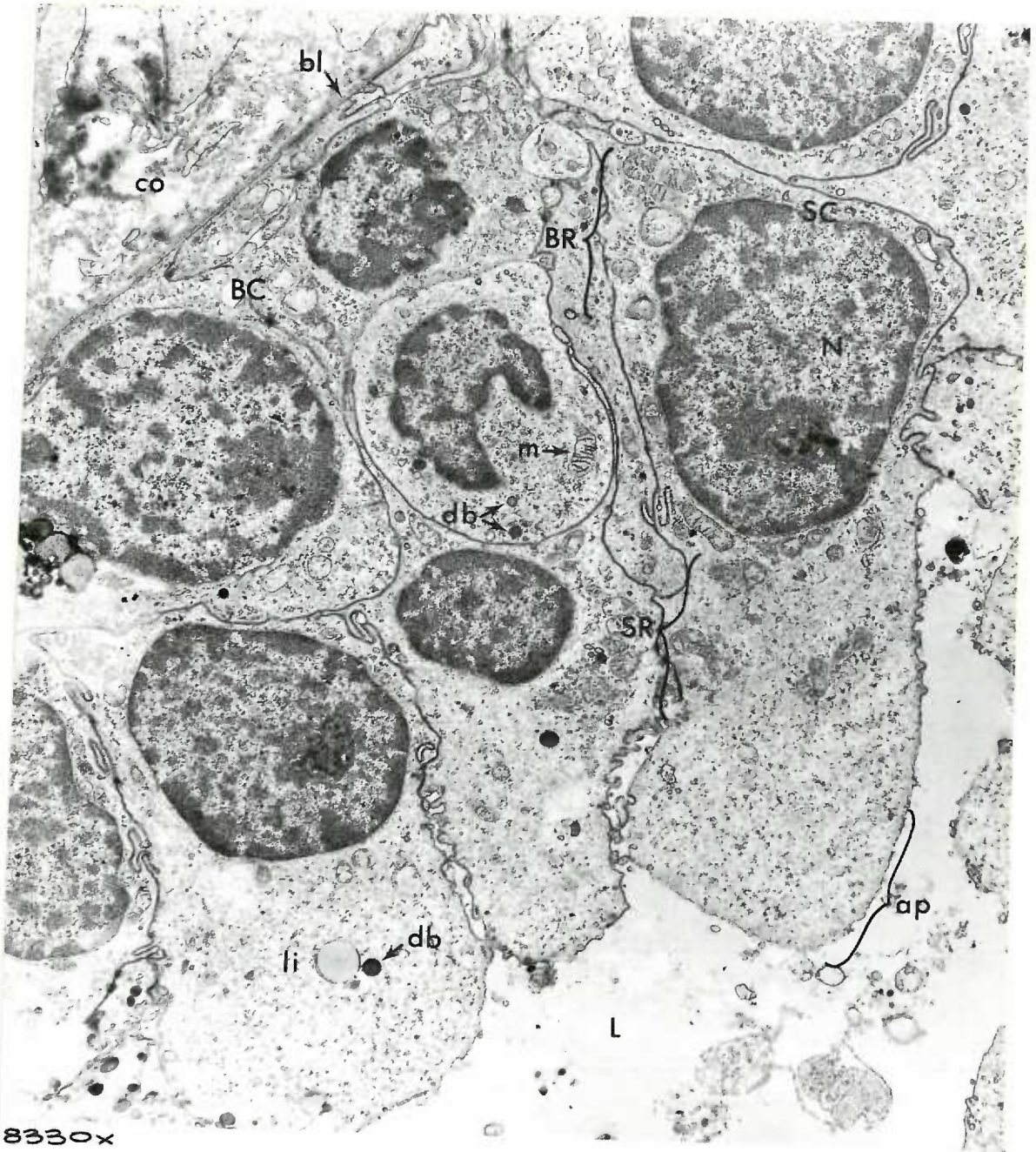
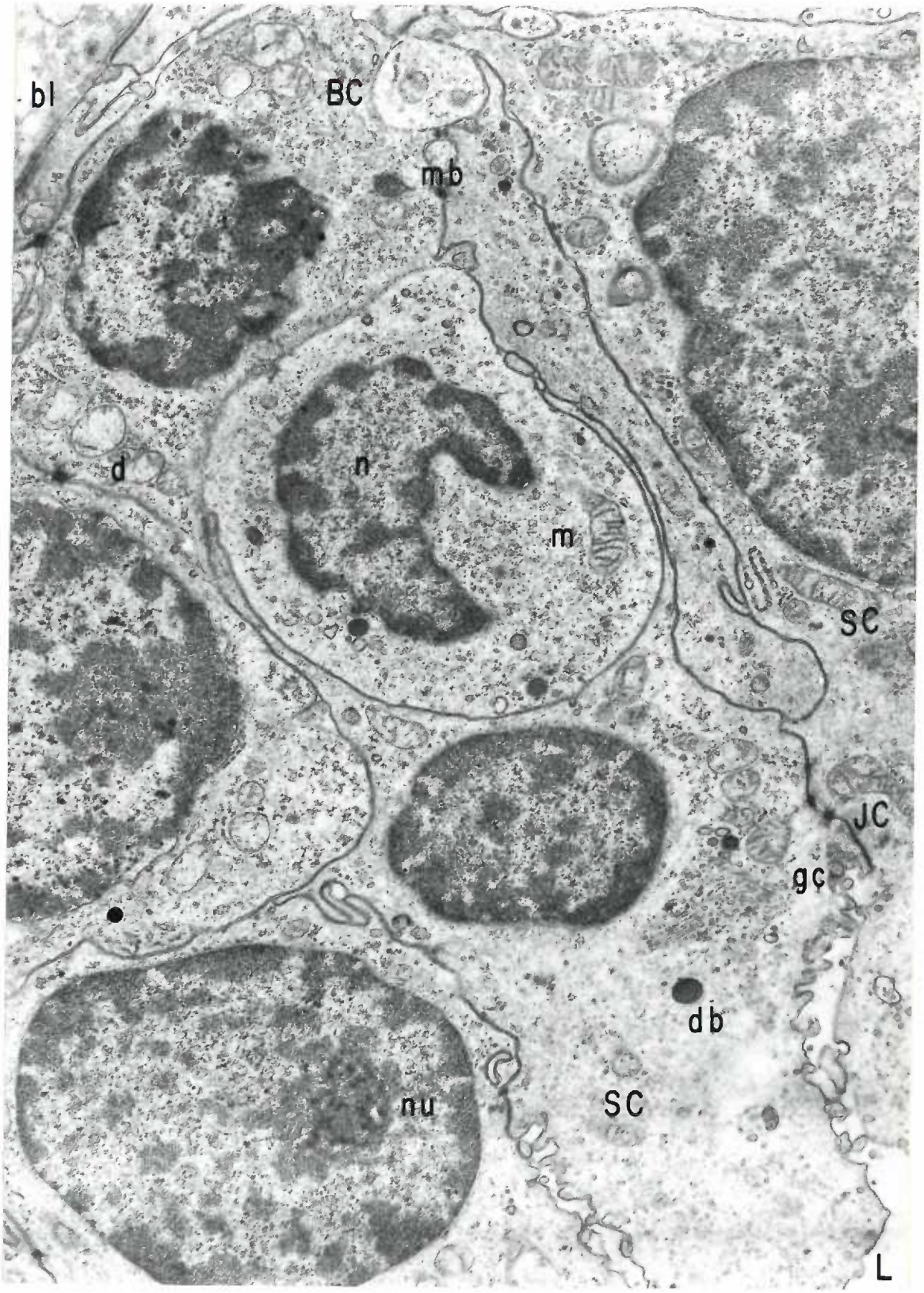


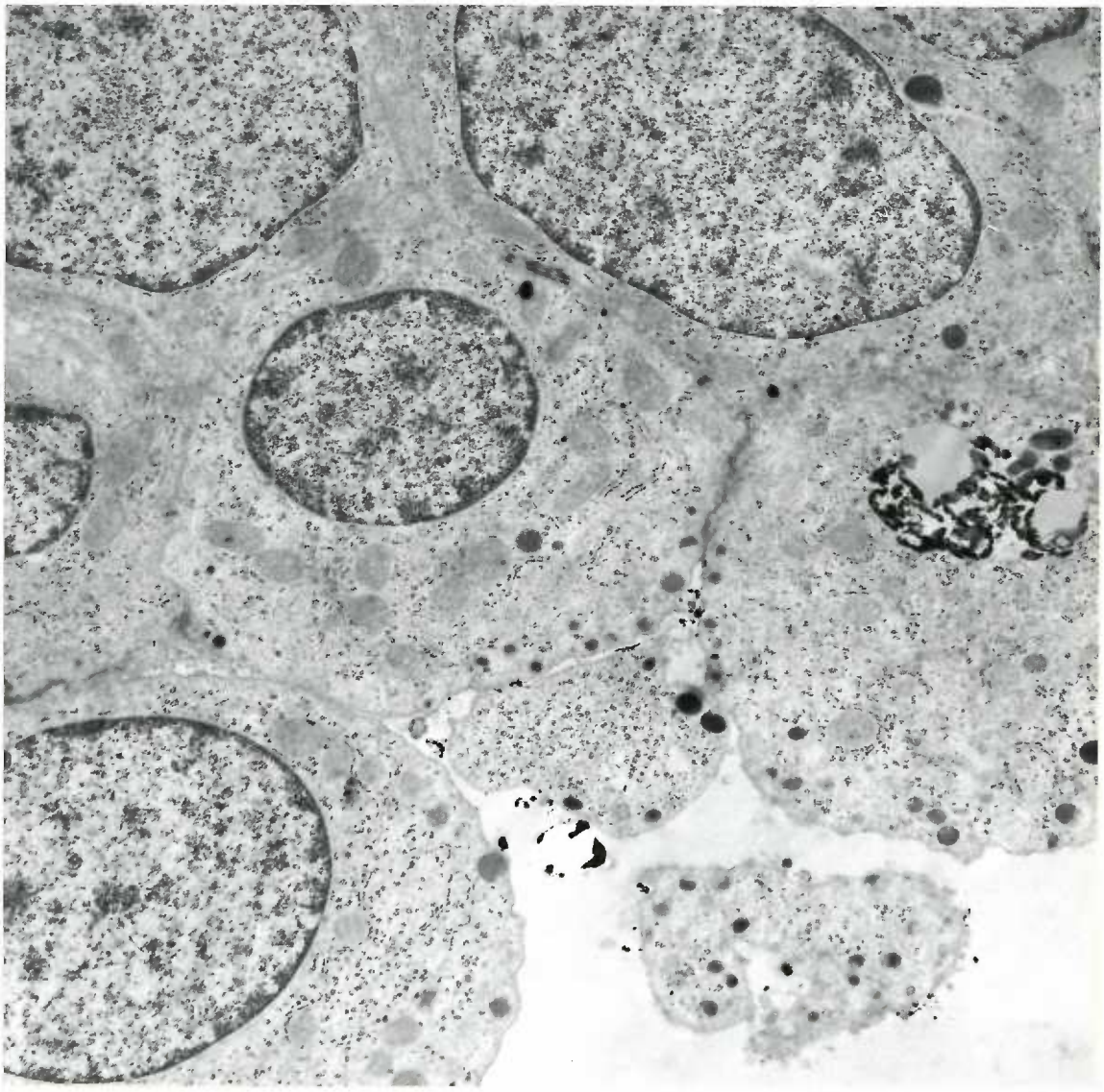
Fig. 14

Figure 15. A higher magnification of Figure 14. The basal cell (BC) contains a multivesicular body (mb), but no other evidence of lysosomes. The cell in the center, between the basal cell and secretory cell, probably represents a cross section of another secretory cell. The Golgi complex is well developed above the nucleus in the secretory cell, but is not present in the basal cell. 14,300 X.



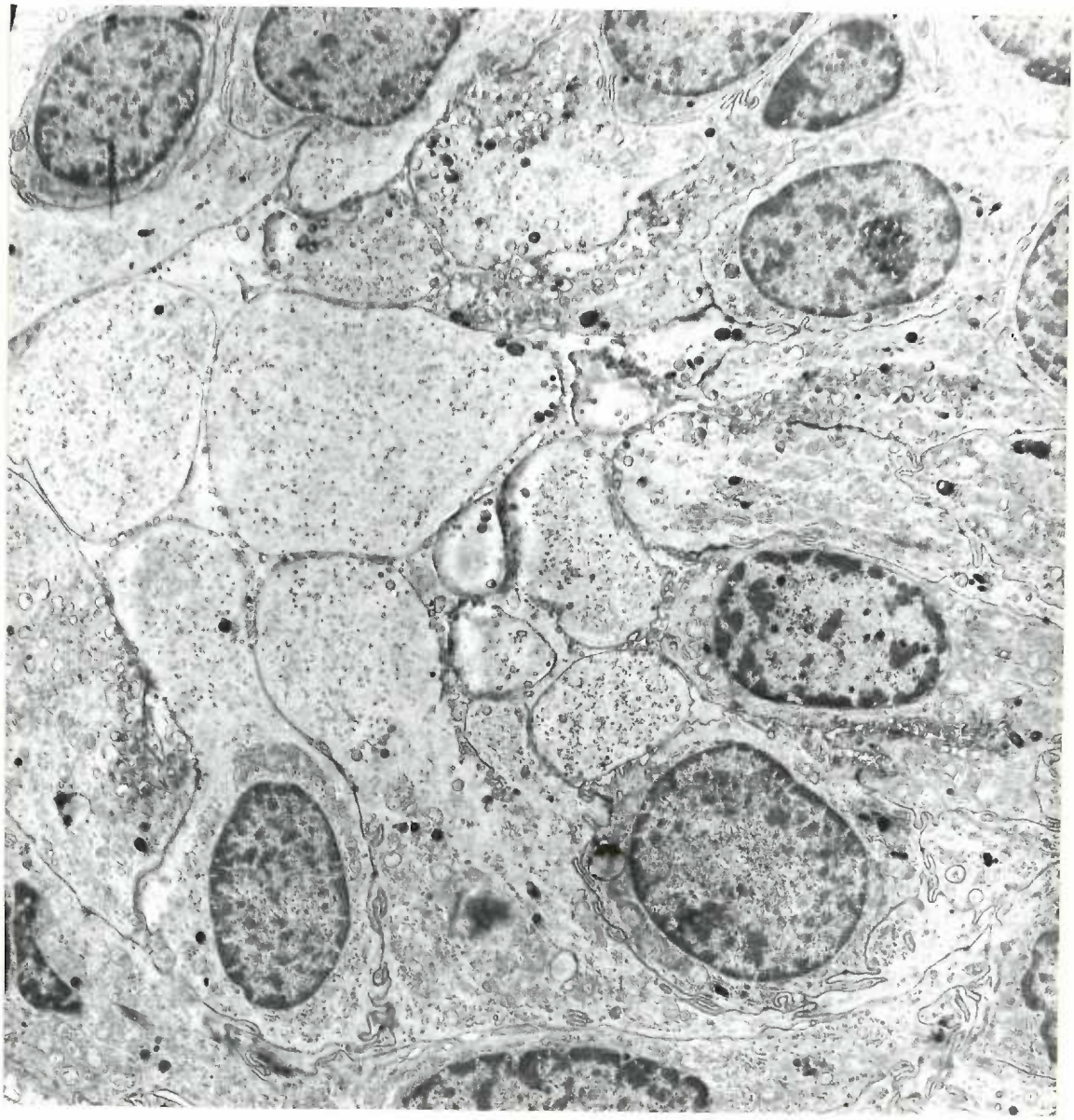
15

Figure 15 A. Low columnar cells fixed in 4% paraformaldehyde and 4% glutaraldehyde for 24 hours, postfixed for 2 hours in 1% Osmium-S-Collidine without a prior washing in buffer. Small dense bodies (SG_1) appear along the apical borde r and in the several cytoplasmic blebs in the lumen (ASB). The large dense body (DB) is similar to the ones found in Figure 18 and 19. 5,000X.



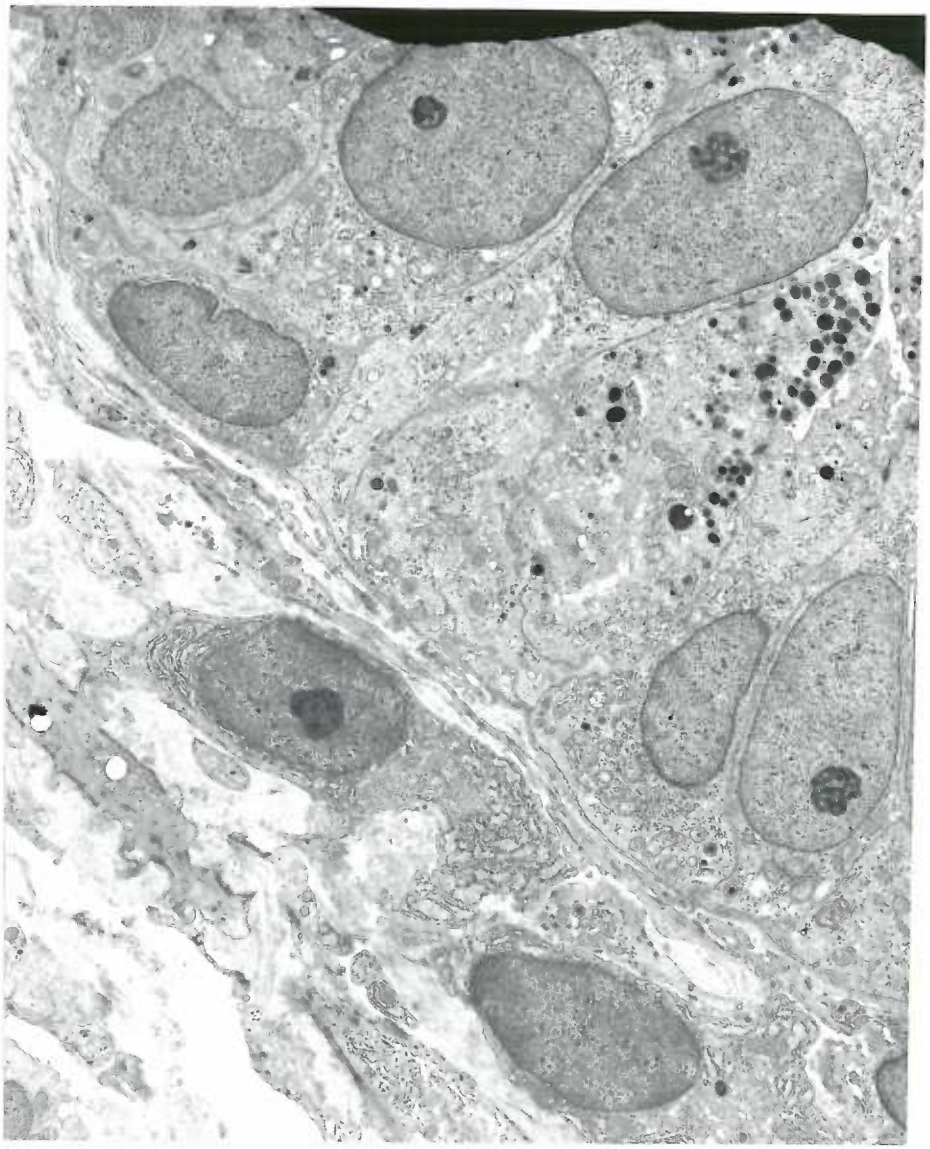
15A

Figure 16. A gland from hyperplastic tissue with low columnar cells prepared the same as the tissue in Fig. 14. The secretory cells have attenuated projections into the lumen; the projections are filled with an electron lucent material containing small flecks of dense material, similar to the blebs in the lumen labelled apical secretory bodies (ASB). The cell organelles, mitochondria and ribosomes are concentrated around the nucleus, rarely situated in the apical region. Several small dense bodies lie near the nuclear region, and at the apical tips of some cells. 5,950 X.



16

Figure 17. Low columnar epithelium fixed with 1% oxmium-S-Collidine for 2 hours for morphological study. The basal lamina separates the epithelium on the right from the stroma on the left. The secreting cells contain small dense granules (Sg_1) concentrated in the luminal tip of the cell. The basal cell has a small amount of cytoplasm filled primarily with ribosomes. The nucleoli in the secreting cells are very prominent, but they are not present in the basal cell. The white blood cell with abundant rough endoplasmic reticulum is probably a plasma cell. 5,000 X.



17

Figure 18. A tangential section through several low columnar secretory cells from hyperplastic tissue fixed for 1 hour in 2% osmium buffered with veronal acetate. Free ribosomes, r, and mitochondria fill the cytoplasm. The remnants of a large vacuole (v) is contained in one cell. There is no evidence of secretory activity. Cytoplasmic filaments, and membrane infoldings are present and are characteristics often seen in other inactive prostatic cells. 7,750 X.

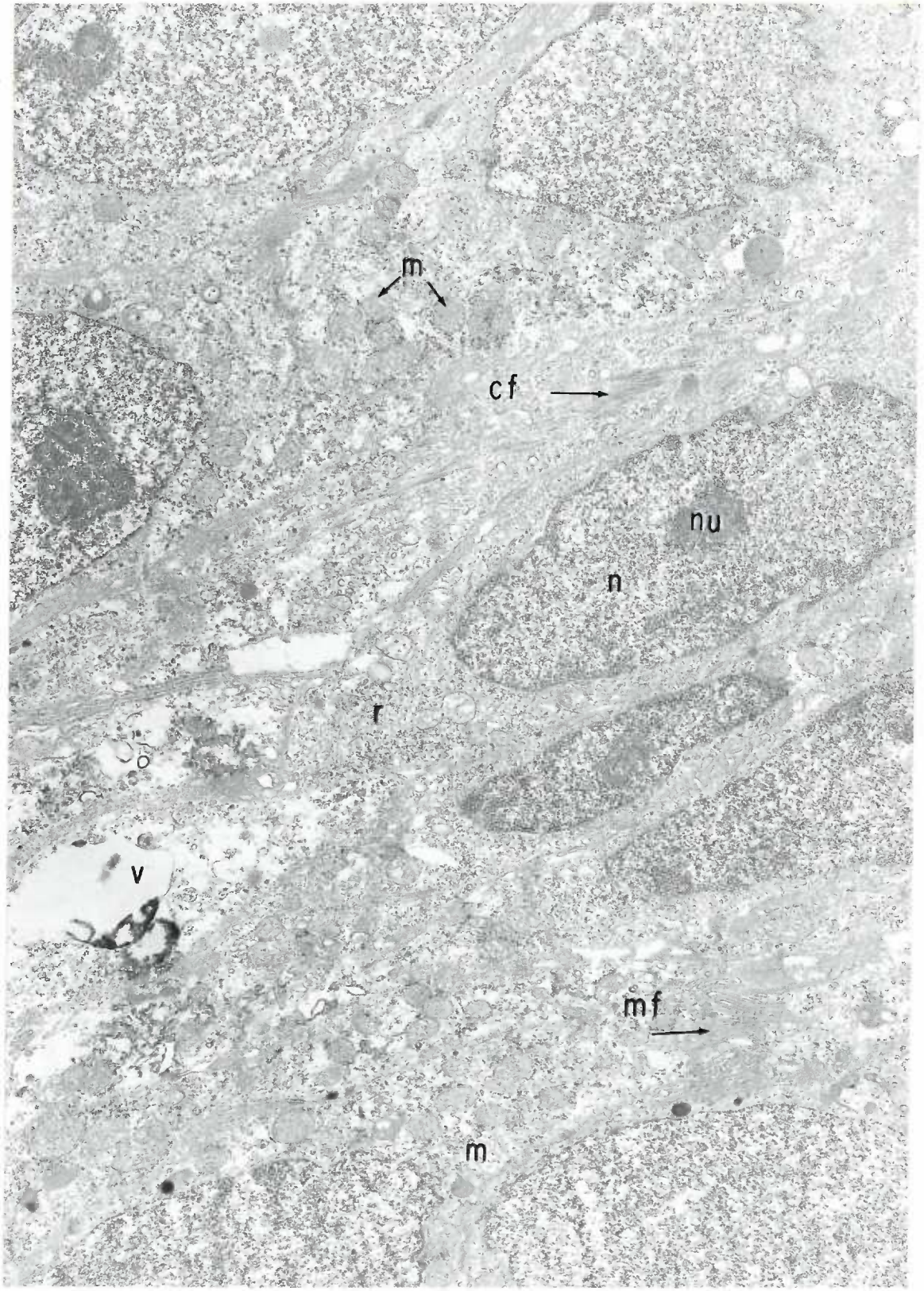

m
cf
nu
n
r
o
v
mf
m

Figure 19. Two basal cells and the base of several secretory cells from hyperplastic tissue prepared identical to that in Fig. 18. The nucleus of the basal cell has an irregular contour as compared to that of the secretory cell nearby. Chromatin is clumped around the periphery of the basal cell nucleus and generally denser than the nucleus of the secretory cell. A large vacuole fills the base of the secretory cell. 11,570 X.

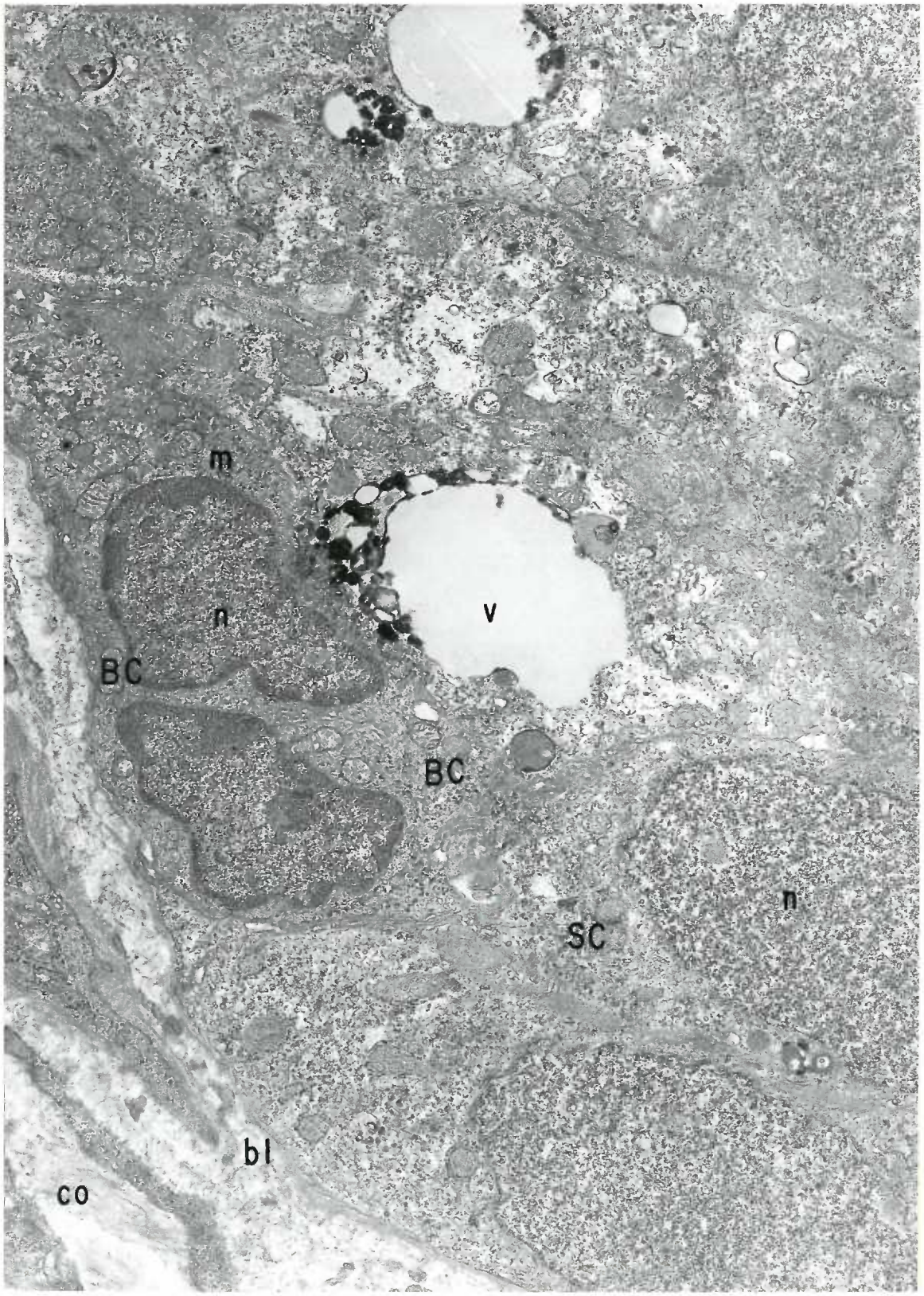
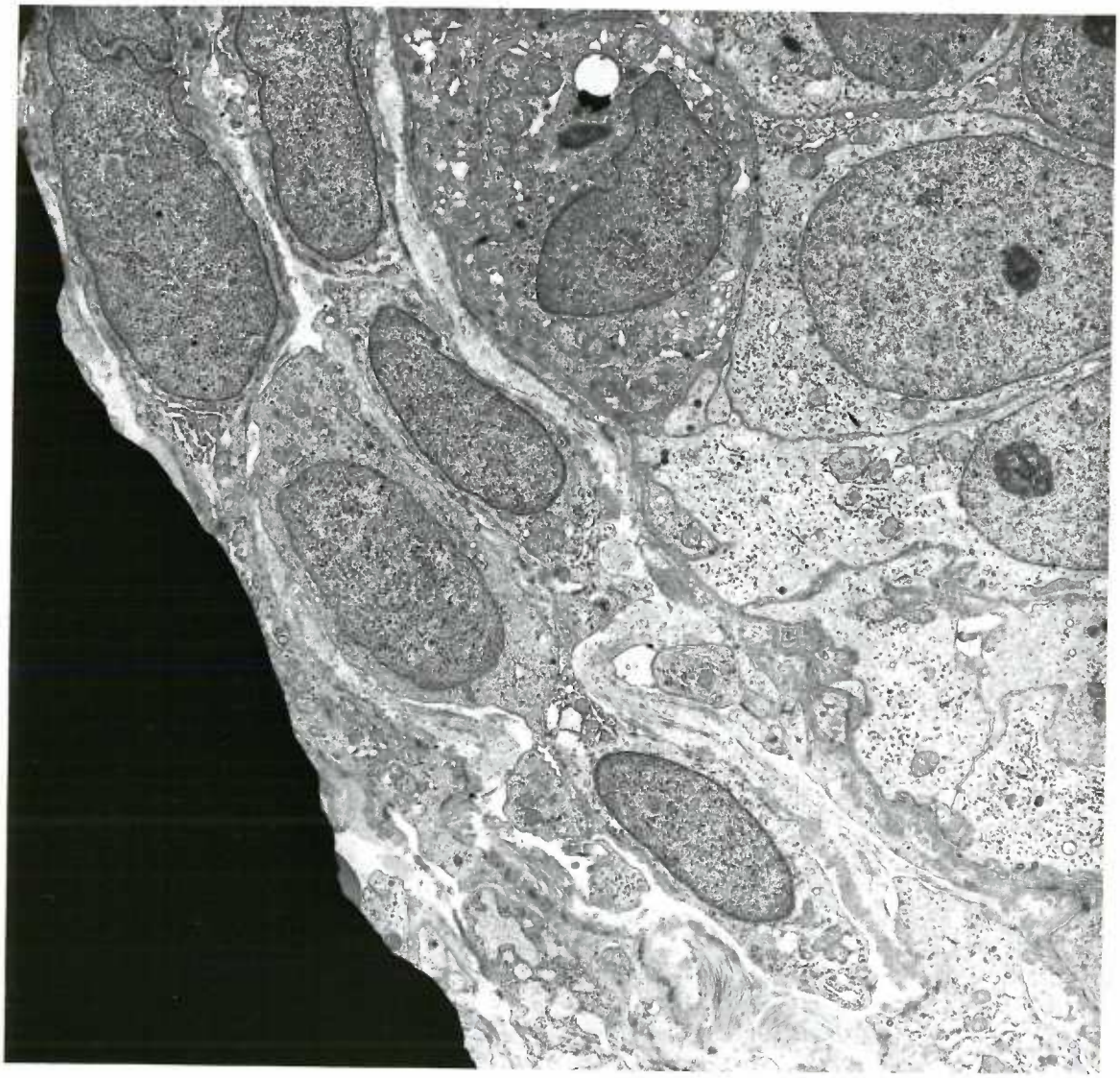
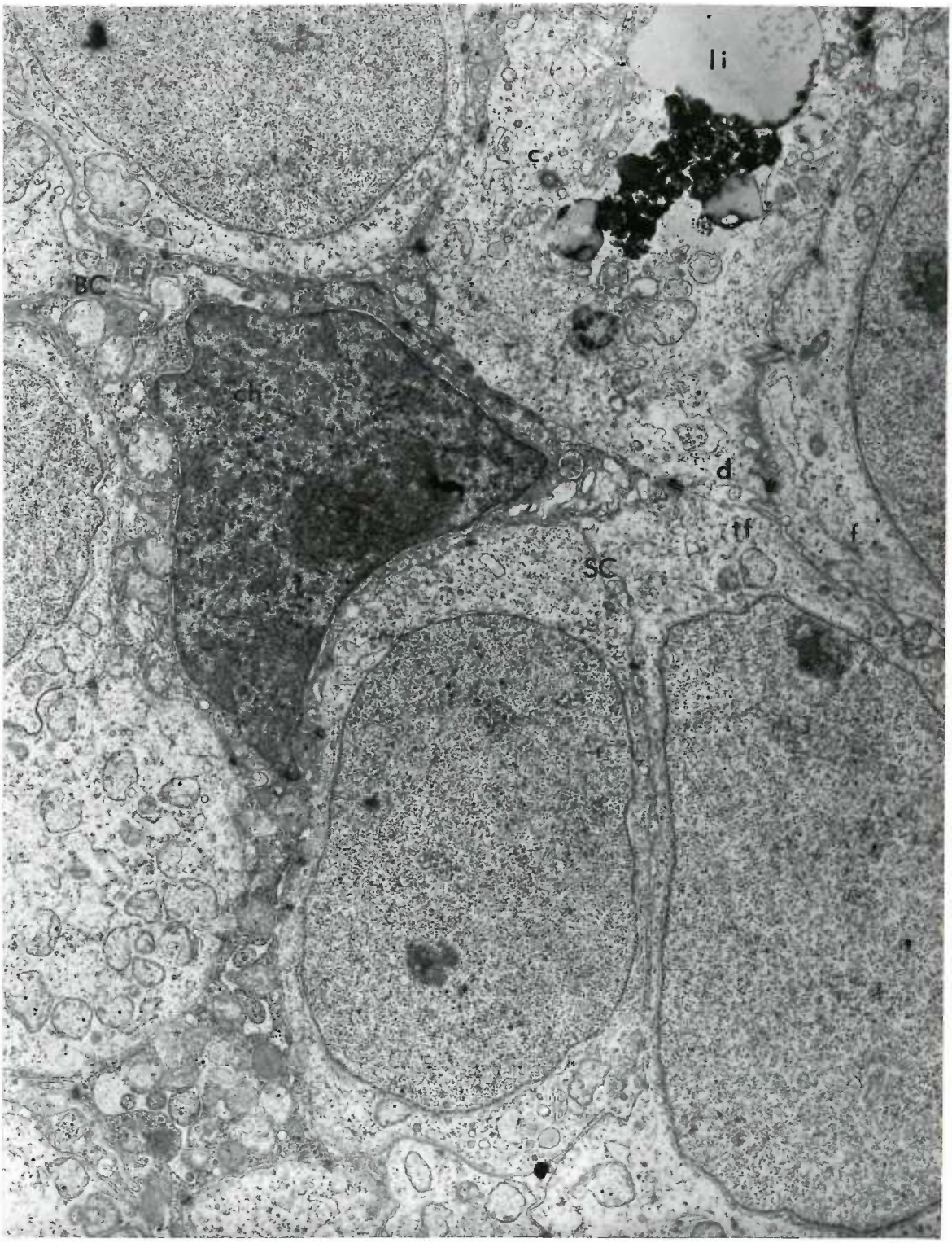

m
n
BC
v
BC
n
SC
bl
co

Figure 20. Low columnar cells and stroma from hyperplastic tissue prepared for morphological study with fixation for 2 hours in 1% osmium buffered with S-Collidine. A large basal cell with electron dense cytoplasm lies between the basal lamina and several secretory cells. The cytoplasm is filled with many ribosomes unassociated with cytoplasmic membranes, the ribosomes in the secretory cells are scattered in an electron lucent cytoplasm, but occasionally attached to endoplasmic reticulum. Several fibroblasts lie in the stroma separated from the epithelium by bundles of collagen and the basal lamina. 5,960 X.



20

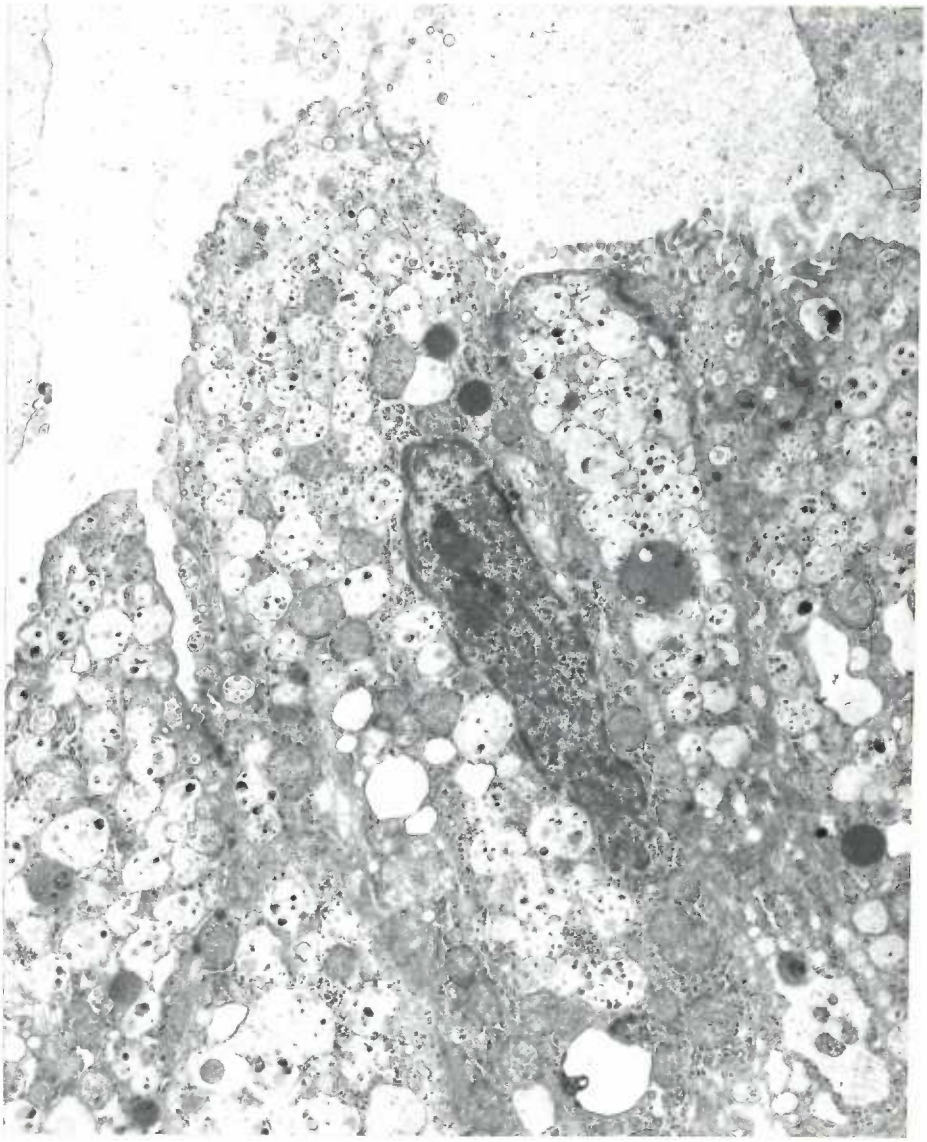
Figure 21. Hyperplastic tissue fixed with 1% osmium-S-Collidine for 2 hours for morphological observation. There are two distinct cell types, light secretory cells (SC) and a dark cell (BC) with an immature cytoplasm chiefly composed of mitochondria and ribosomes. The cells are attached to each other with several desmosomes that contain tonofibrils (tf) similar to those found in other kinds of epithelial cells. 10,180 X.



10,184

Fig. 21

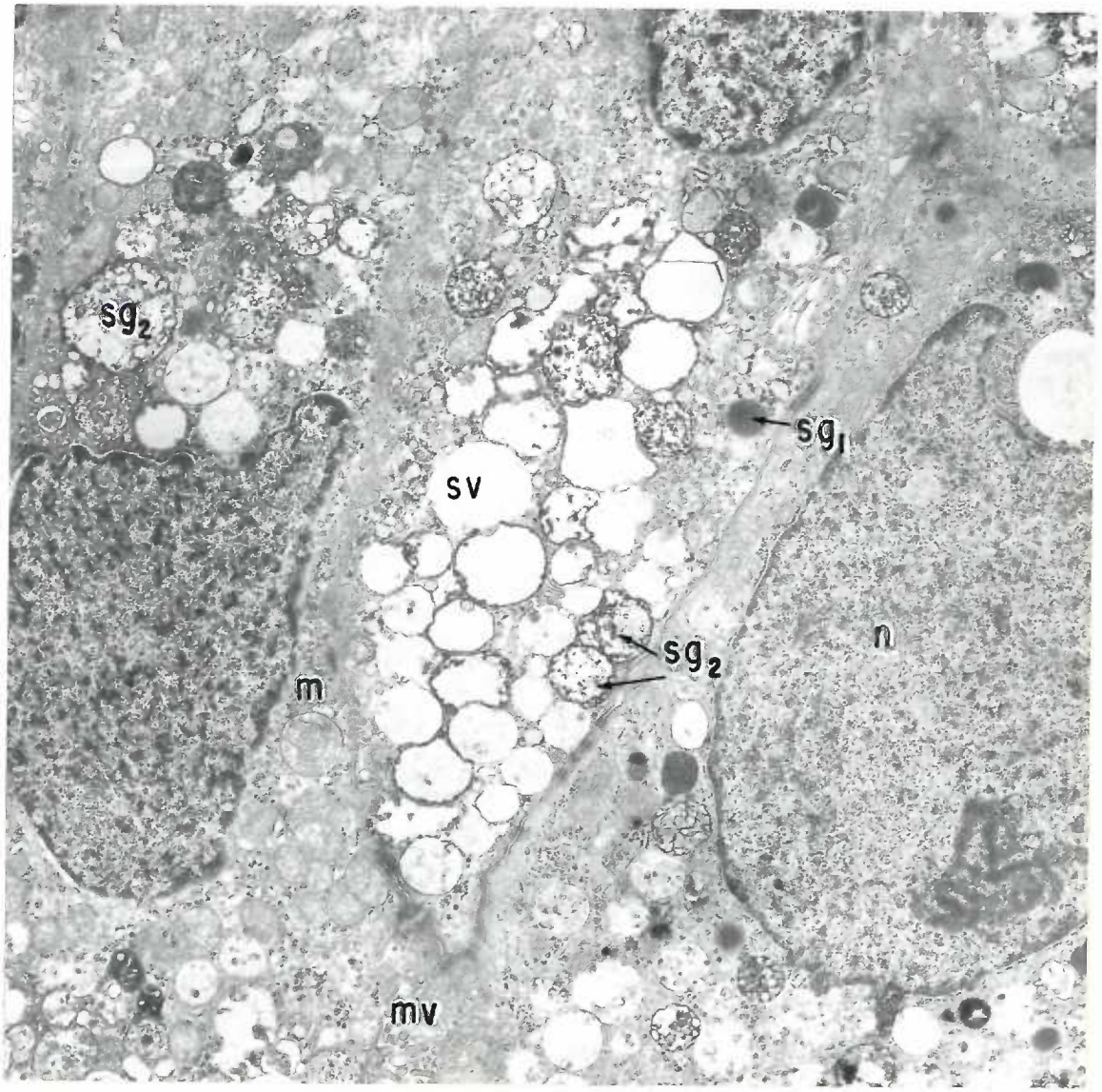
Figure 22. Tall columnar cells fixed with 1% osmium-S-Collidine for 2 hours for morphological study. The tips of several cells project into the lumen of the gland, their cytoplasm is filled with vacuoles containing small vesicles and droplets. Occasional dense bodies are associated with the secretory vacuoles. The nucleus of this cell appears more pyknotic than is usually seen in osmium fixed material. 7,400 X.



22

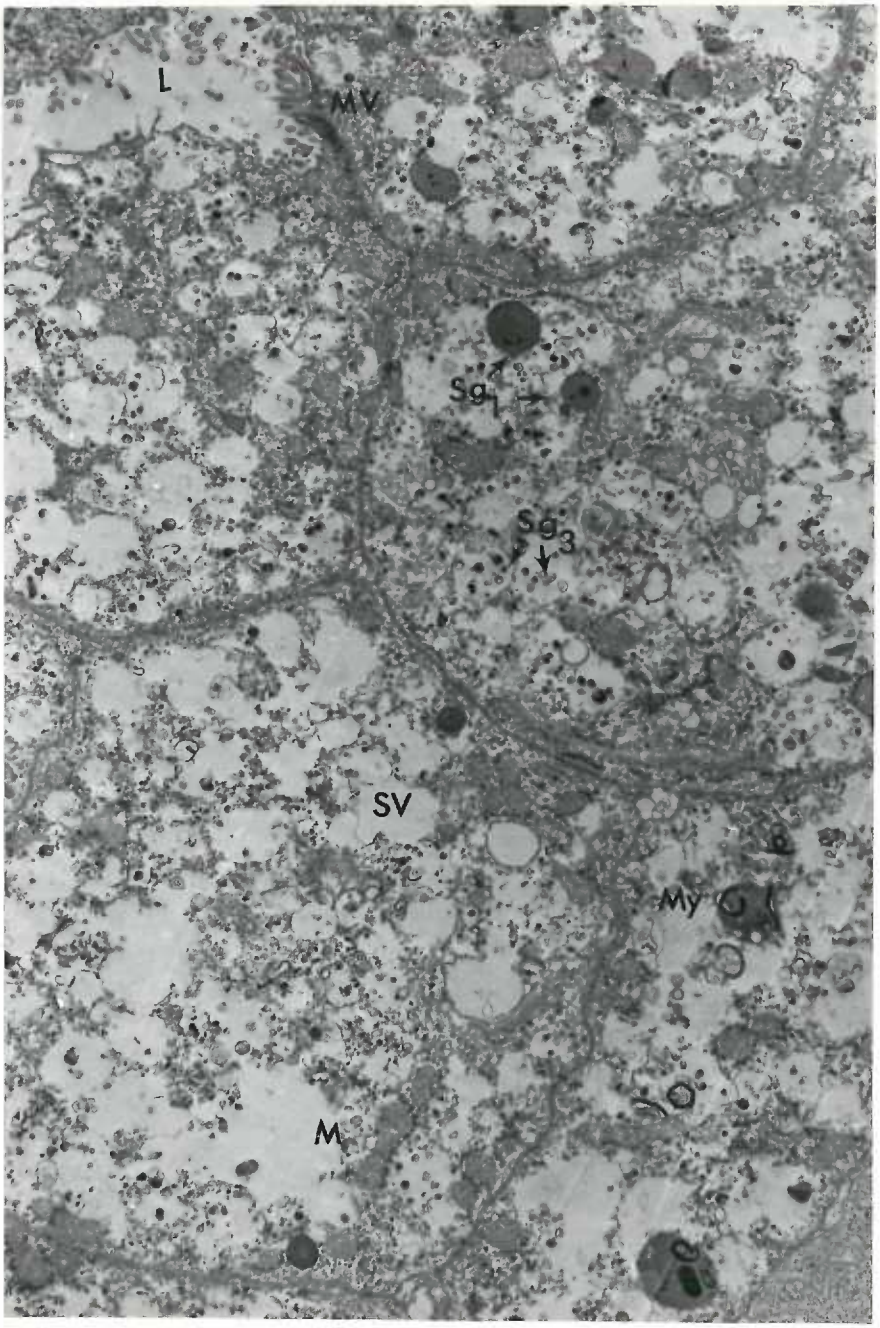
Figure 23. Tall columnar cells fixed in 3.5% glutaraldehyde buffered with .1M cacodylate for 1 hour; stored two days in cacodylate buffer with 1% gum acacia added; post-fixed for 2 hours in 1% osmium-S-Collidine. The tips of the cells are filled with secretory vacuoles, many of which are empty. Others contain a granular type of electron dense material (Sg_2), some have small droplets and vesicles (Sg_3) similar to those in Fig. 22. A few dense homogeneous bodies are mixed in with the secretory products (Sg_1). The cell tip on the right may represent an apocrine secretory body, or the tip of an attached secretory cell that projects into the lumen. 29,600 X.

Figure 24. Tall columnar cells prepared the same way as tissue in Figure 23. The cytoplasm contains many empty secretory vacuoles; they probably correspond to the vacuoles seen in the cytoplasm of the light microscope preparations in Fig. 3, 5, and 6. 28,960 X.



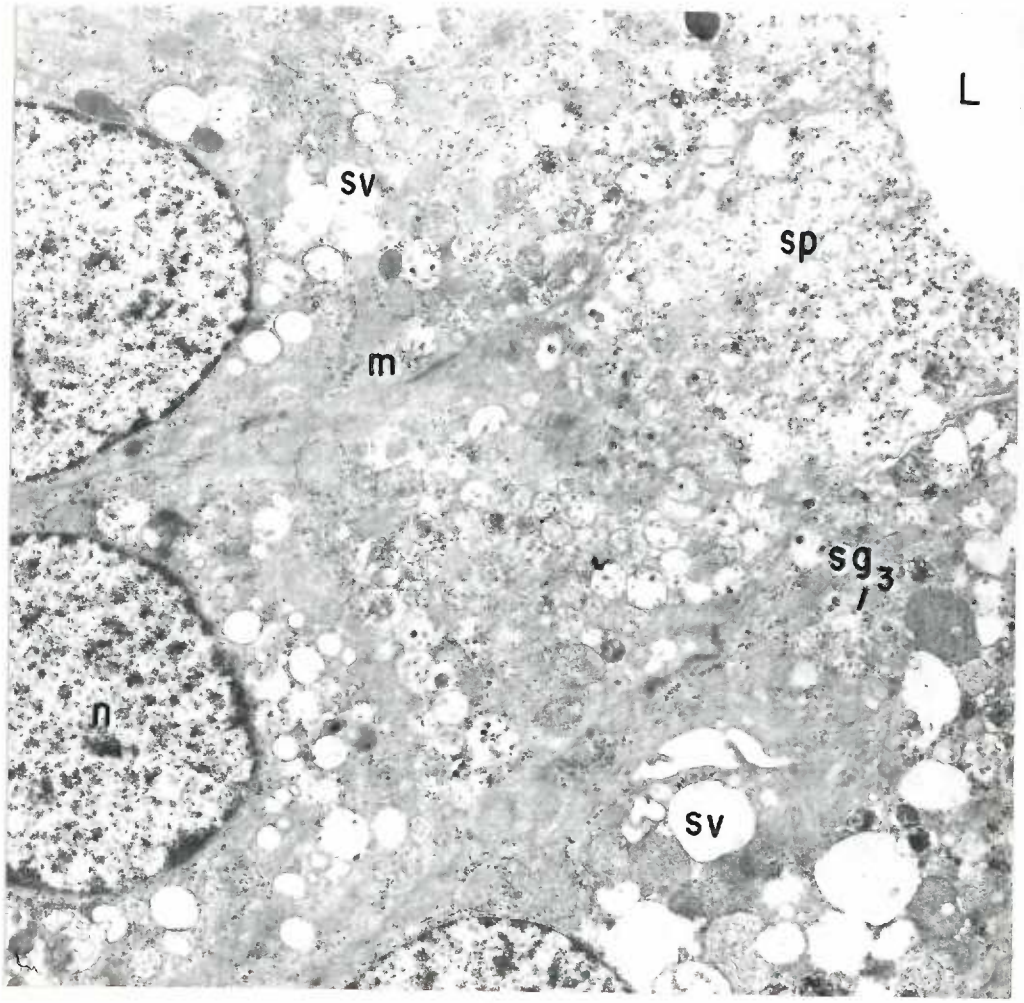
24

Figure 25. The tips of several tall columnar cells fixed 2 hours in 10% glutaraldehyde buffered in .1M cacodylate, rinsed briefly in buffer and post-fixed 1 hour in 1% osmium-S-Collidine. The cytoplasm is vacuolated more than usual and contains myelin figures (my) often associated with poorly fixed material. 7,300 X.



25

Figure 26. Tall columnar cells from hyperplastic tissue fixed 2 hours in 15% glutaraldehyde, rinsed briefly and post-fixed 1 hour in 1% osmium-S-Collidine. The cytoplasm has empty secretory vacuoles, but in general contains more electron dense material than tissue fixed in less concentrated glutaraldehyde. 7,310 X.



26

Figure 27. Hyperplastic tissue fixed in 20% glutaraldehyde for 2 hours. The cytoplasm is full of secretory products that contain numerous myelin figures. The mitochondria have lost their cristae and are difficult to distinguish from the secretory products. The smooth muscle cells (sm) and collagen bundles (co) appear shrunken. 12,240 X.

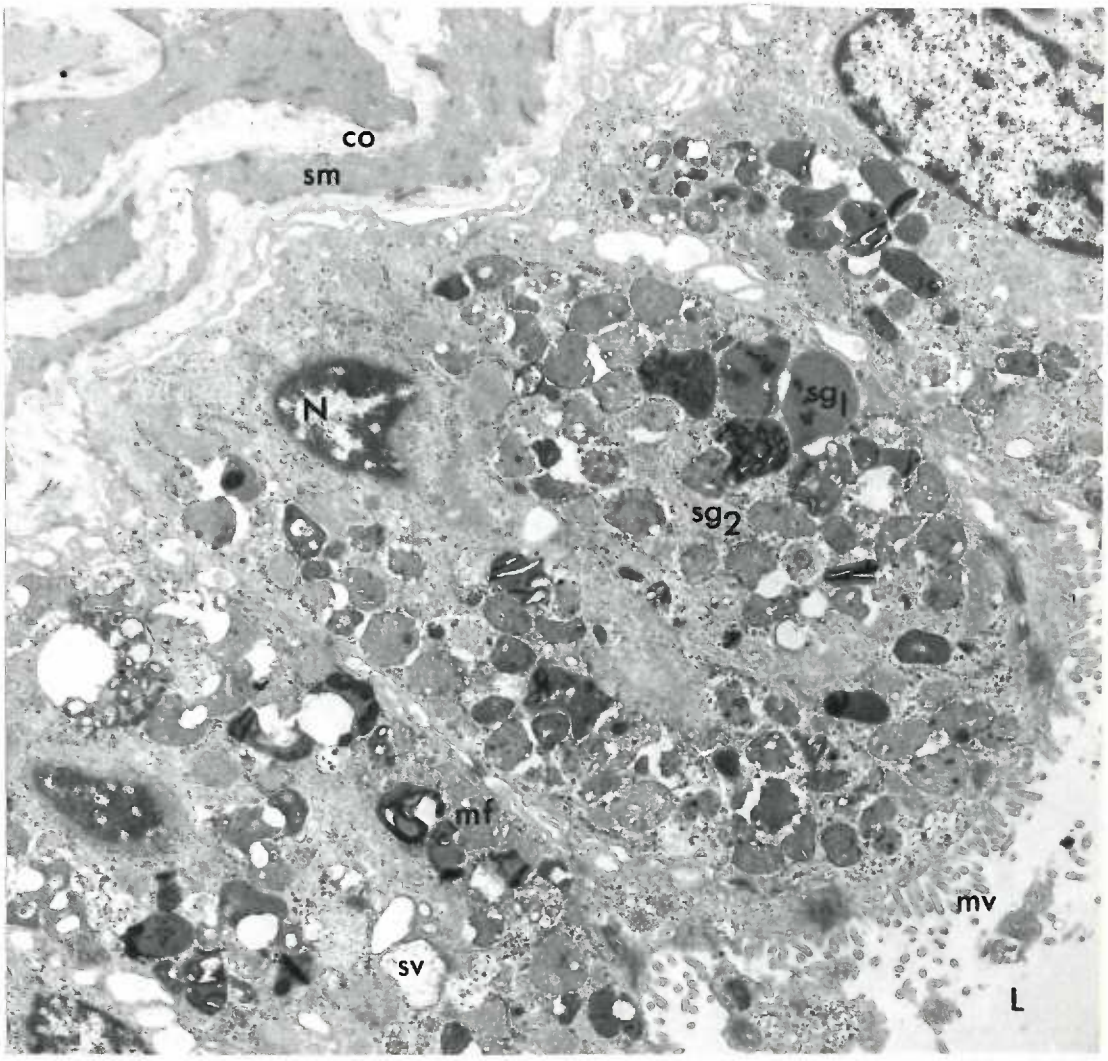
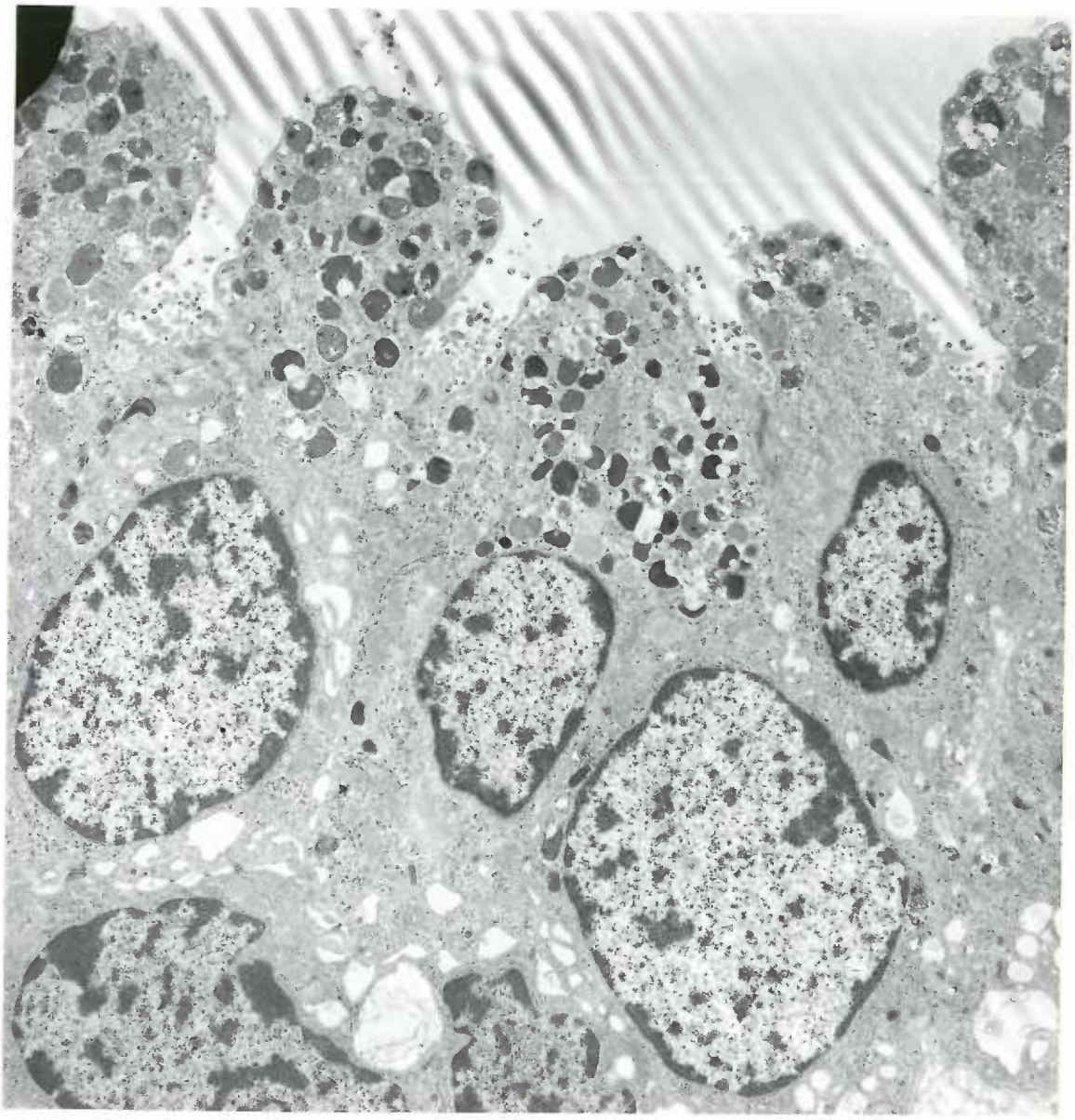


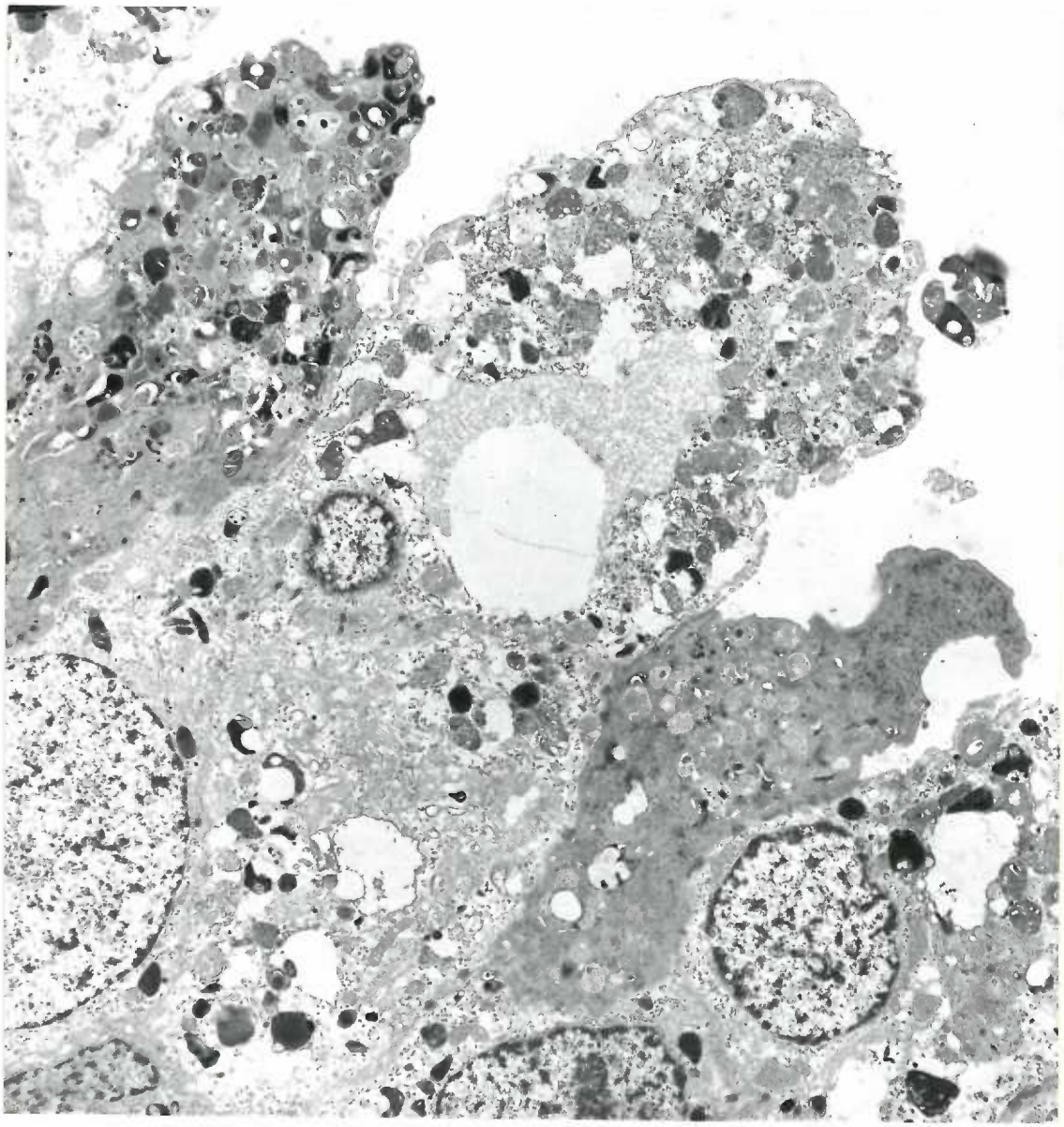
Fig. 27

Figure 28. Tall columnar cells fixed for 2 hours in 20% glutaraldehyde, rinsed briefly and post-fixed 1 hour in 1% osmium-S-Collidine. The secretory vacuoles are filled with dense homogeneous secretions. There are very few empty vacuoles, but large intercellular lacunae give the appearance of cytoplasmic vacuoles similar to those seen with less concentrated glutaraldehyde. 7,930 X.



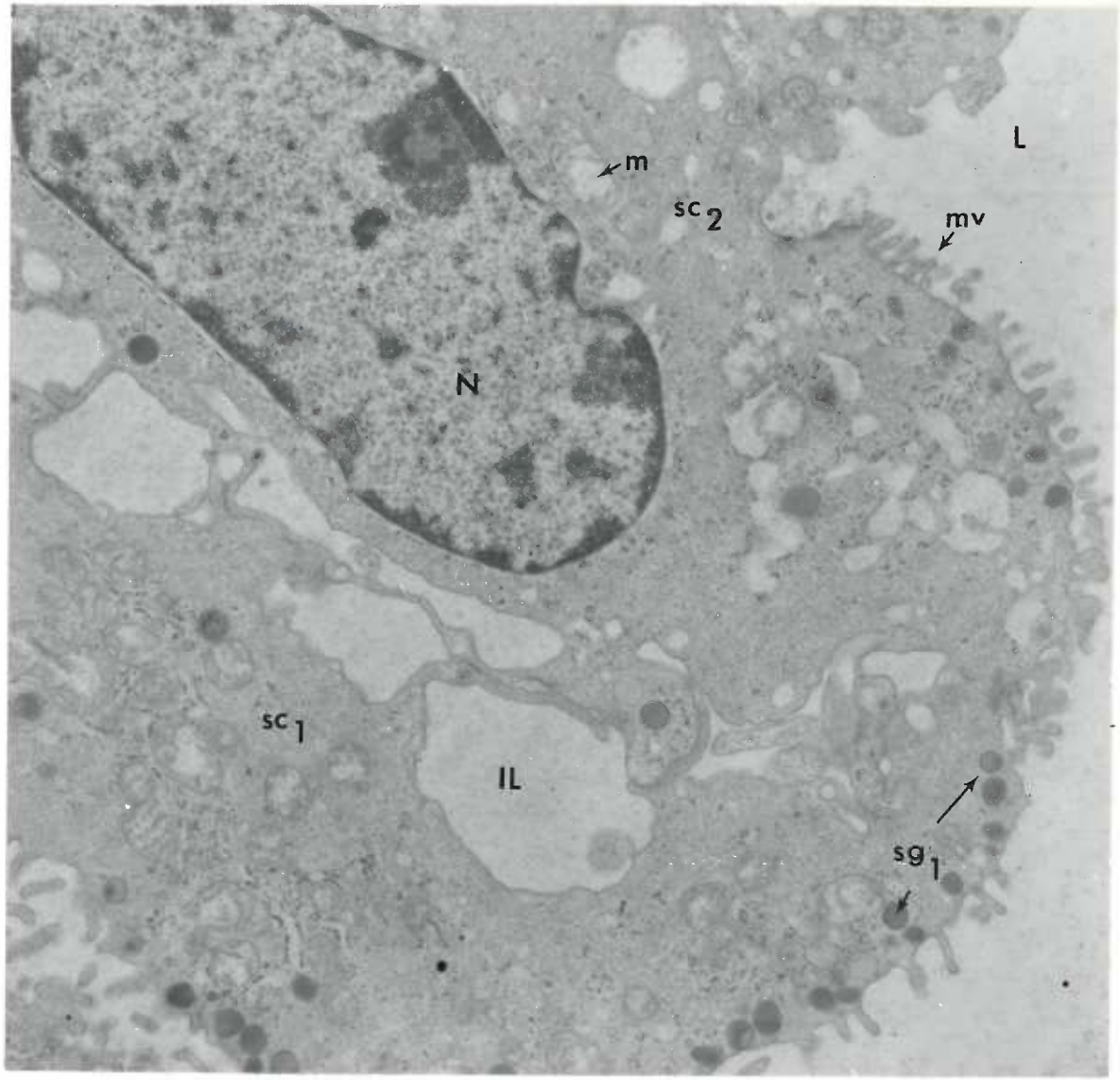
28

Figure 29. Tall columnar cells prepared the same as the tissue in Fig. 28. The electron dense dark cells correspond to the dark cells present in the 1u sections used for light microscopy as shown in Fig. 8. The cytoplasm of the dark cell appears much more compact than the adjacent light cell; the tip of the cell on the right is attenuated similar to those in low columnar cells, and probably represents an apocrine secretory body. The matrix of the light cell is electron lucent, it appears similar to cells fixed in less concentrated glutaraldehyde as in Fig. 62. 7,930 X.



29

Figure 30. A cuboidal cell from hyperplastic tissue, fixed in 5% glutaraldehyde for 1 hour and postfixed in 1% osmium-S-Collidine after a brief rinse in buffer. Small dense bodies are lined up along the cell periphery. Large intercellular lacunae exist between the two cells, similar to those observed after 20% glutaraldehyde fixation, in Fig. 28.
15,700 X.



30

Figure 31. Cuboidal cells with an inactive appearing cytoplasm. Fixed in 1% osmium buffered in veronal acetate with .005M uranyl nitrate added. The cytoplasm contains filaments similar to those in low columnar cells fixed in 2% osmium in Fig. 18. There are several small dense bodies, but no evidence of secretory activity. 17,370 X.

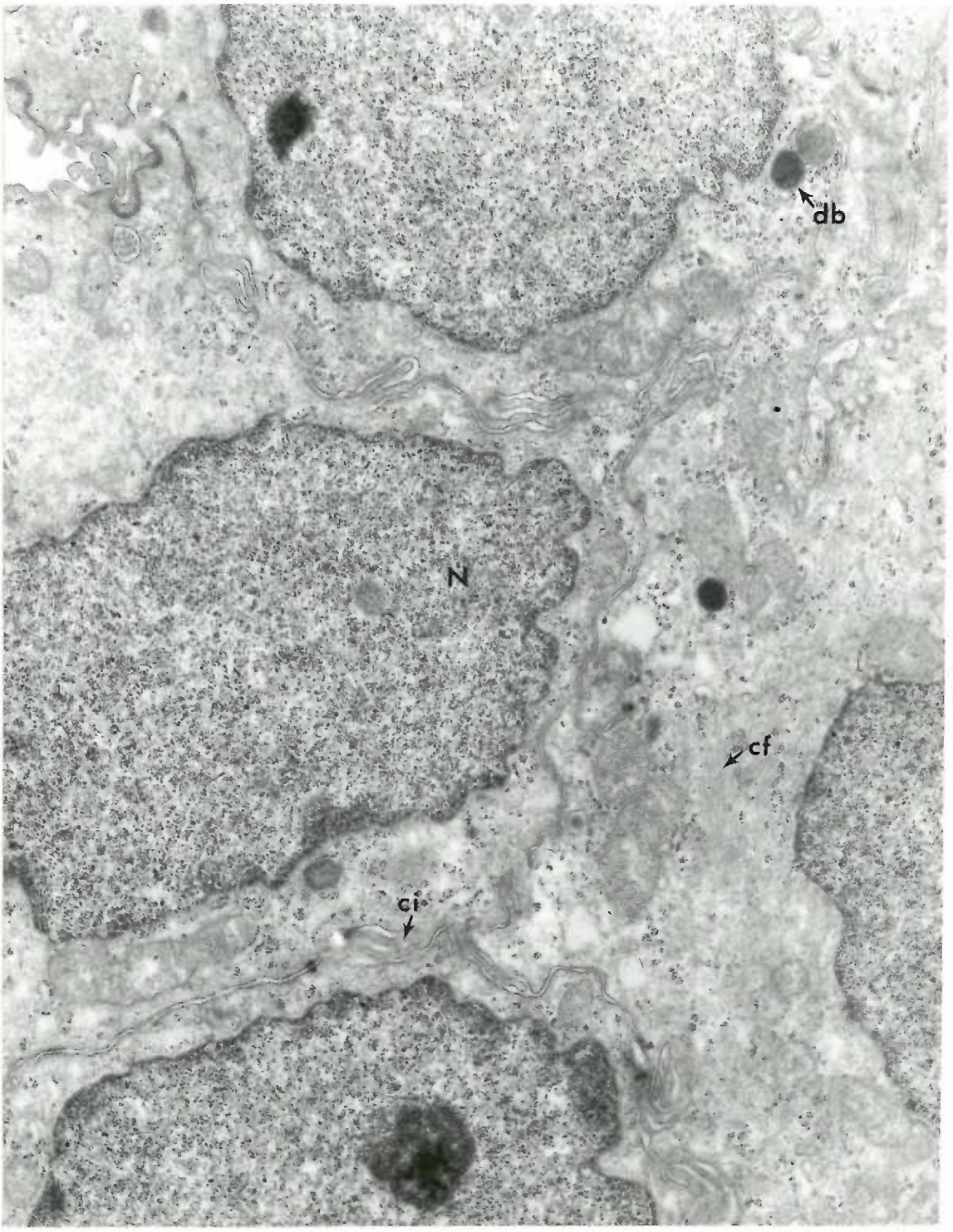


Fig. 31

Figure 32. Cuboidal cells from hyperplastic tissue fixed in 3.5% glutaraldehyde, washed for 24 hours and postfixed 1 hour in 1% osmium-S-Collidine. The small dense bodies (Sg_1) appear both in the cell cytoplasm and in the cell lumen as secretory products (SP). One of the secretory granules contains the ghost of a crystalline like structure (arrow). 20,000 X.

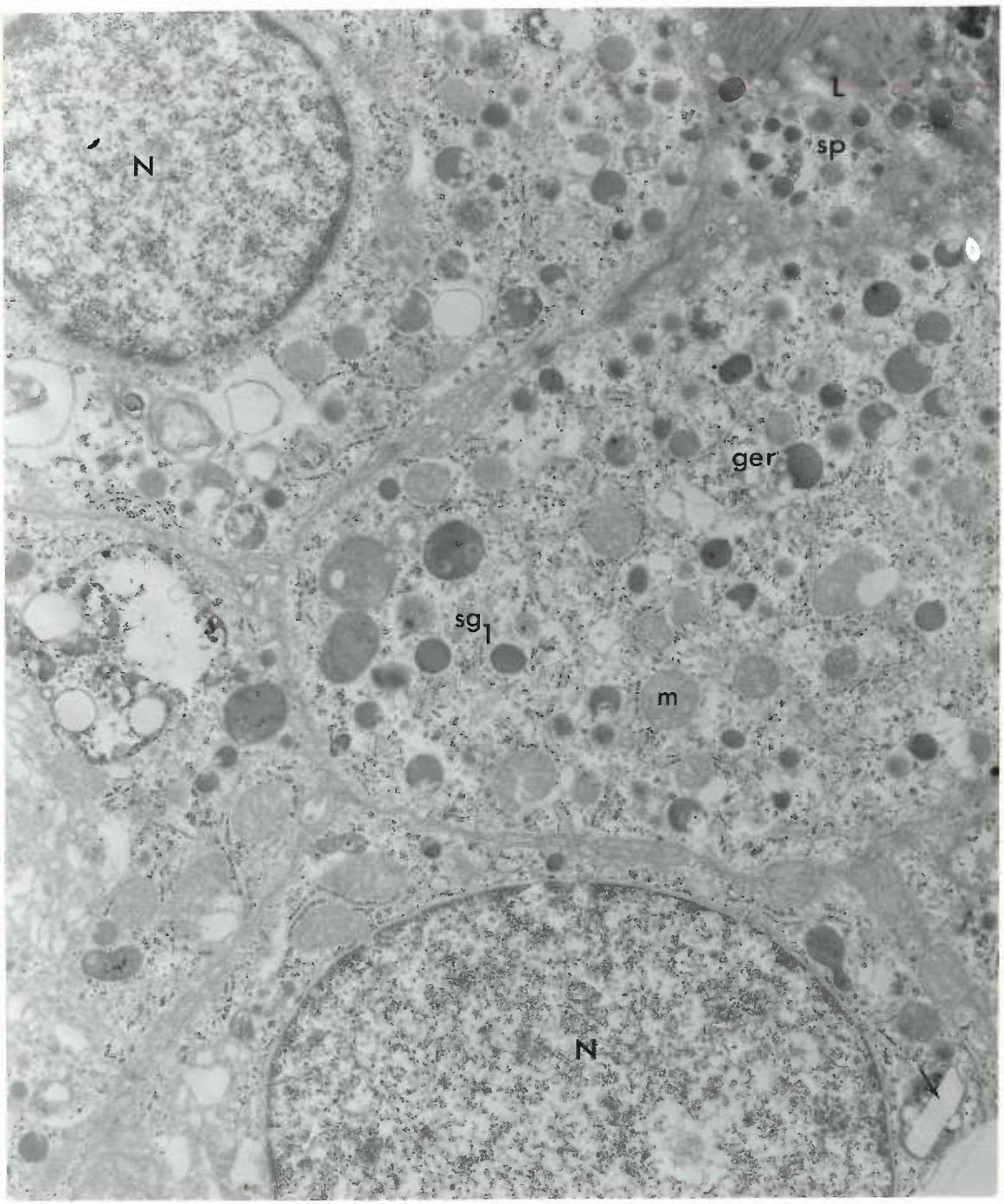
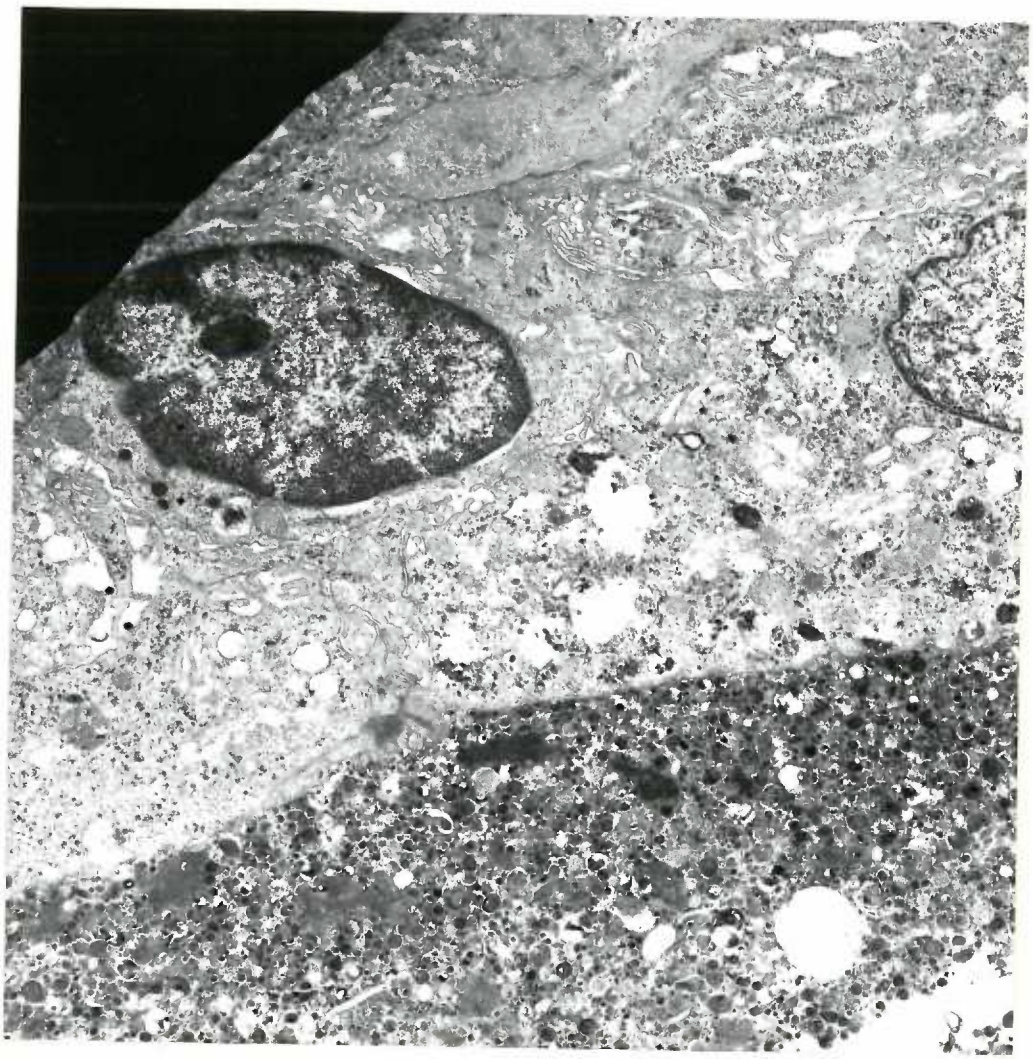
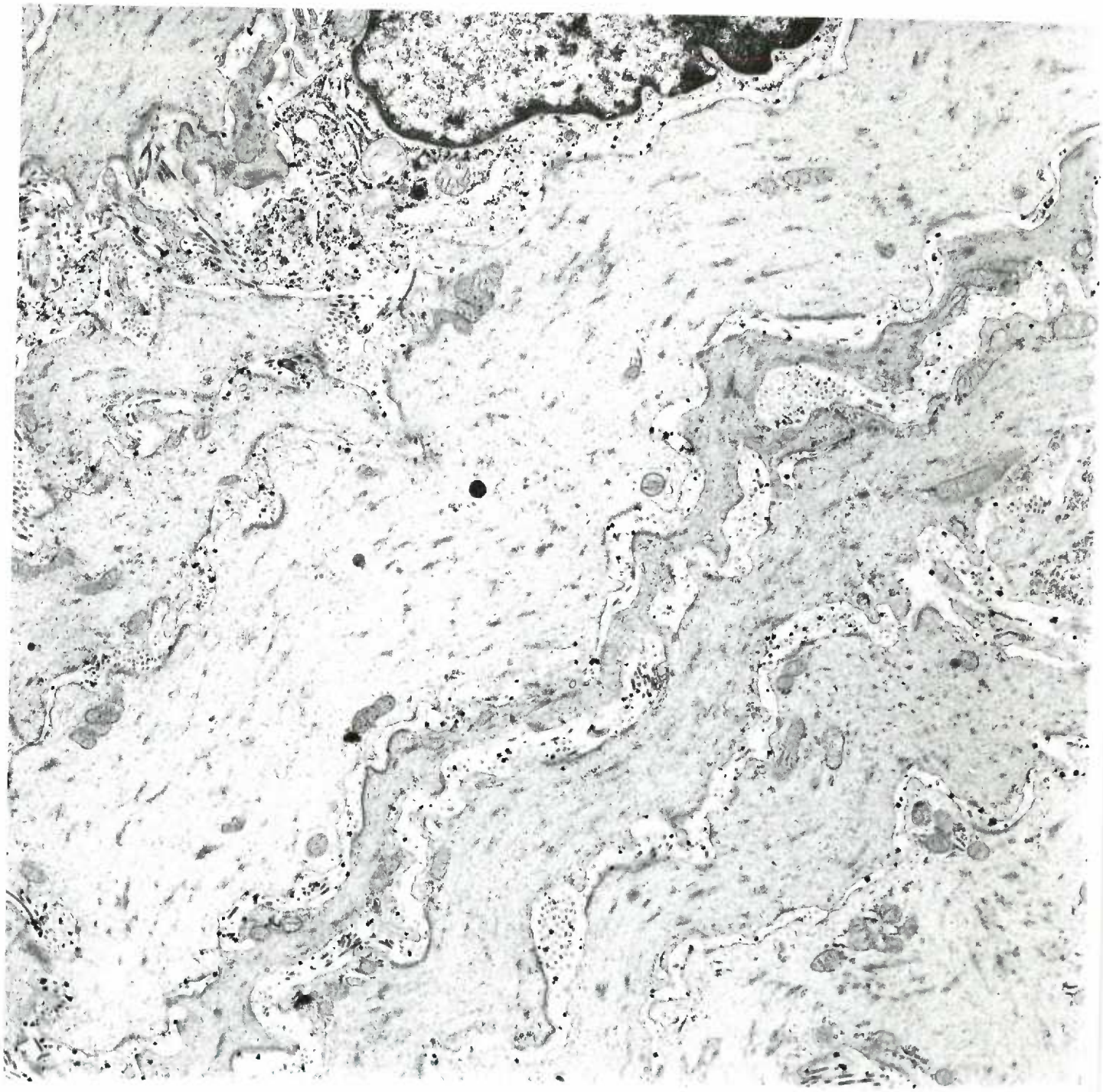

N
L
sp
ger
sg1
m
N

Figure 33. Secretory product situated in a prostatic duct, fixed in 1% osmium-S-Collidine for morphological study. The cuboidal duct cell has several vacuoles in the cytoplasm but no evidence of secretory granules or other secretory activity. The small dense droplets in the lumen are probably secretory products from secretory cells nearby. 10,180 X.

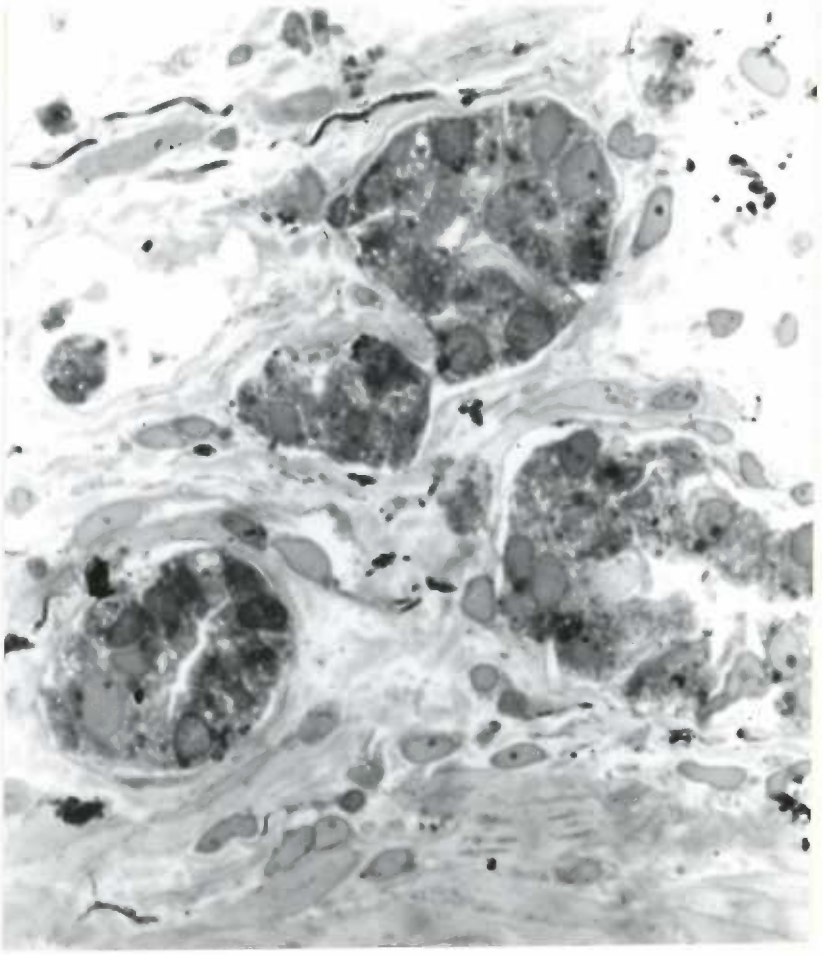


33

Figure 34. Small glands from a histological Grade II cancer specimen. Prepared with 1% osmium-S-Collidine for 2 hours. The electron micrograph associated with this area is shown in the next figure. 750 X.



Guinea Pig Control Isth ATP 11,600X



34

Figure 35. Cancer cells from the specimen in Fig. 34. The cytoplasm is filled with lipid droplets, smooth vesicles containing an electron lucent material, abundant mitochondria, and Golgi complexes. There is no evidence of secretory products or dense bodies. The nucleus appears to be lobulated and cut through a fold, exposing the nuclear pores. 6,860 X.

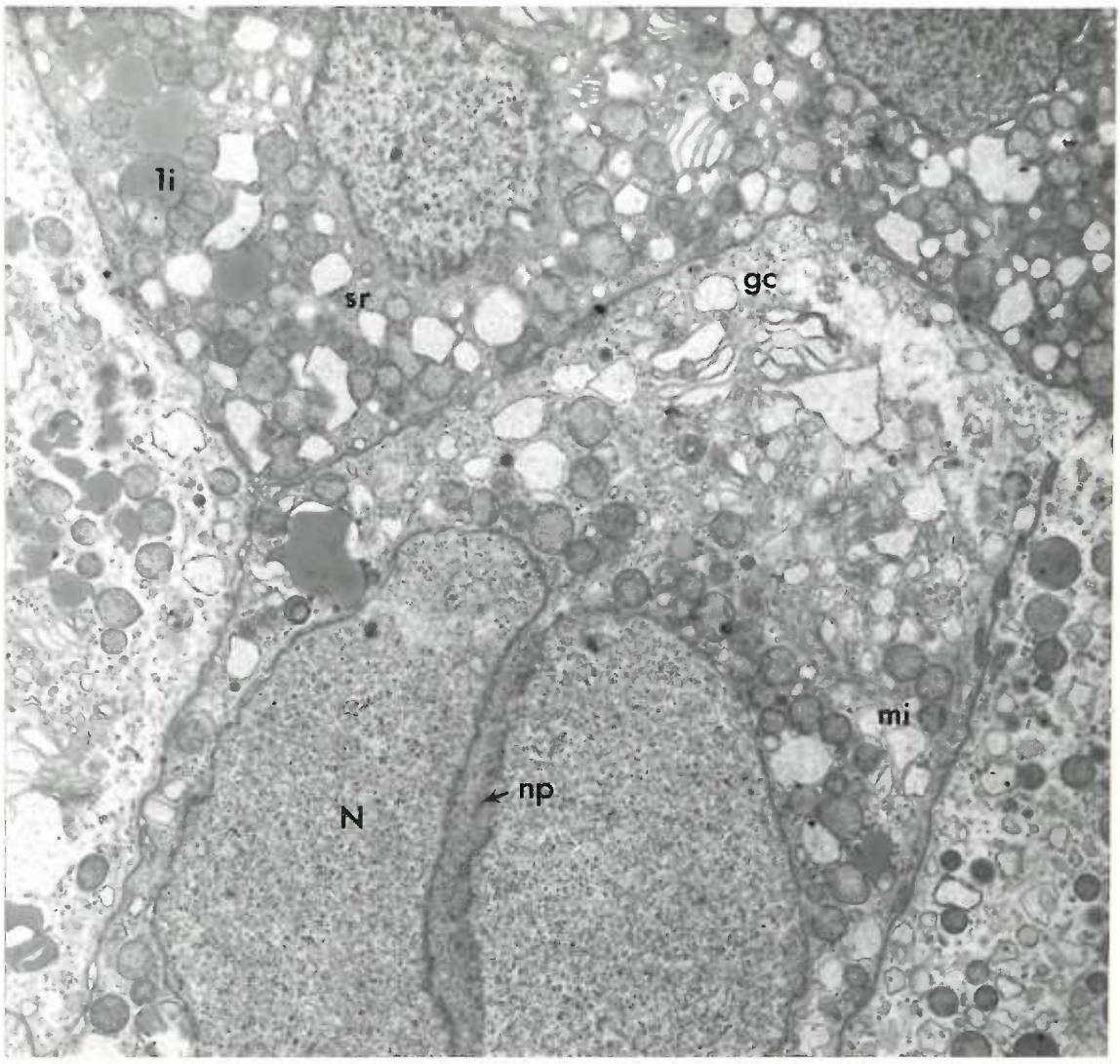


Fig. 35

Figure 36. Cells from a histological Grade II cancer as shown in Fig. 34. The nucleus is large and contains a prominent nucleolus. The cytoplasm contains several large Golgi complexes, lipid droplets surrounded by a rippled membrane, and many small dense bodies. A few of the mitochondria are large and electron dense; they have a fibrillar matrix similar to that shown by Mao.[71]

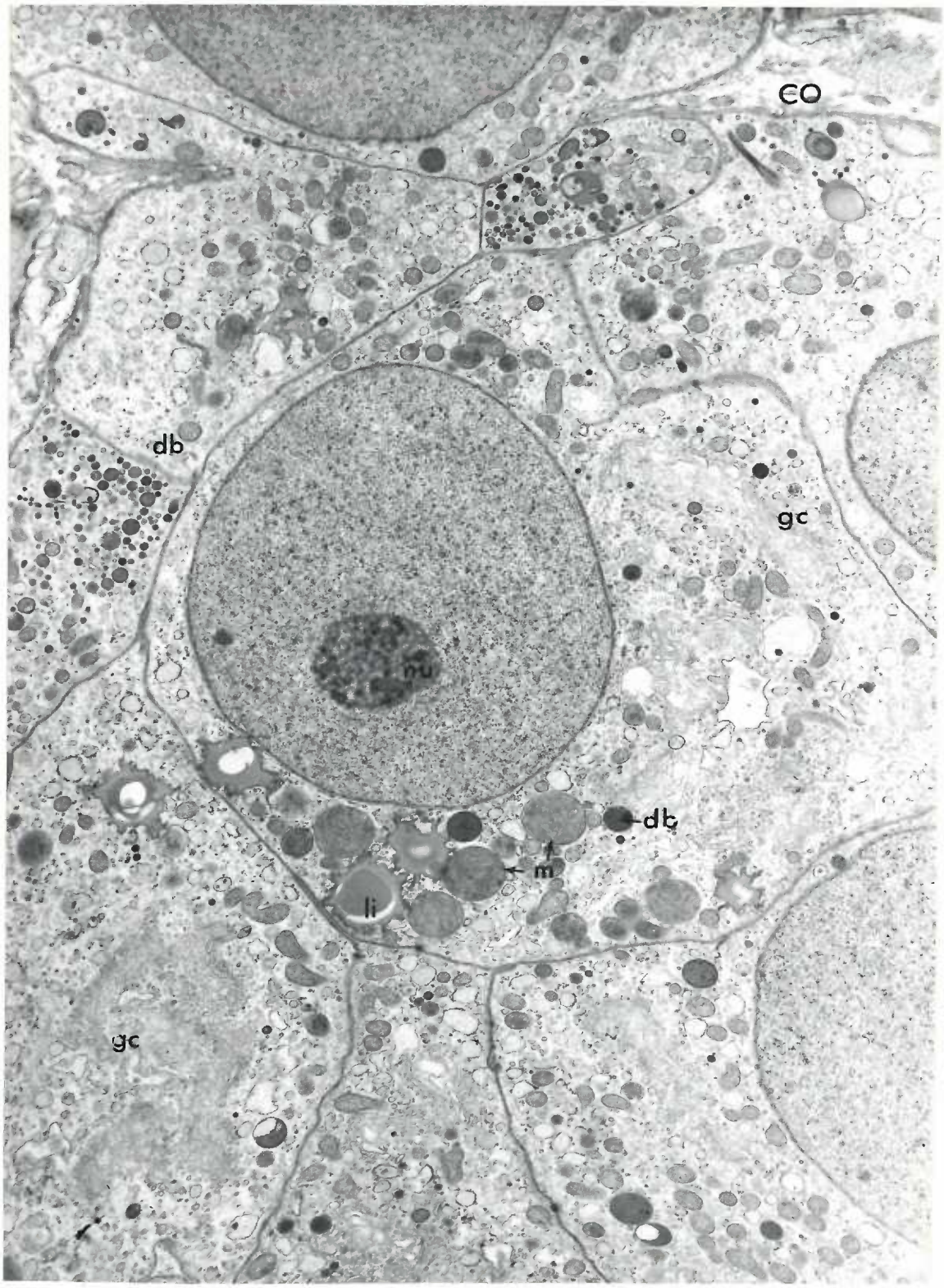
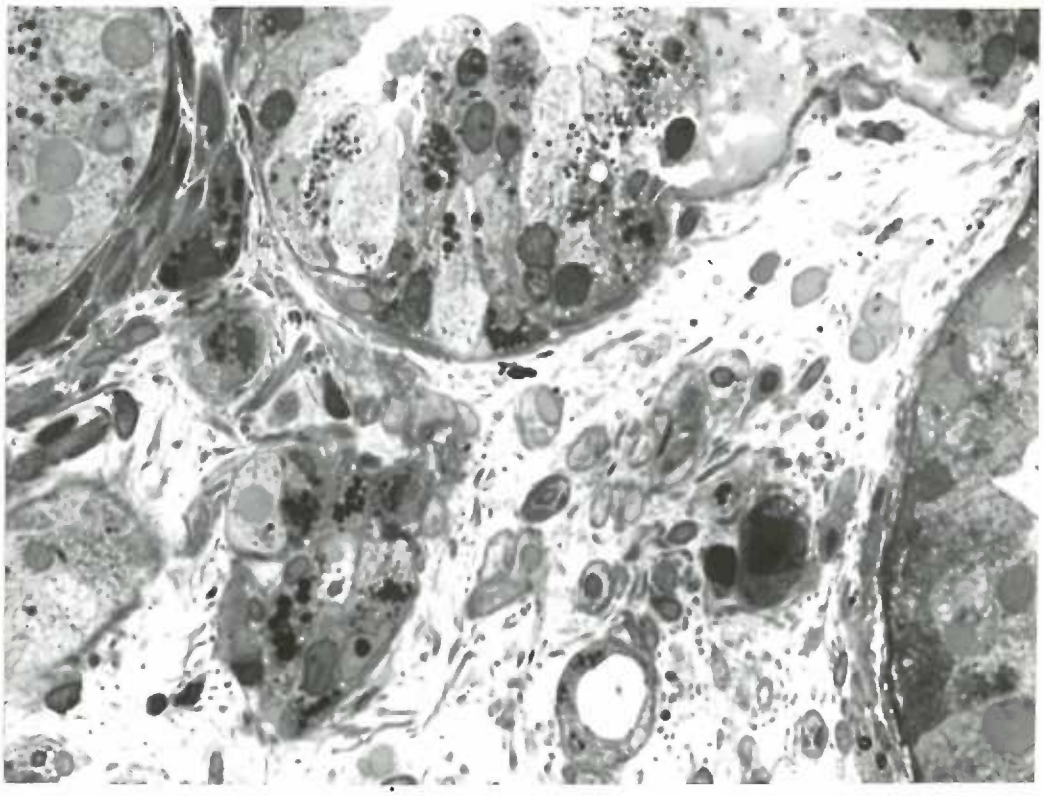
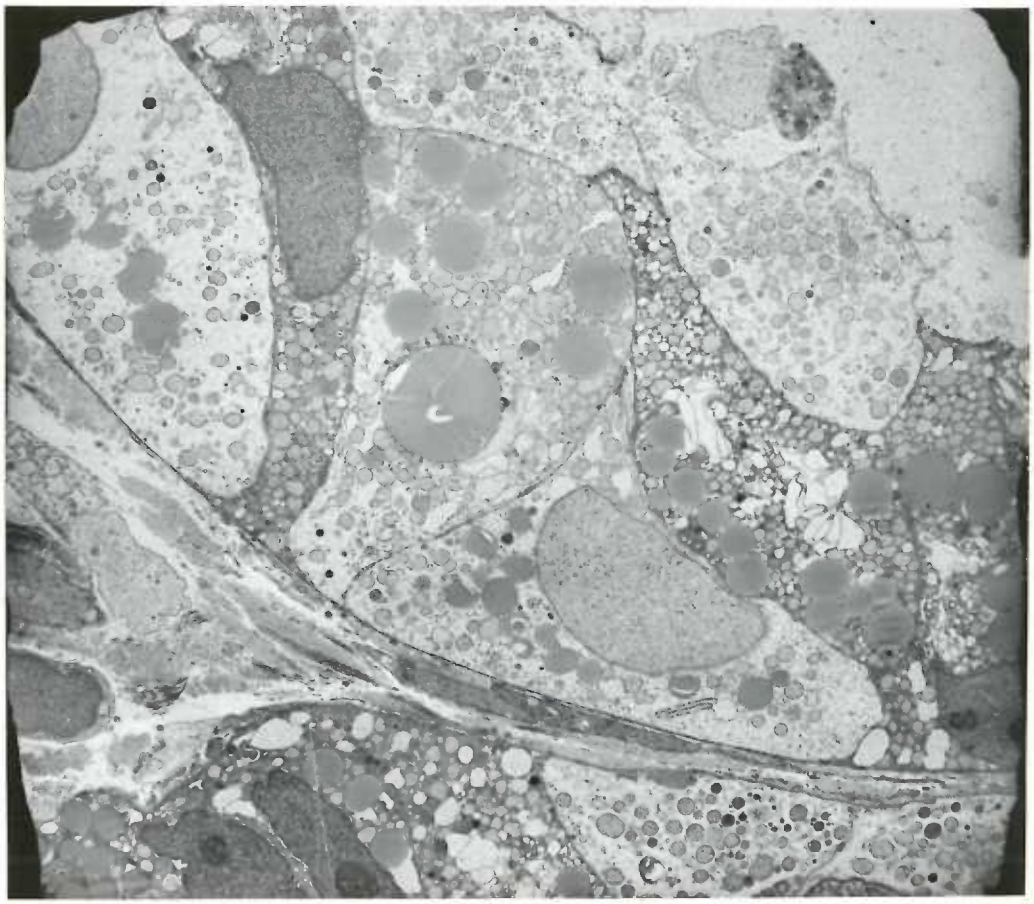

CO
db
gc
nu
db
m
li
gc

Figure 37. A light micrograph of Grade II cancer tissue prepared for morphology with fixation in 1% osmium-S-Collidine for 2 hours. The cells are very heterogenous; there are no recognizable basal cells, but dark and light cells are present along with cells of intermediate density. An electron micrograph of cells from this area follows in Fig. 38. 615 X.



37

Figure 38. Grade II cancer cells from a specimen similar to that in Fig. 37. Prepared for morphological study by fixation with 1% osmium-S-Collidine for 2 hours. Dark and light cells both have large lipid droplets and smaller round vacuoles filled with moderately electron dense material. The cytoplasm between the vacuoles is electron dense in the dark cells, and lucent in the light cells. A basal lamina surrounds the cells and appears intact, but cells from another gland, at the bottom of the picture, lie close by with only a small amount of collagen and two small smooth muscle cells between the two. These would be considered "back-to-back" glands with the light microscope. The arrow points to a unique structure which probably is an annulate lamellae. 2,500 X.



38

Figure 39. Grade III cancer cells prepared for morphological study by fixation in 1% osmium-S-Collidine for 2 hours. The cell membranes of the light and dark cells appear to fuse with the outer membrane of the smooth muscle cell. The arrow points out a possible membrane barrier between the two cells, but it is poorly developed. The mitochondria of the dark and light cell are large with a very electron lucent matrix. They contain only a few short cristae. 8,300 X.

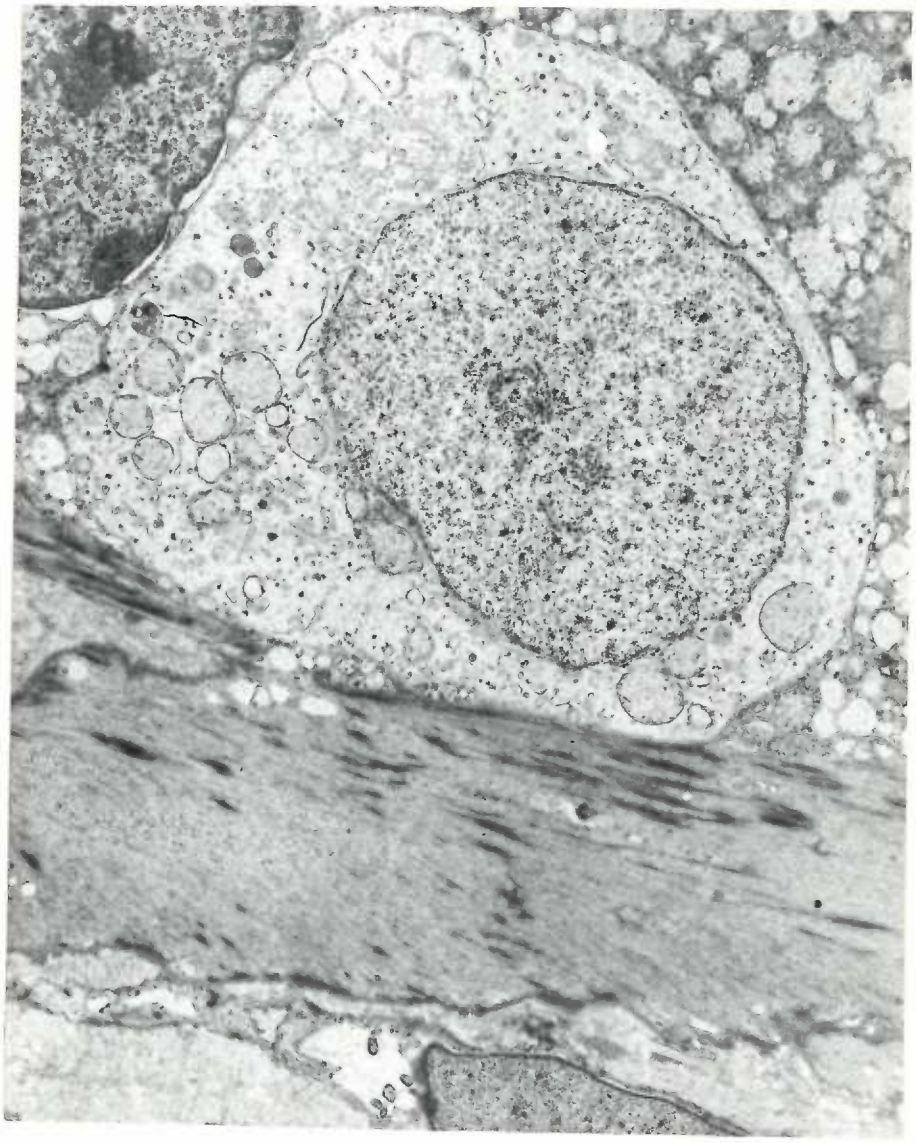
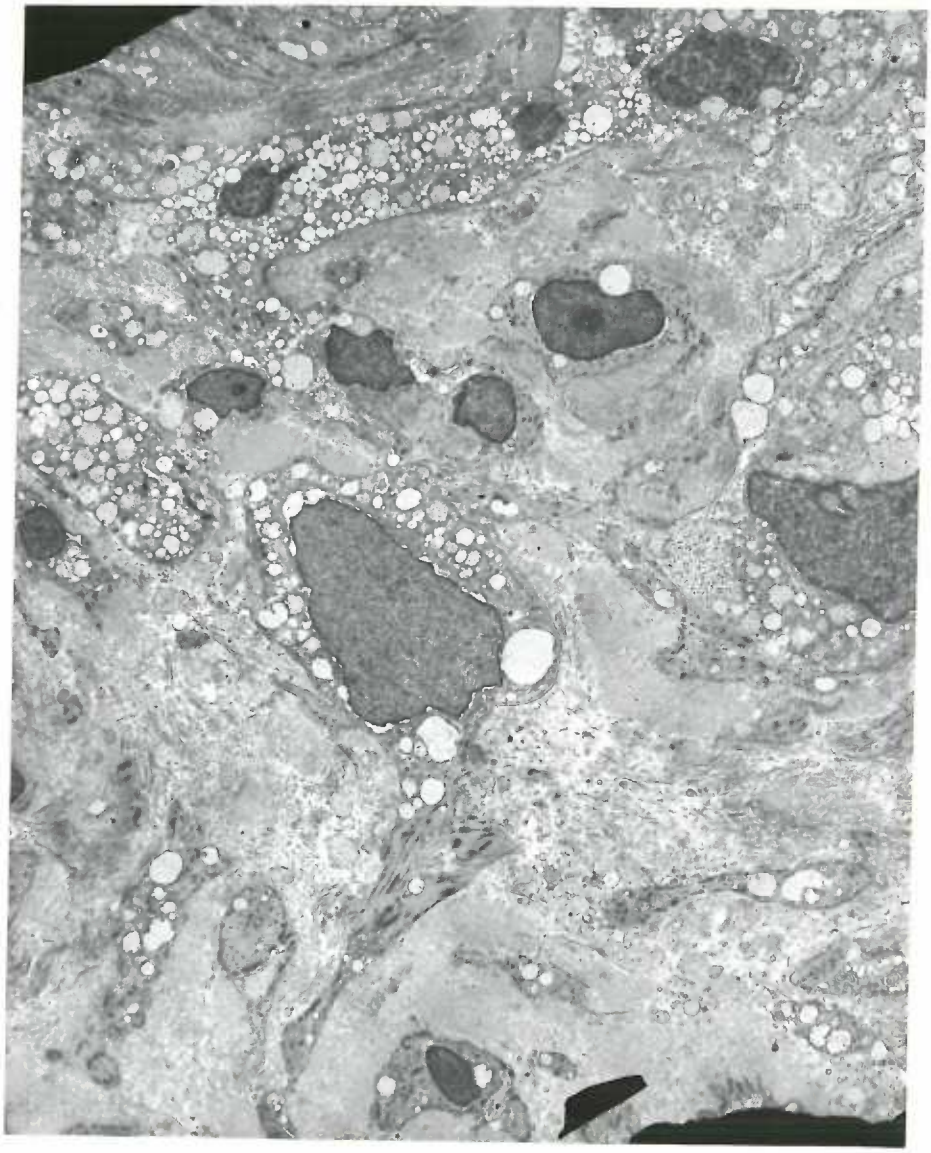
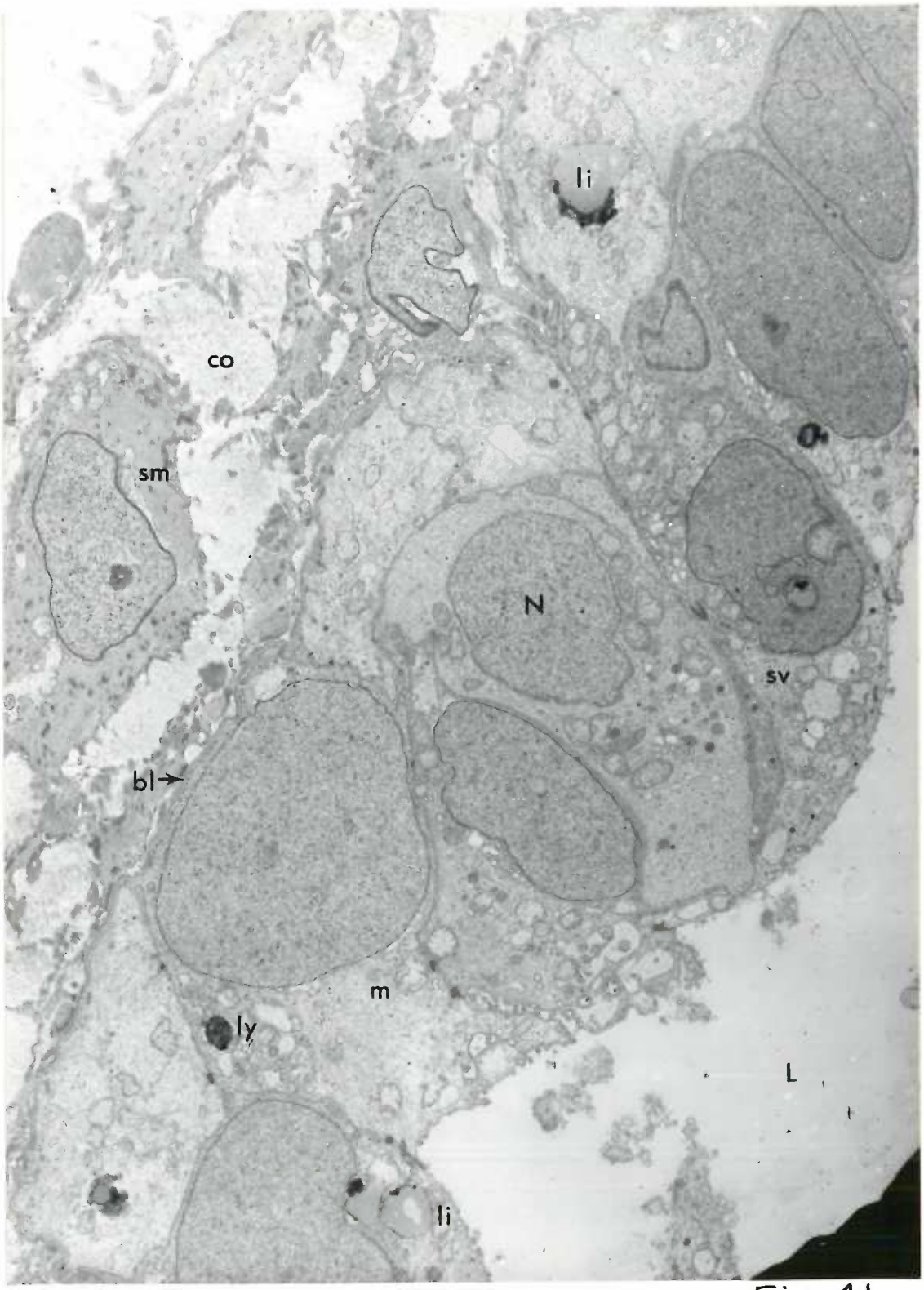


Figure 40. Grade III cancer cells invading the stroma in a preparation fixed for morphological study with 1% osmium-S-Collidine for 2 hours. The cancer cells are surrounded by a basal lamina, but in some instances the lamina is disrupted and the muscle and cancer cells are intimately associated with each other, as pointed out with the arrow. Both cancer cells and smooth muscle cells have similar cytoplasmic vacuoles, but they are more numerous in the cancer cells. 3,800 X.



40

Figure 41. Well differentiated cancer cells from a specimen that contained predominately Grade II and III tissue. The nuclei are large but without prominent nucleoli as expected in cancer cells from light microscope observations. There is a distinct basal lamina separating the epithelium from the stroma, but there is less collagen between the smooth muscle and basal lamina than is usually seen in hyperplastic tissue. At some points, the smooth muscle cells appear to touch the basal lamina. Several large lysosome s with lipid droplets are present in each cell. 6,560 X.



6563X

Fig. 41

Figure 42. Grade III cancer cells fixed for morphological study with 1% osmium-S-Collidine for 2 hours. Two large cancer cells with markedly vacuolated cytoplasm are surrounded by stromal cells. The outer membranes of the cancer cells are indistinct. They appear to be formed by short sections of fibrous material, as pointed out by the arrows, similar to a basal lamina. 6,300 X.

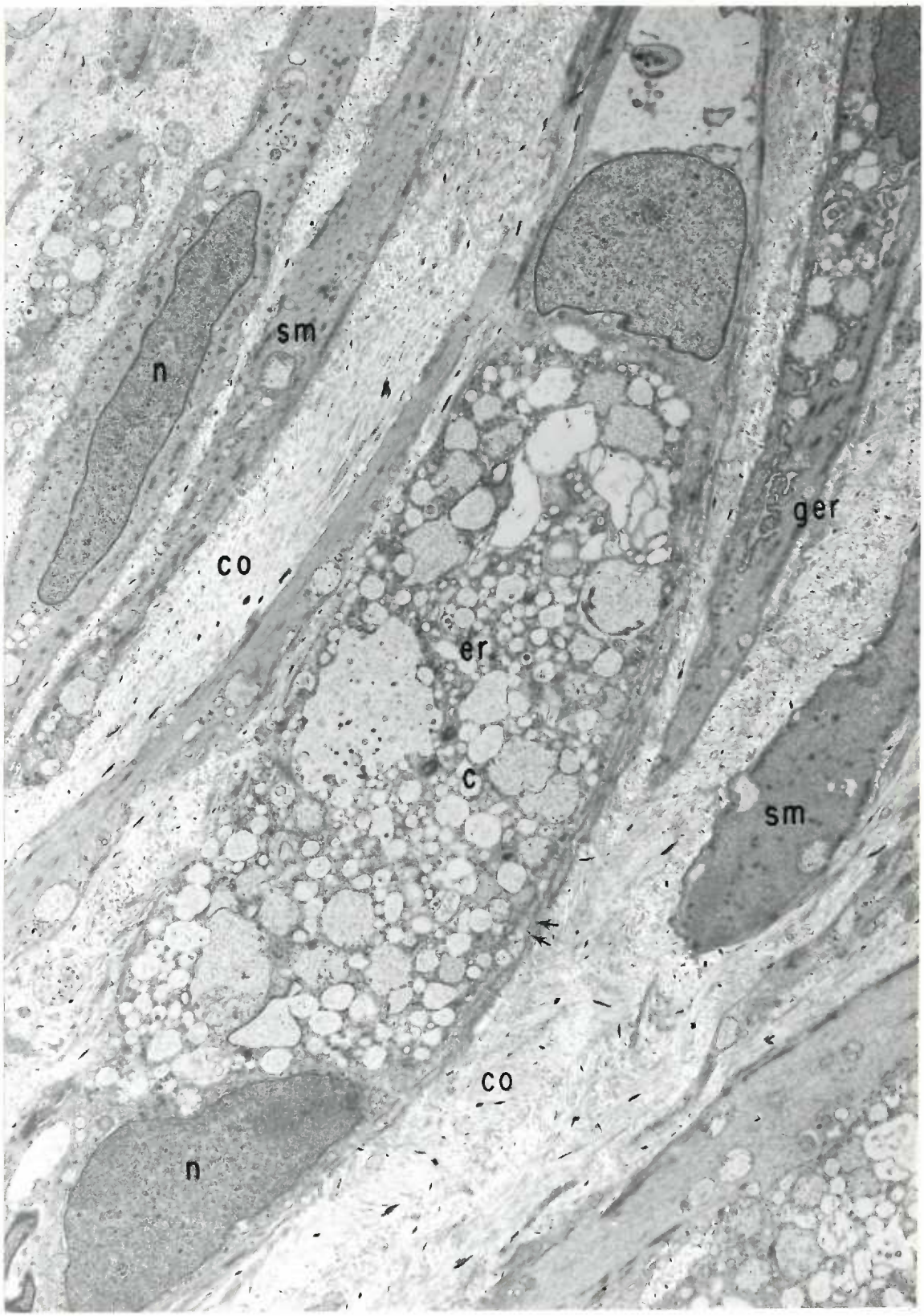
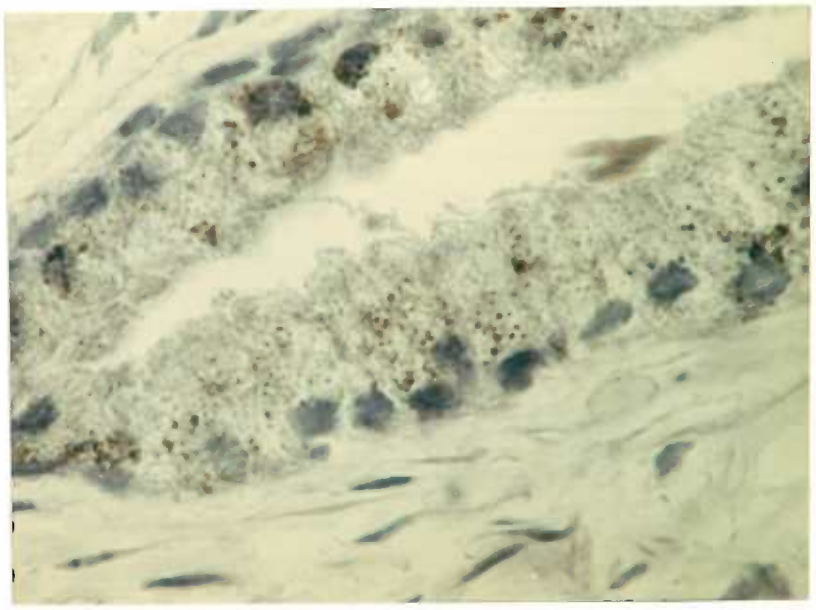

n
sm
co
ger
er
c
sm
co
n

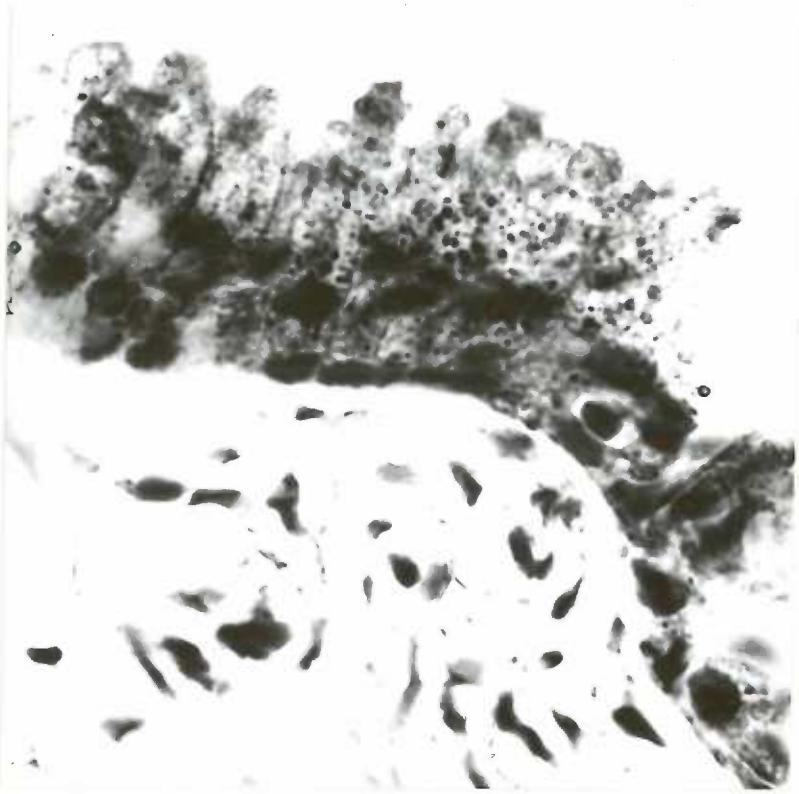
Figure 43. Tall secretory cells from hyperplastic tissue, incubated for 2 minutes for acid phosphatase activity in the modified Gomori's medium, counterstained with Harris's hematoxylin for light microscopy. The reaction product is a fine, brownish-black precipitate located in the supra-nuclear region and around the nucleus. Both the stroma and the nuclei are free of reaction product. 600 X.



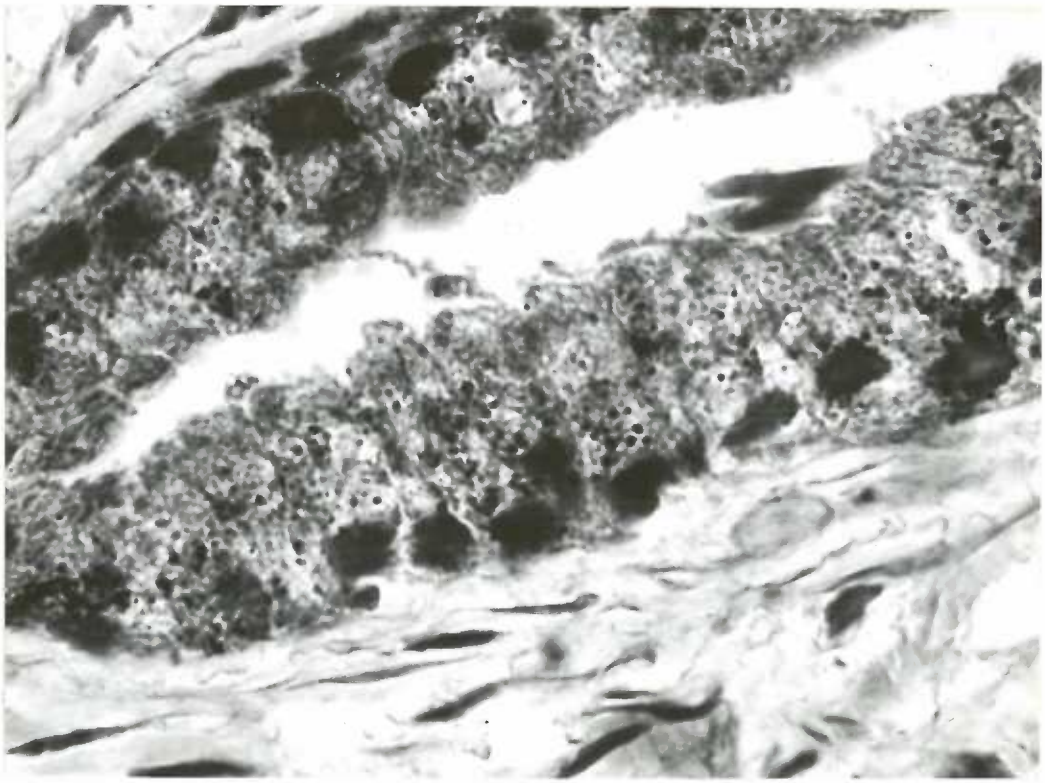
43

Figure 44. Frozen sections reacted for esterase activity with thiolacetic acid substrate for 10 minutes. The lead sulfide reaction product is located in large granules in the supranuclear region, the stroma and nuclei are free of reaction product. 1150 X.

Figure 45. A black and white light micrograph of the material shown in Fig. 43, to compare the localization of the acid phosphatase reaction product with that achieved for esterase activity. Both appear to be localized in the supranuclear and perinuclear regions of the tall secreting cells. 1150 X.



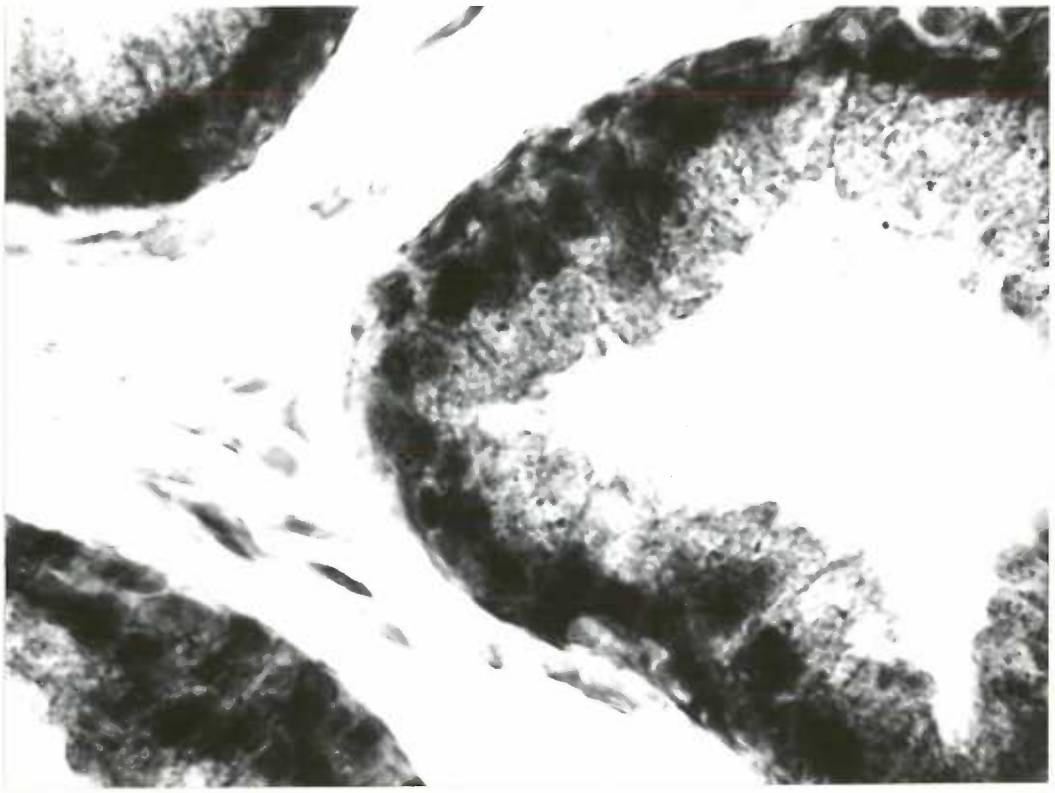
44



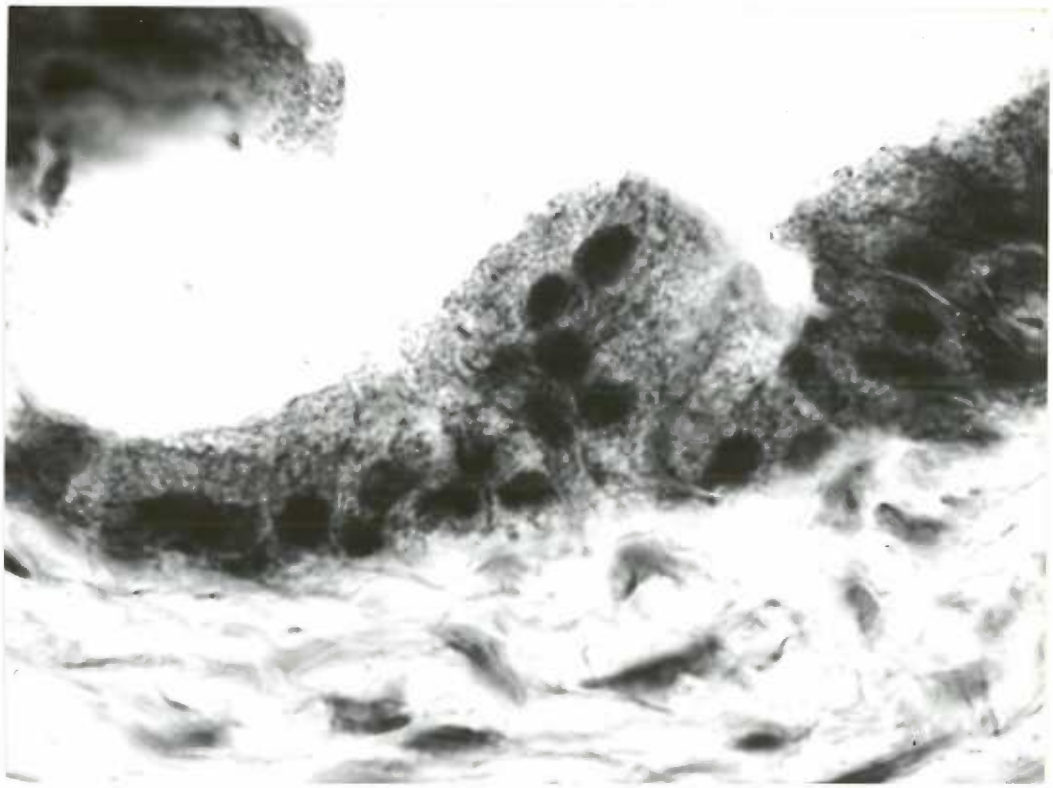
45

Figure 46. Hyperplastic tissue preincubated with 10^{-7} M E-600 for 10 minutes, and then incubated for 10 minutes with the thiolacetic acid substrate and the inhibitor together. There are a few dense reaction granules similar to those in Fig. 44, but in general, there is less evidence of enzyme activity than in the untreated tissue in Fig. 44. 1150 X.

Figure 47. Control tissue prepared by incubating for 10 minutes for esterase activity without the substrate added to the medium. There are a few dense cytoplasmic granules but they are smaller and less dense than those seen in Fig. 44 and 45. 1150 X.

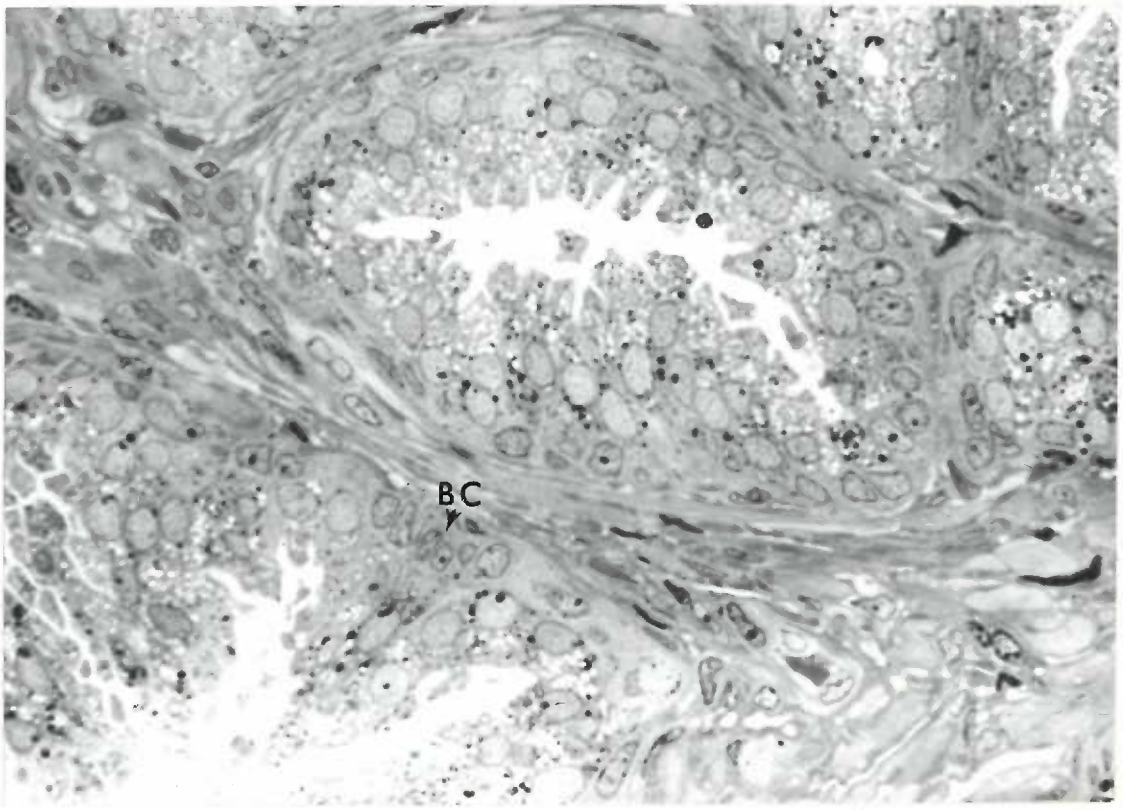


46



47

Figure 48. A one micron thick section of hyperplastic tissue incubated for esterase activity for the electron microscope. The tall cells have a vacuolated cytoplasm and small dense granules similar to that found in unincubated tissue, (Fig. 5 and 6). 720 X.



48

Figure 49. Tall columnar cells fixed for enzyme cytochemical study with 5% glutaraldehyde for 2 hours, washed in buffer for 1 hour, incubated for esterase activity for 15 minutes and post-fixed 1hour in 1% osmium-S-Collidine. Thin sections for electron microscopy were stained with lead citrate and uranyl acetate. Reaction product granules are visible in the granular secretory products (Sg_2) but the dense homogeneous secretions are so electron dense, it is difficult to define reaction precipitate in them. Secretory products in the lumen contain a small amount of reaction product unassociated with specific organelles, this may represent a non-specific staining artifact. The mitochondria have disrupted matrices, but are distinguishable by the arc of ribosomes associated with them. They remain free of reaction product. The nuclei and cell membranes also lack evidence of enzymatic activity. 5,300 X.

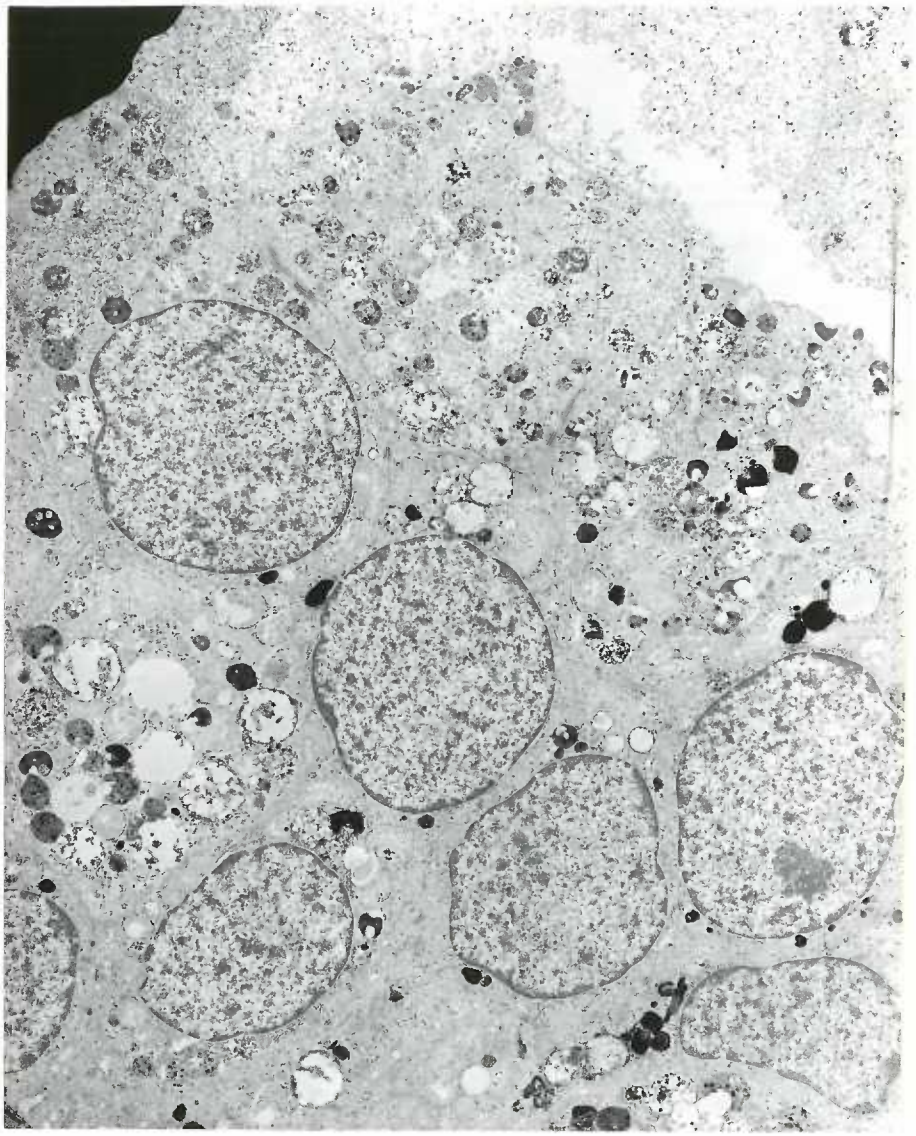


Figure 50. Tall columnar and basal cells prepared for enzymatic study the same as tissue in Figure 49. The basal cells contain small lysosomes with moderate amounts of enzyme activity. A large lysosome with a small lipid droplet in it also contains activity; the large lysosome was present only in this section, and was not seen in other hyperplastic specimens. The lipid droplet (li) in the secretory cell has a fine rim of reaction precipitate. 7,000 X.

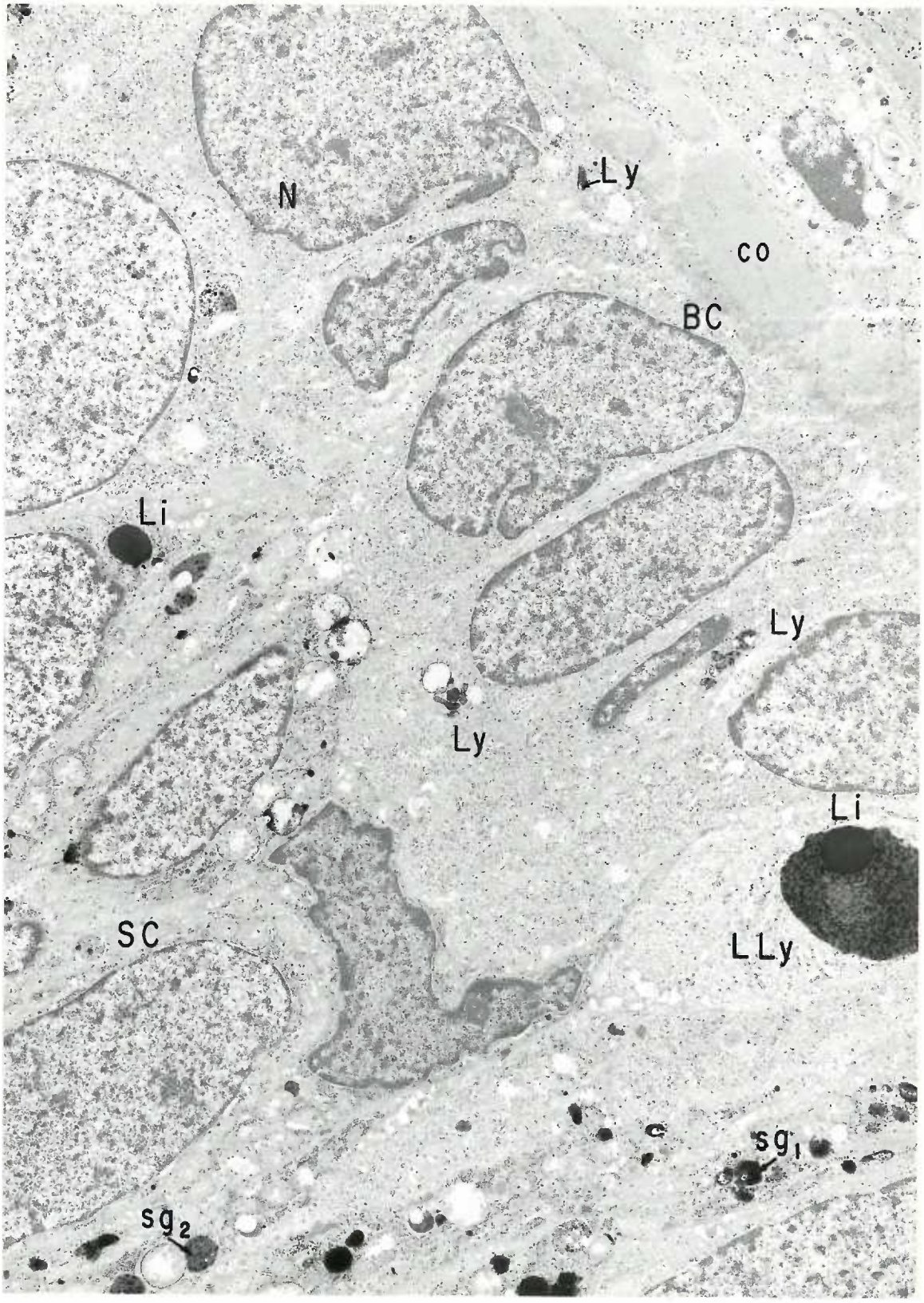

N
LLy
co
BC
c
Li
Ly
Ly
Li
SC
LLy
sg1
sg2

Figure 51. Hyperplastic tissue fixed initially in 10% glutaraldehyde for 2 hours, but otherwise prepared for enzyme activity the same as tissue in Fig. 49. Enzyme reaction product (rp) lies in the granular secretions and around the edge of lipid droplets (li). The area outlined is shown at higher magnification in the next figure to illustrate the amount of reaction product found in the dense homogeneous secretions. (Sg_1). 7,000 X.

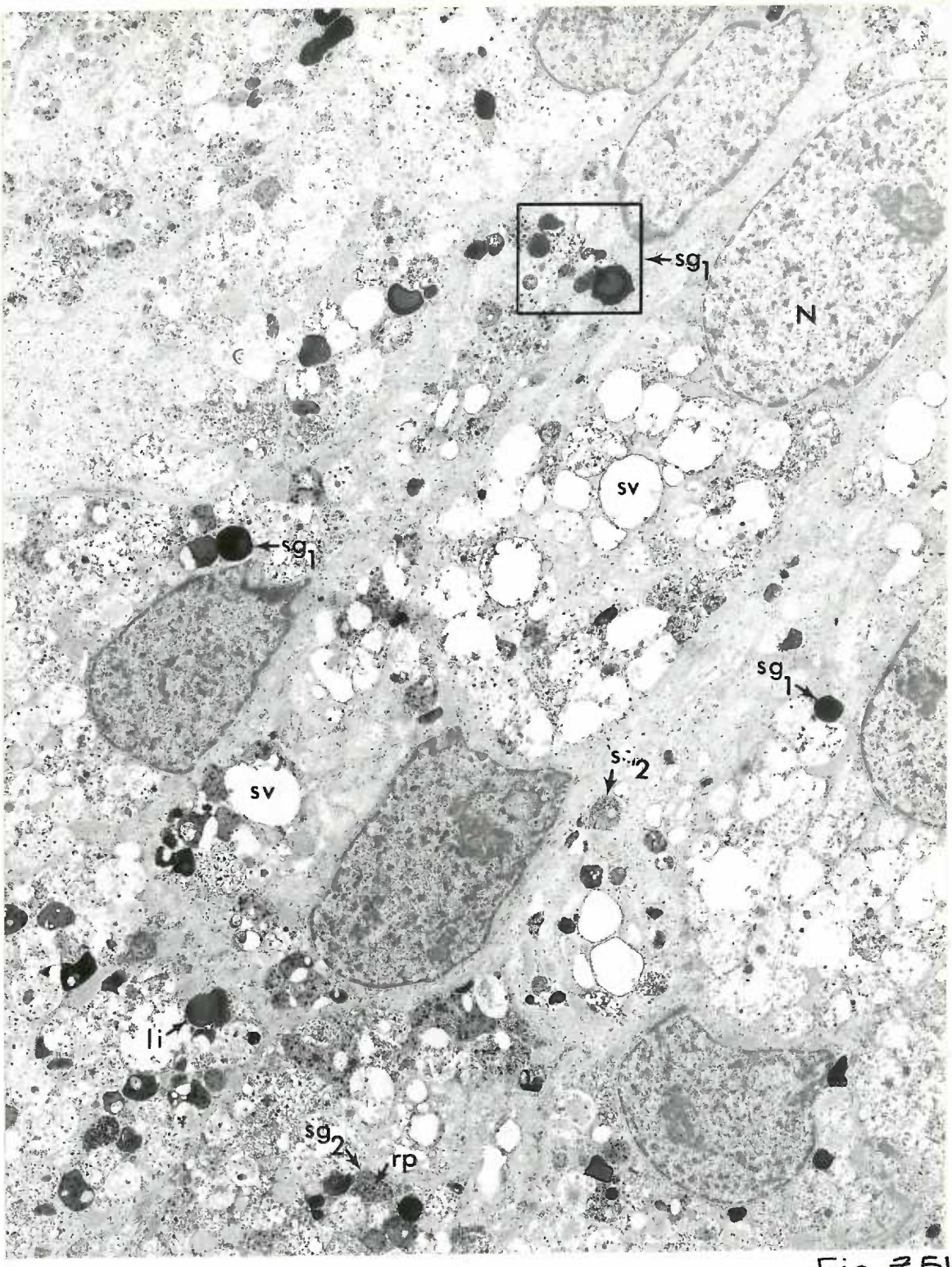


Fig. 251

Figure 52. A higher magnification of the area outlined in Fig. 51. The reaction deposits in the dense homogeneous secretions are visible in about the same concentration as in the more electron lucent secretions nearby. The reaction granules are more discernible when compared with the control section in Fig. 61. 40,800 X.

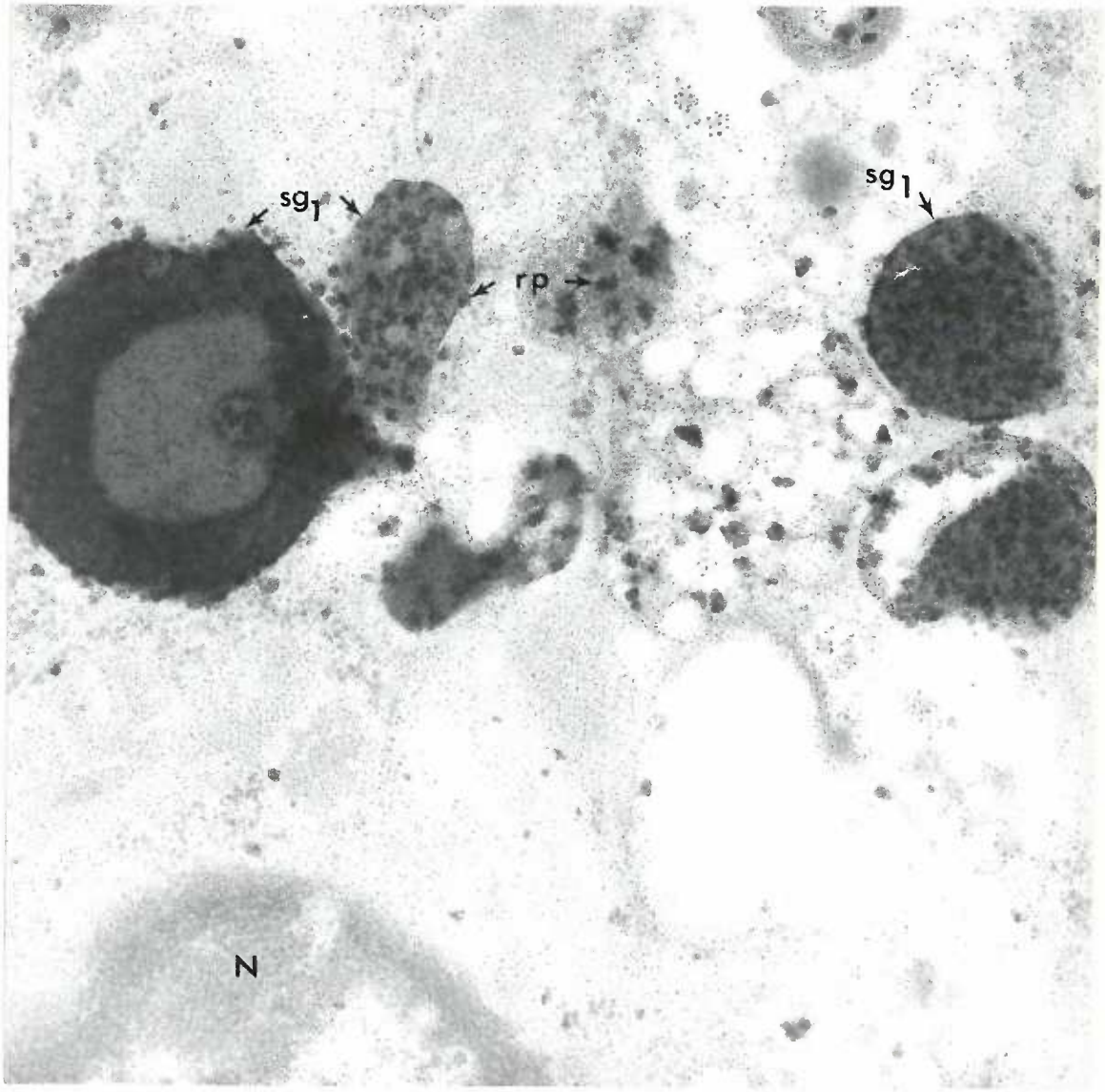
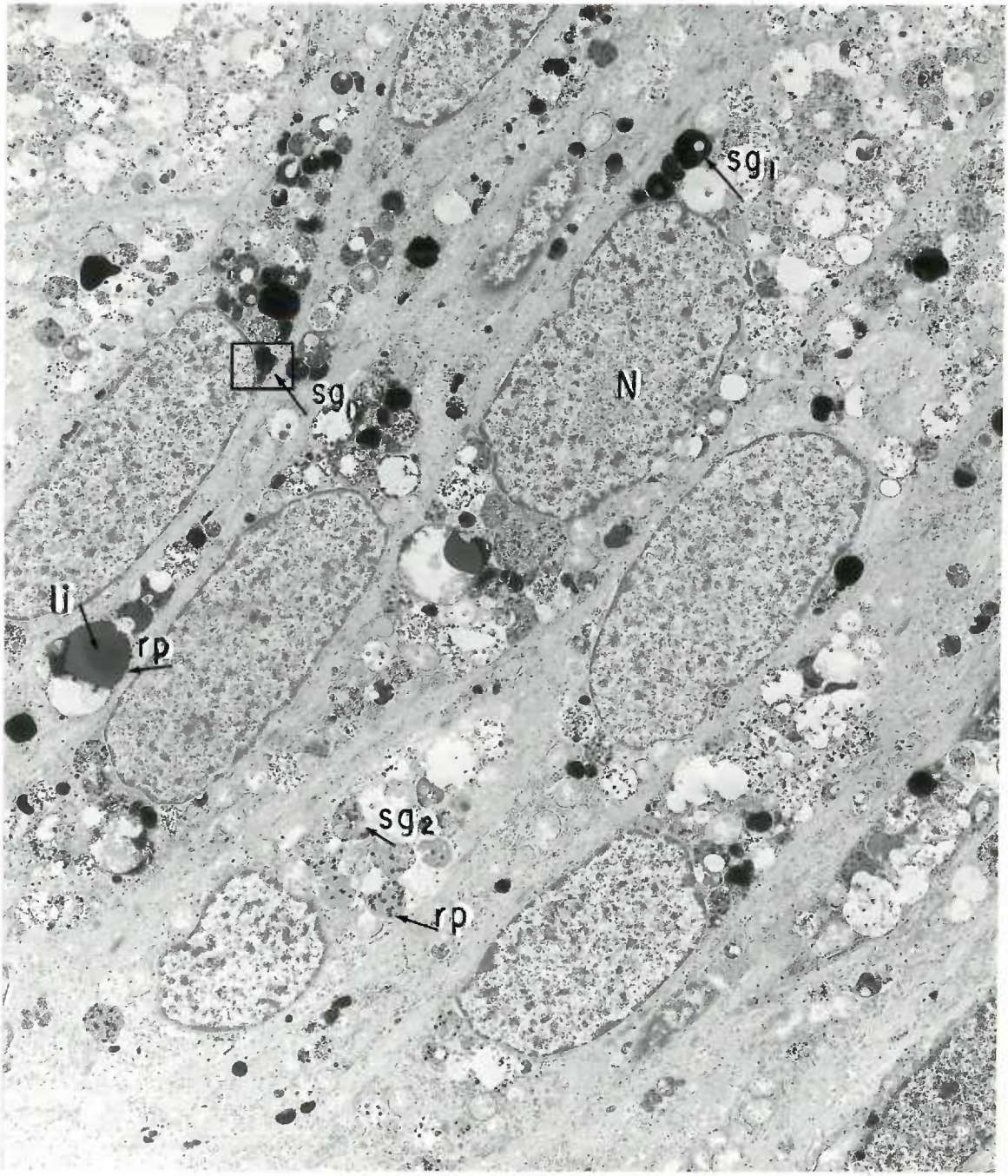


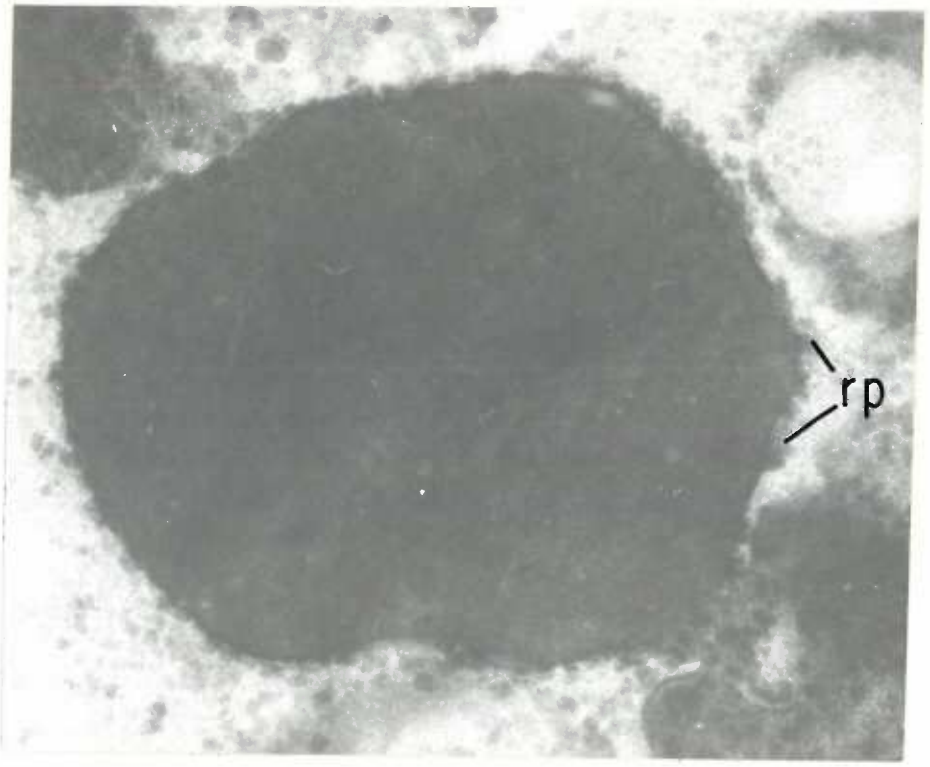
Fig. 52

Figure 53. Tall secretory cells prepared the same way as tissue in Fig. 49. The cytoplasm is filled with secretory products and empty vacuoles. The granular secretions (Sg_2) have reaction product that is easily visible, but the matrix of the dense secretions have obscured the fine reaction particles. The area outlined is shown on the next page at higher magnification. 6,300 X.

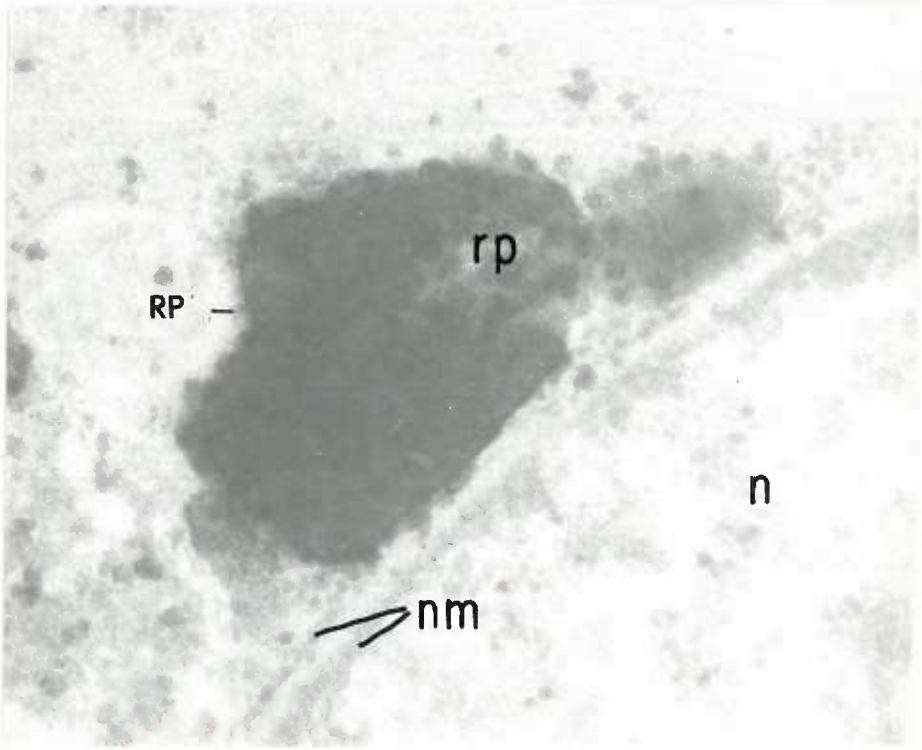


53

Figure 54 and 55. Higher magnification of the dense secretory products outlined in Fig. 53. The reaction product situated along the edge of the secretion is clearly distinguishable from the secretory material, but that in the matrix blends in with the dense secretion. In Figure 55, the reaction product appears to overly the secretions as well as lie along the edge. Both figure are magnified 62,900 X.



54



55

Figure 56. Tall secretory cells incubated for 15 minutes for esterase activity. The secretory products contain reaction deposits; small dense bodies in basal cells also have enzyme reaction product localized in them. 20,360 X.

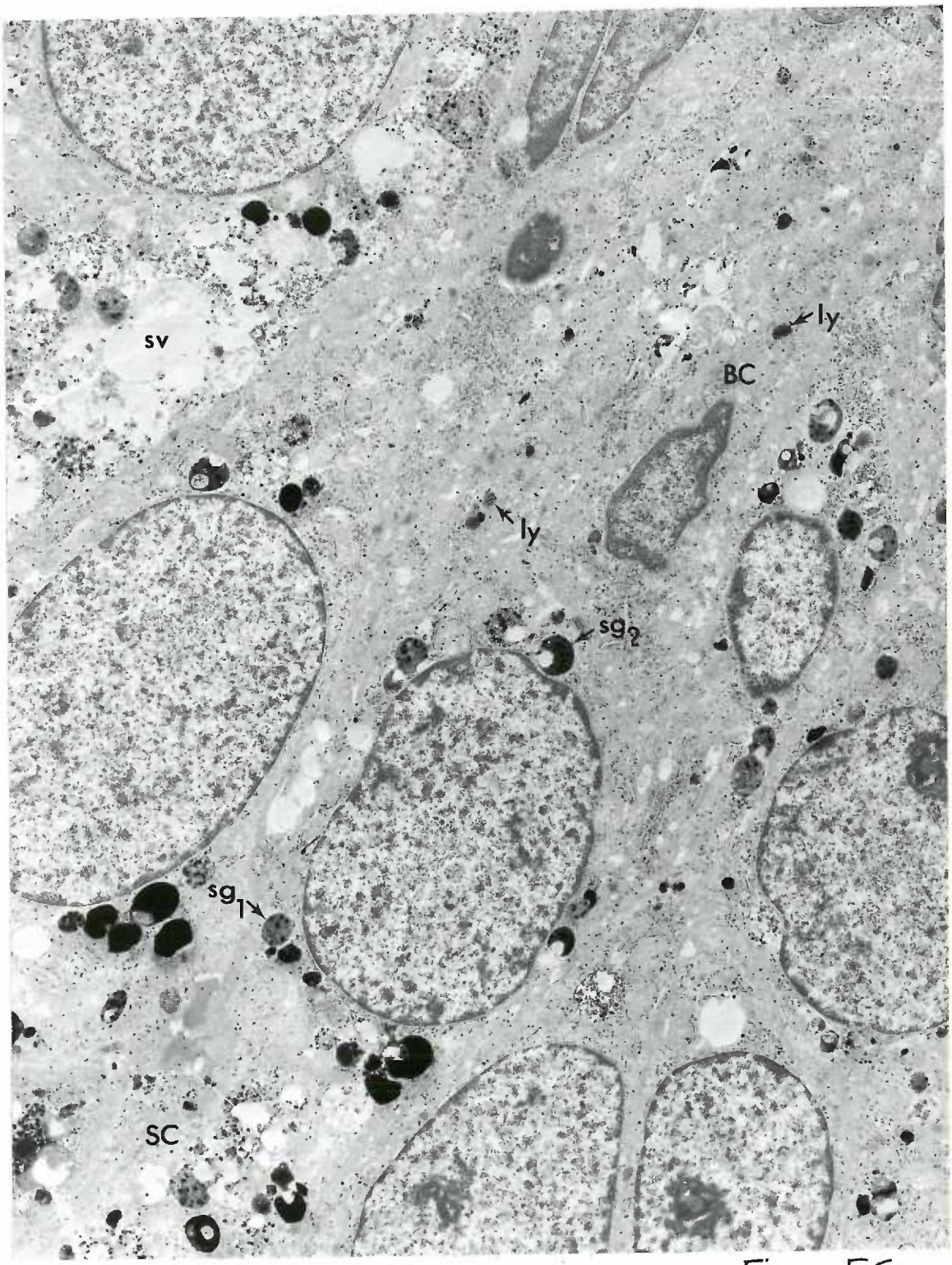
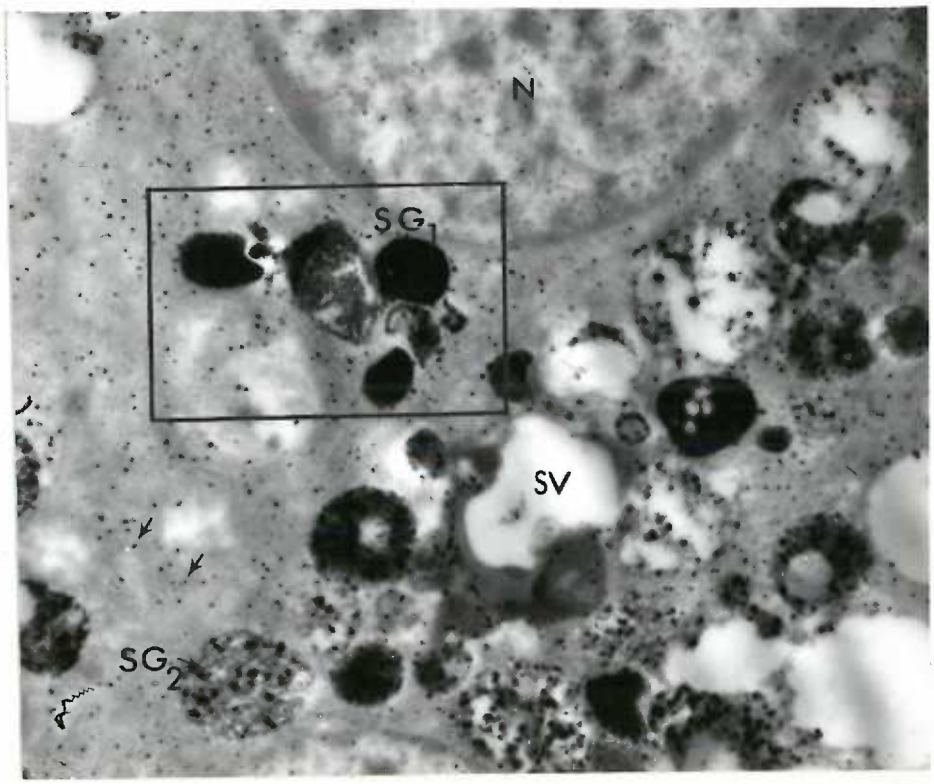
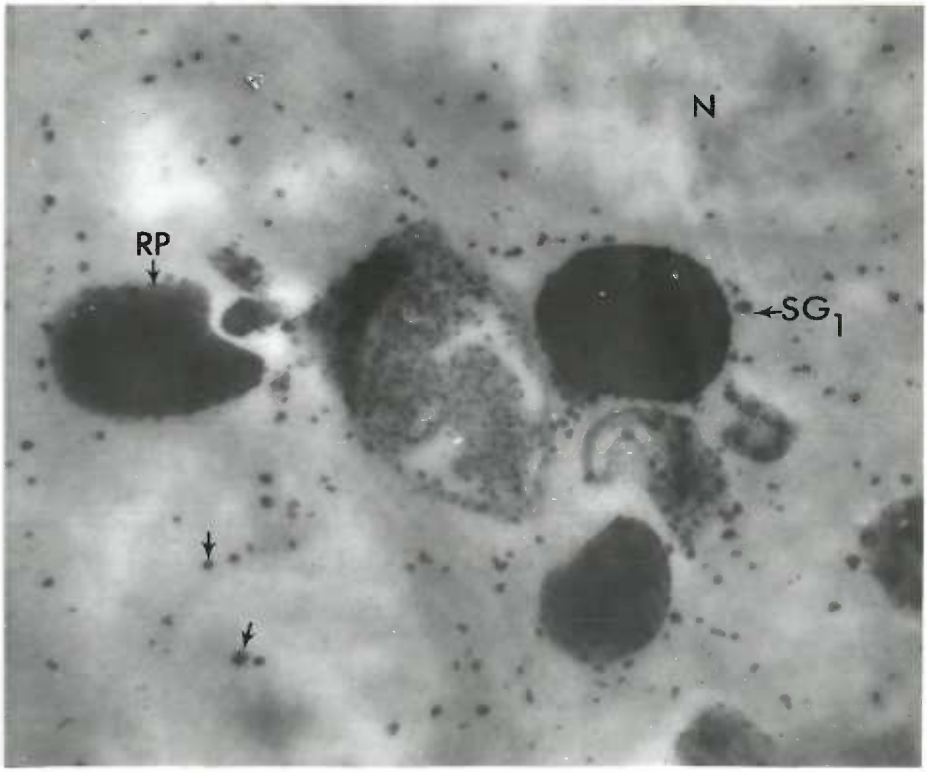


Fig. 56

Figure 57 and 58. A portion of a tall secretory cell prepared the same as tissue in Figure 49, but unstained with lead citrate and uranyl acetate. The reaction product is sharply contrasted to the cytoplasm of the cell, but still not distinct in the dense secretory granules. Figure 58 is a higher magnification of the outlined area, a small amount of reaction granules lie over the dense secretions, but most of the density appears to be due to the secretory material, and not to the reaction product. The arrows point out granules unassociated with particular organelles and apparently represent nonspecific reaction artifact. Figure 57: 11,000 X. Figure 58: 37,700 X.



57



58

Figure 59. Low columnar cells prepared for enzyme study with fixation in 3.5% glutaraldehyde for 1 hour, washed 2 hours in buffer and incubated for esterase activity for 15 minutes, post-fixed in osmium-S-Collidine for 1 hour. Thin sections were not stained with lead citrate or uranyl acetate. The enzymatic product is localized to small lysosomes around the nucleus and at the cell periphery. There is a fine scattering of activity in the apical secretory products. 19,100 X.

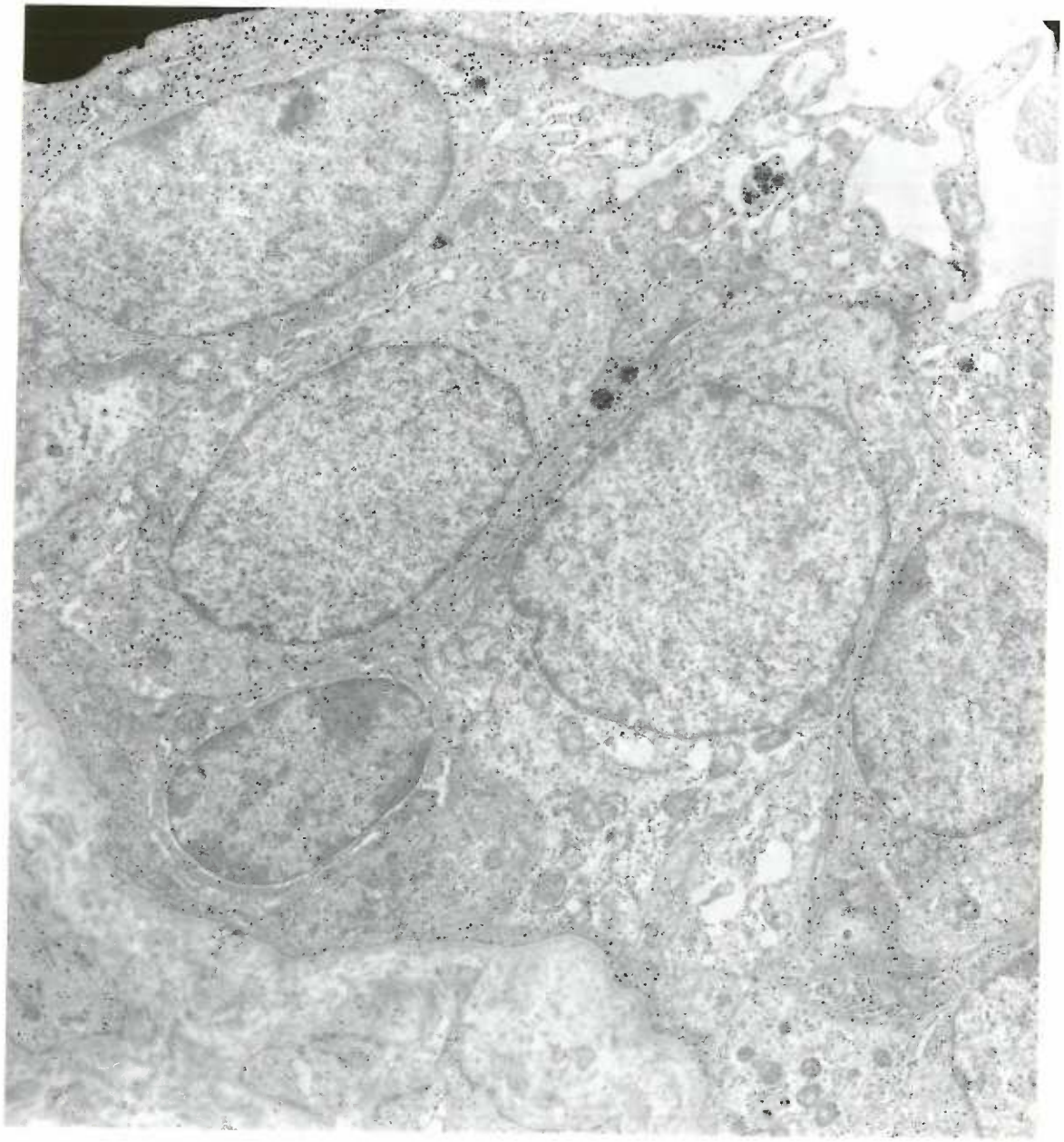
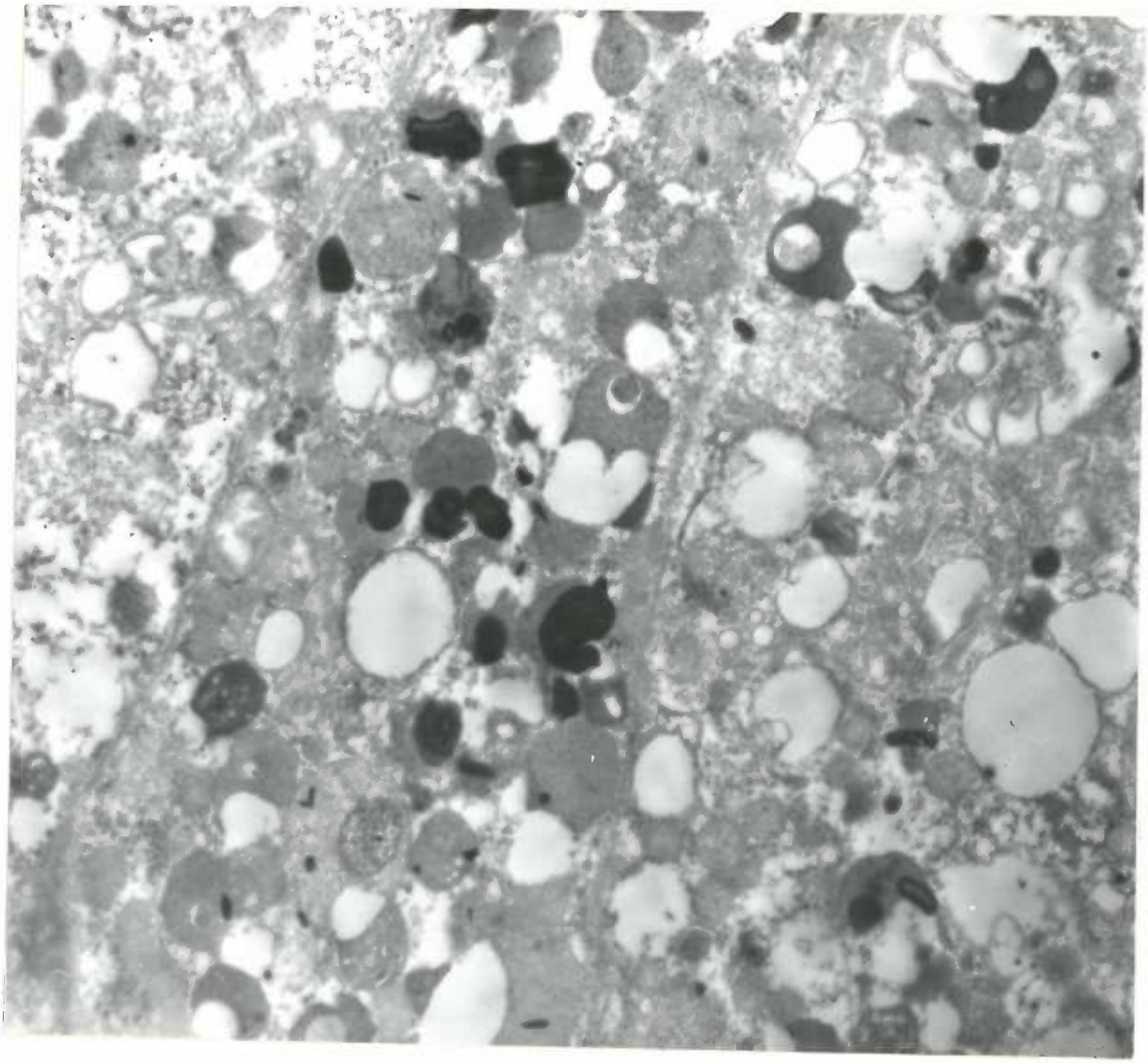
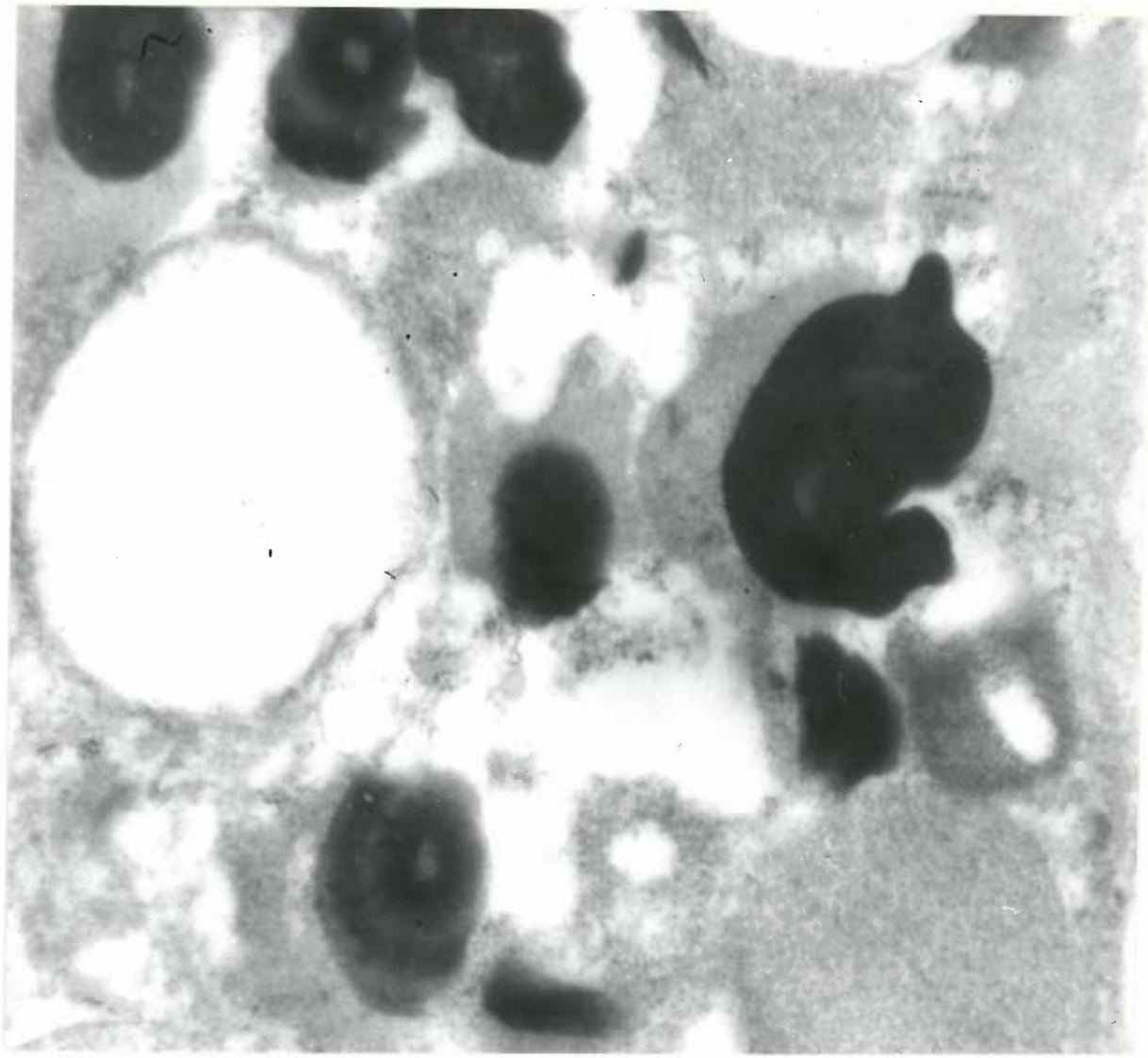


Figure 60. Controls prepared the same as tissue in Fig. 49, except the thiolacetic acid substrate was not included in the incubation medium. Thin sections were stained with uranyl acetate and lead citrate before viewing with the electron microscope. There is no evidence of reaction product in the cytoplasm or in the secretions. The dense secretions outlined are shown at a higher magnification in Fig. 61. 15,700 X.



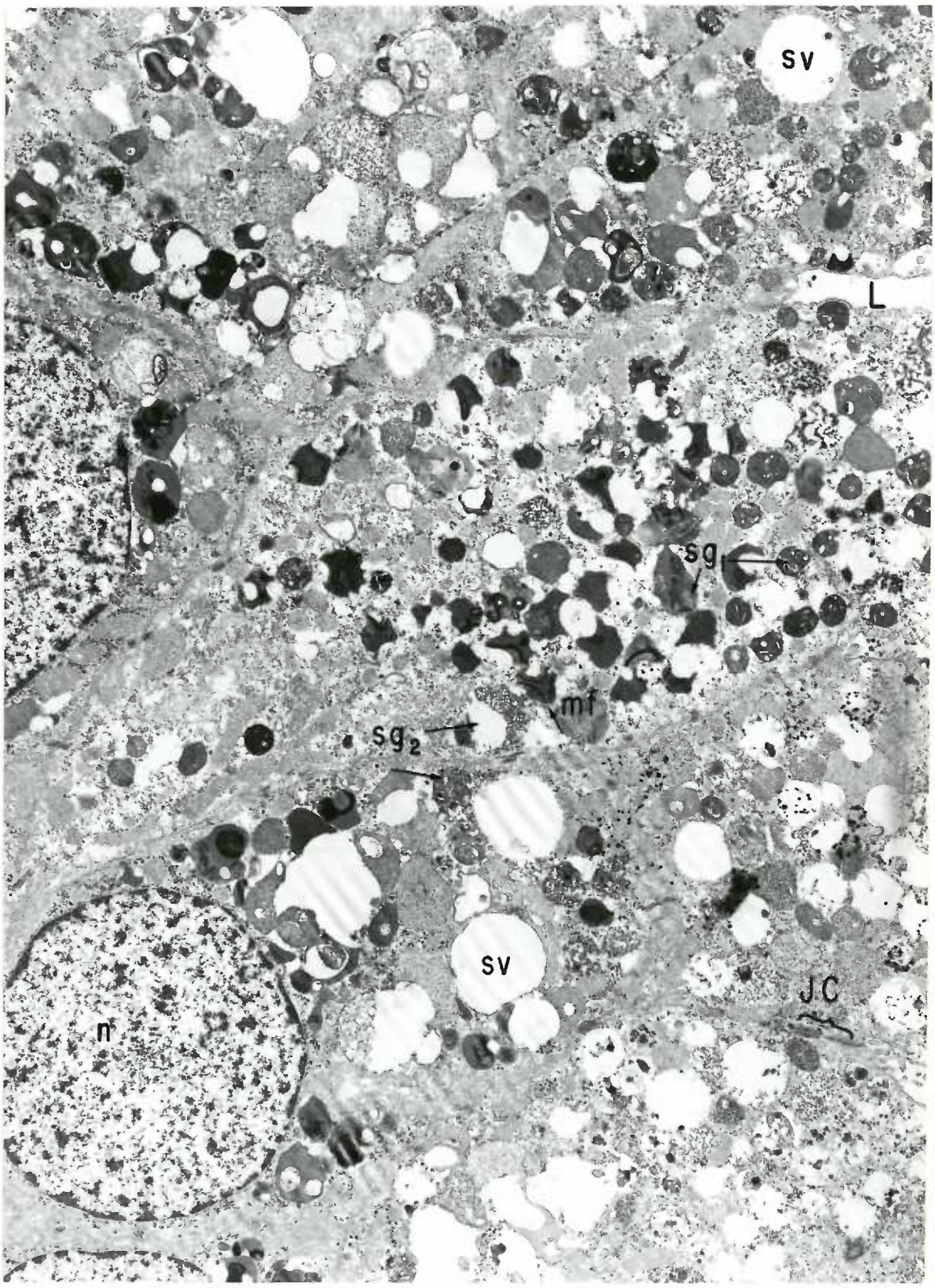
60

Figure 61. A higher magnification of the area outlined in Fig. 60. The dense secretions have smooth membranes in contrast to the experimental tissue where the secretions are studded with lead sulfide granules, as in Fig. 55. The dense secretions are homogeneous and free of reaction products. 34,700 X.



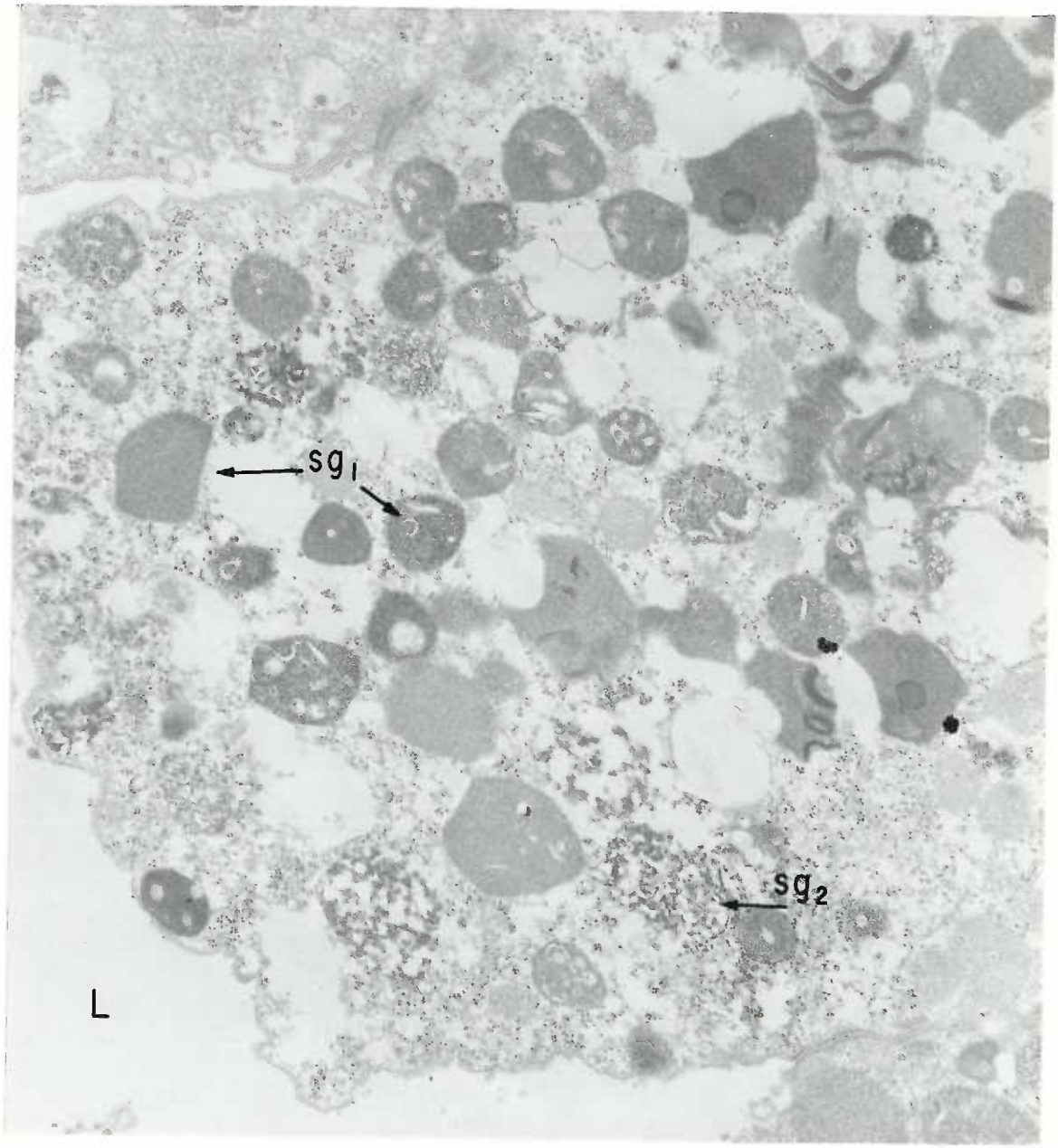
61

Figure 62. A control section of tall secretory cells prepared the same as the tissue in Fig. 60. The cells contain dense secretions free of enzymatic activity. 11,300 X.



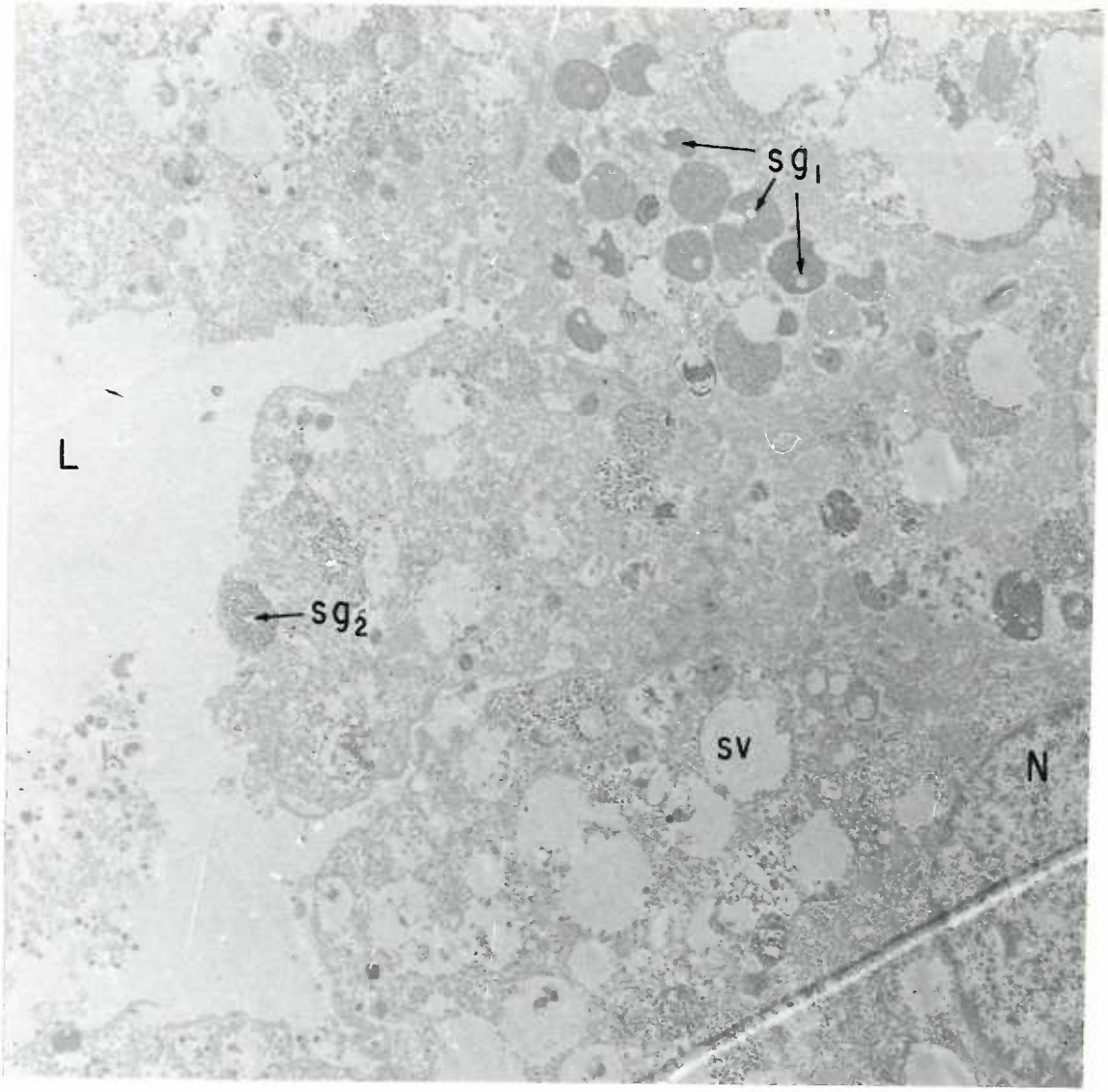
62

Figure 63. A control section prepared the same as tissue in Fig. 60, showing the tip of a columnar cell with dense homogeneous secretions and granular secretions. They are free of reaction products. 21,800 X.



63

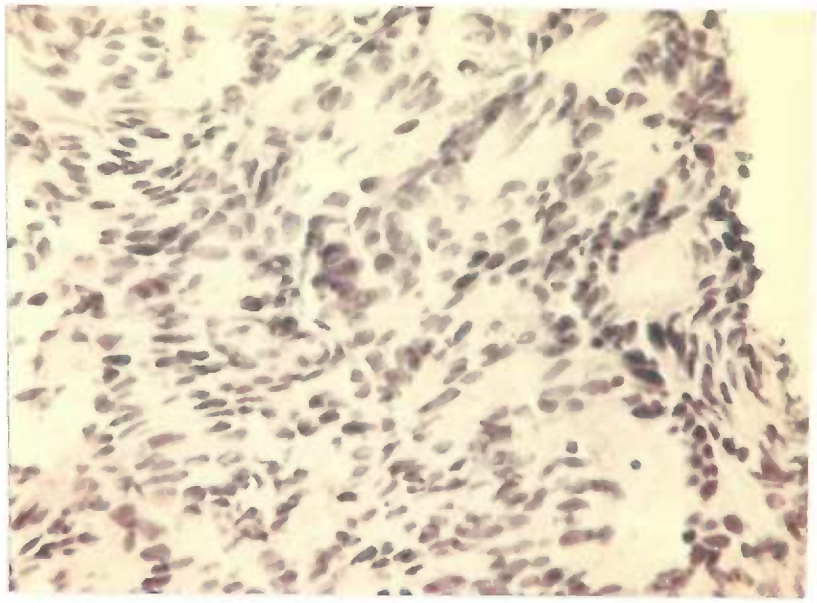
Figure 64. Control tissue prepared the same as tissue in Figure 60, except it is not stained with uranyl acetate and lead citrate. There is no evidence of specific or nonspecific enzyme activity. 9,350 X.



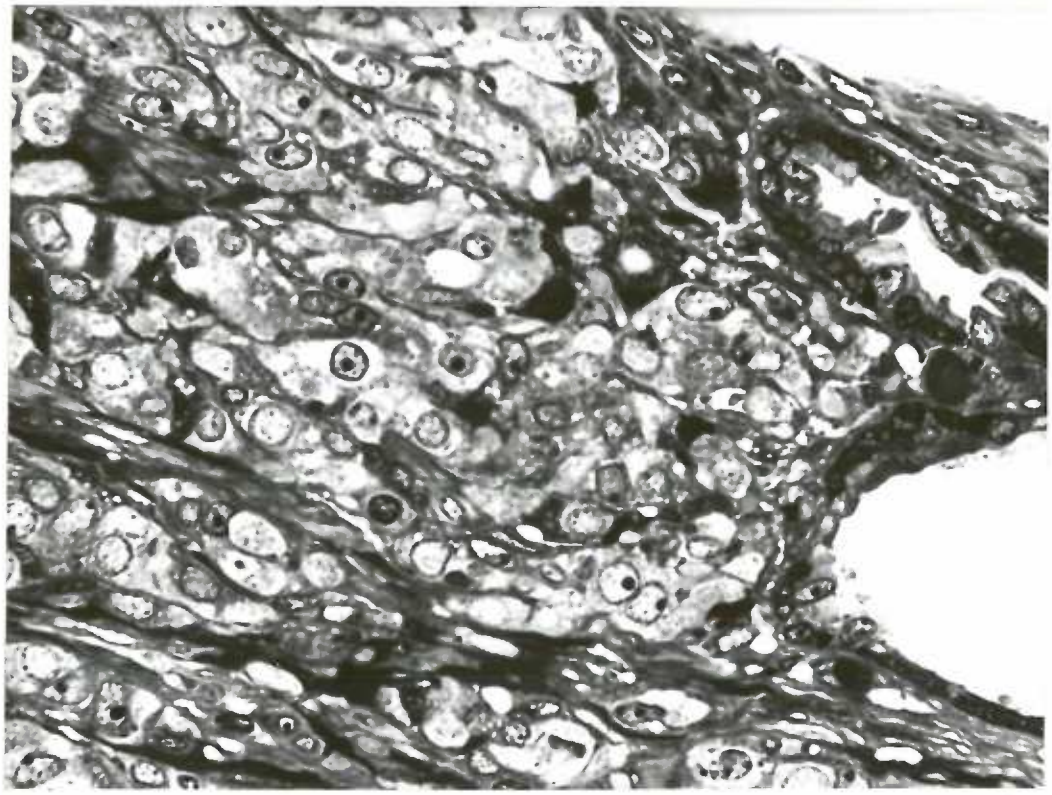
64

Figure 65. A frozen section from a prostatic biopsy of a Grade III prostatic cancer stained with hematoxylin and eosin. The cancer cells are arranged in sheets and there appears to be little attempt to form glands. The nuclei are large, and form most of the cell, but the nucleoli are not prominent in this preparation, as is reported for most undifferentiated cancer cells.[32]
400 X.

Figure 66. Grade III cancer specimen prepared for electron cytochemical observation after incubation for 6 minutes in the thiolacetic acid substrate medium. The cancer cells are large, with prominent nucleoli, and light staining cytoplasm.
615 X.

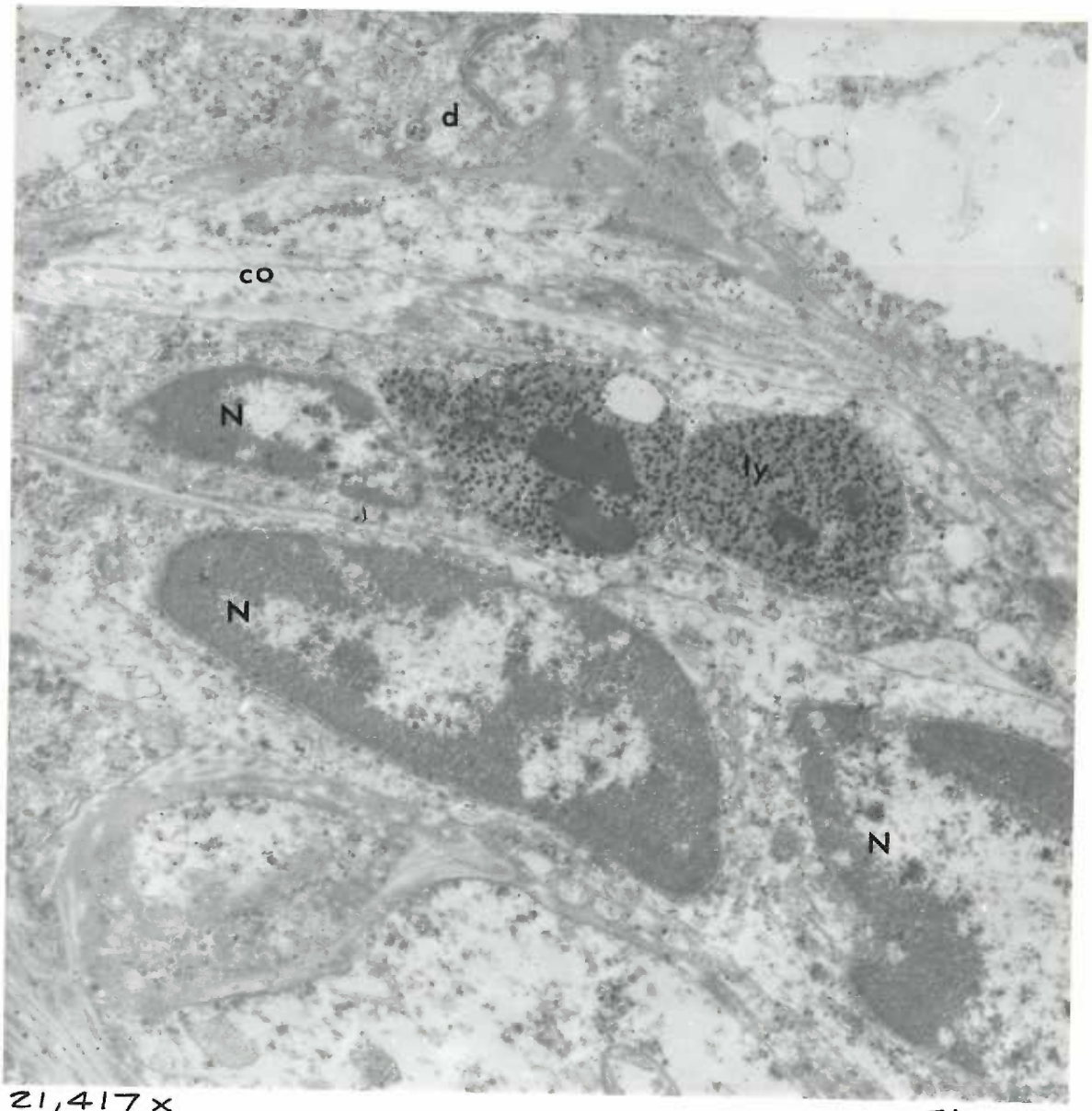


65



66

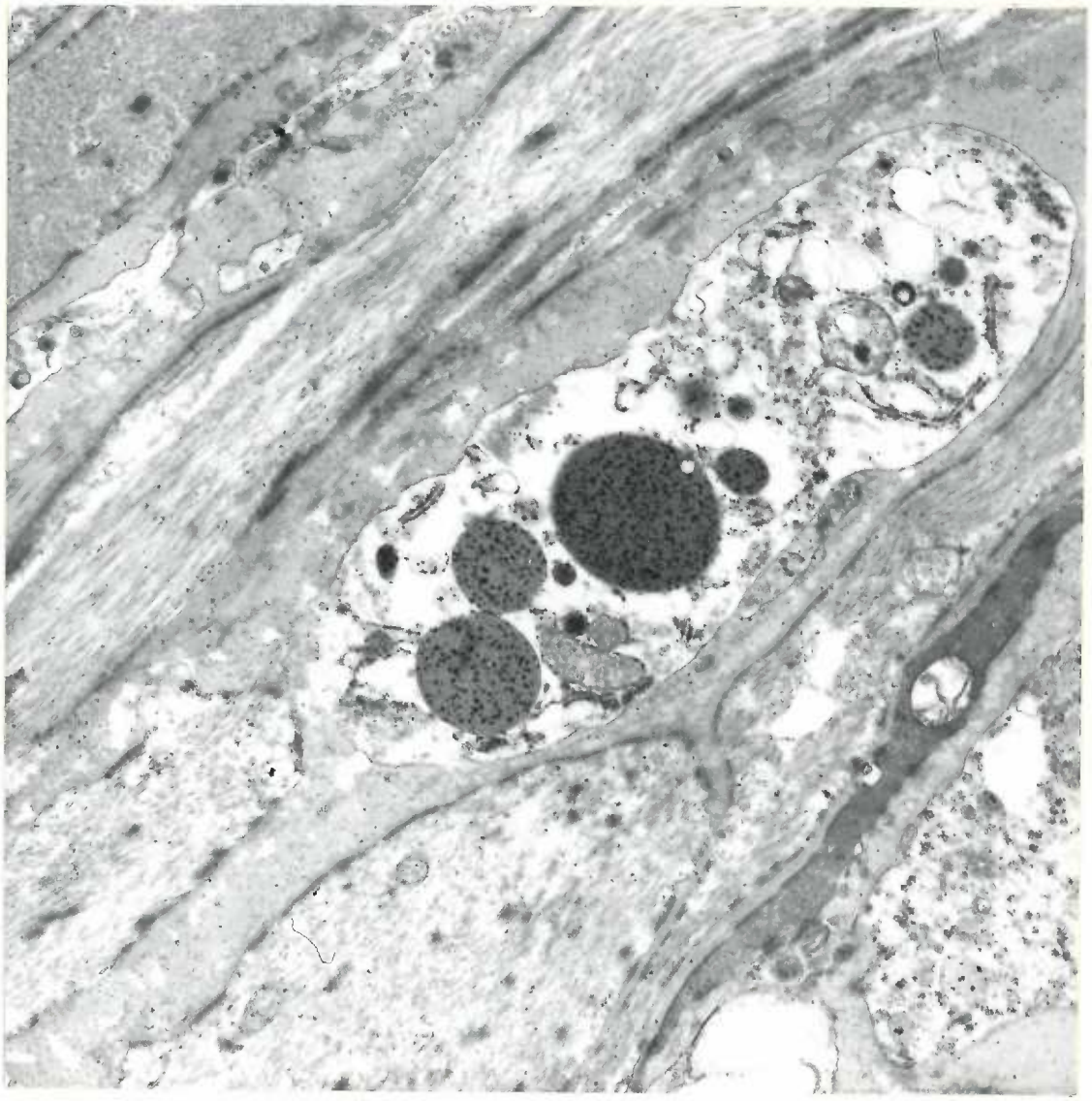
Figure 67. Cells from a Grade III cancer specimen shown in Fig. 66, prepared for esterase localization by fixation for 2 hours in 5% glutaraldehyde, washing 24 hours in buffer and incubating 6 minutes in the thiolacetic acid substrate solution. The tissue was post-fixed 1 hour in 1% osmium-S-Collidine and stained with lead citrate and uranyl acetate for electron microscopy. Two large lysosomes with enzymatic reaction product in them are situated in a cell in the stroma. The nucleus of the cell is similar to the two nuclei in nearby cells; the two mitochondria next to the lysosomes are swollen and disrupted similar to those seen in cancer cells. 21,400 X.



21,417 x

Fig. 67

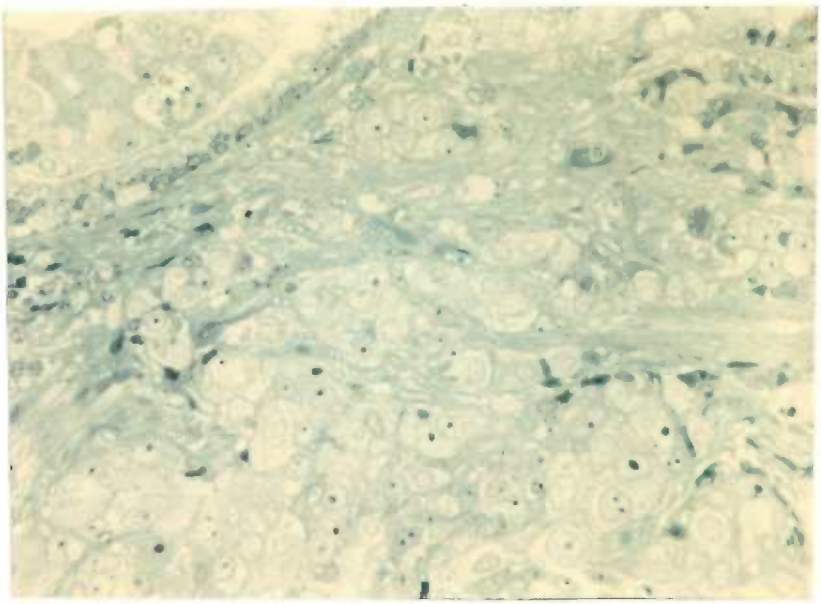
Figure 68. An area from a Grade III cancer specimen as shown in Figure 66 prepared by the same procedure outlined for Figure 67. The moderate sized lysosomes found in the stroma are surrounded by an electron lucent cytoplasm. The mitochondria associated with the lysosomes is slim and has well formed cristae; the endoplasmic reticulum is similar to that seen in other cancer cells, but it is not characteristic of any particular cell type. 15,740 X.



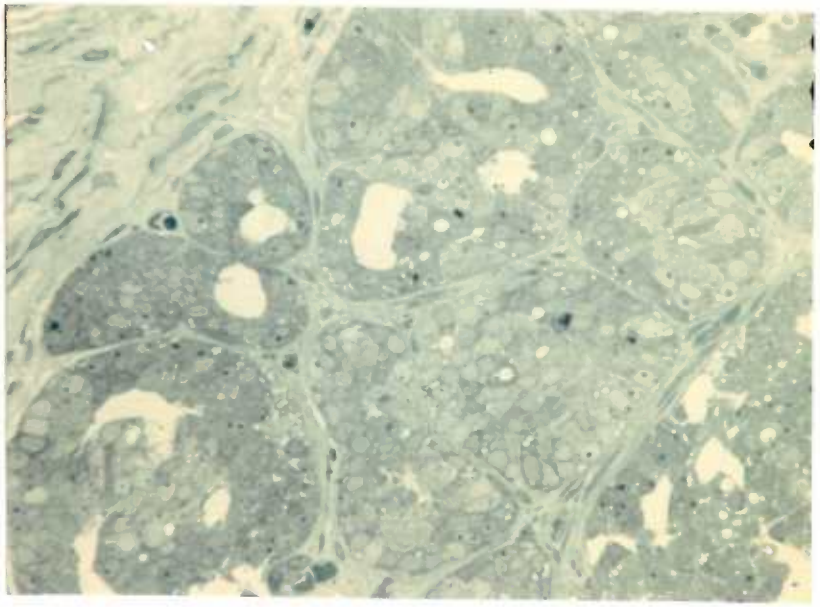
68

Figure 69. Grade II and III cancer cells cut 1 micron thick from epon embedded sections and stained with Richardson's stain for light microscopy. The cancer cells (CC) are mainly light cells with large nuclei and light staining cytoplasm. Atrophic cuboidal cells form a gland at the upper left that contains a group of tumor cells in the lumen. 600 X.

Figure 70. Grade II cancer cells prepared the same as the tissue above. Large cells are crowded into glands with irregular and incompletely formed lumens. 600 X.

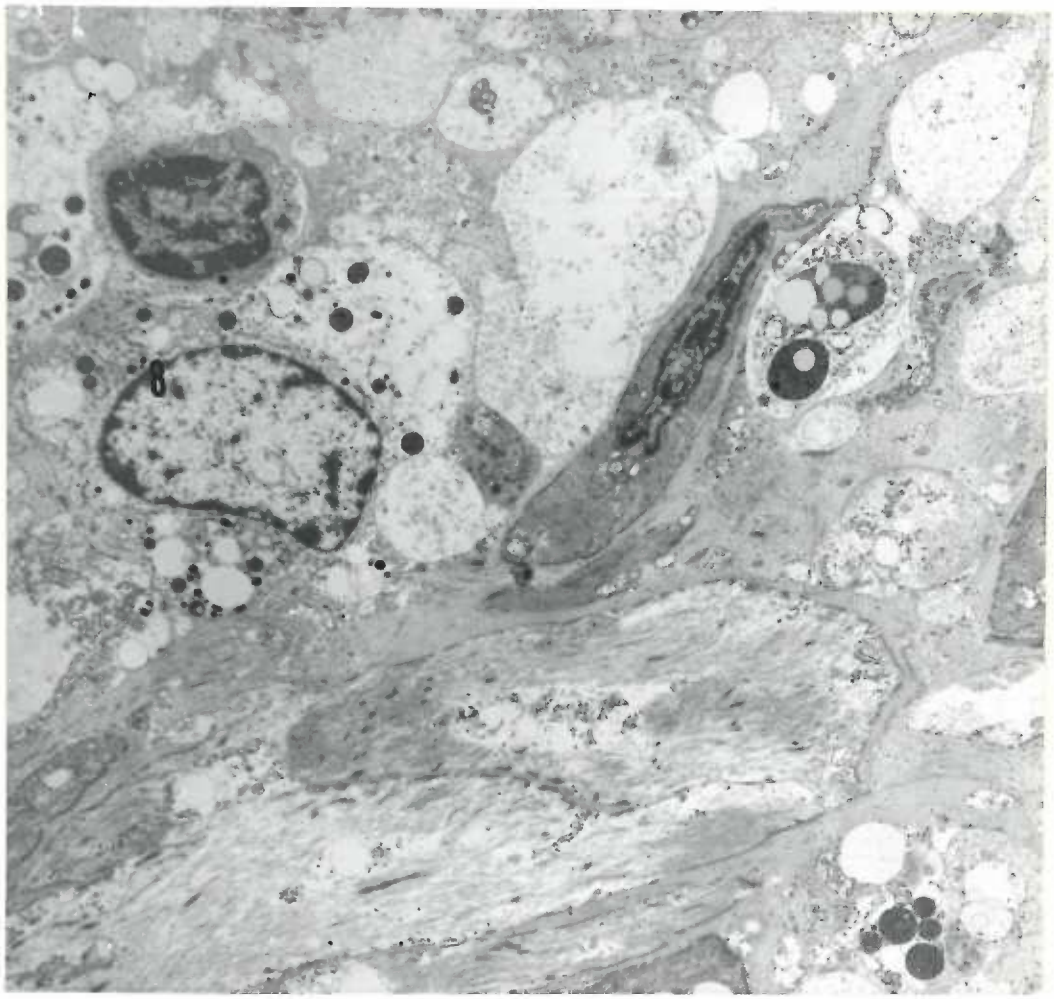


69



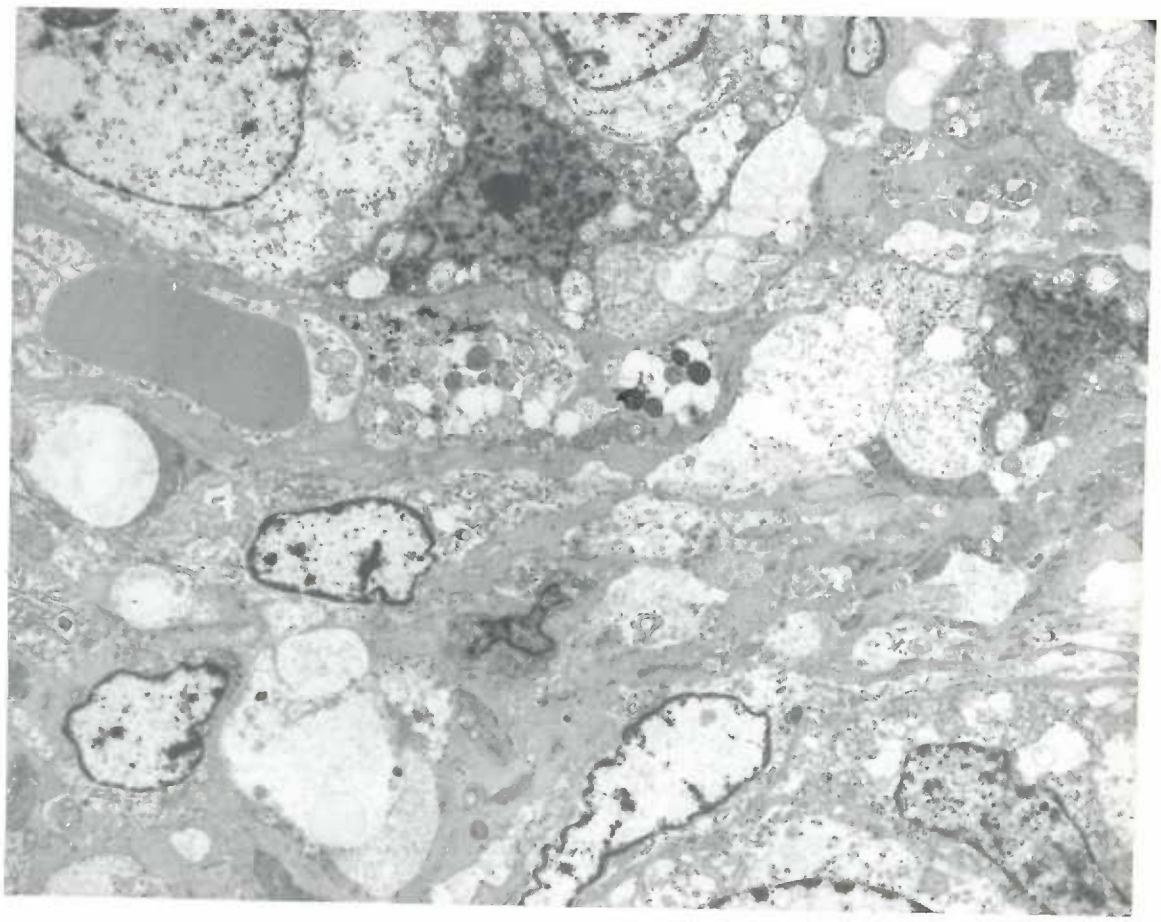
70

Figure 71. Grade III cancer cells from the area shown in the light micrograph in Fig. 69 stained with lead citrate and uranyl acetate for electron microscopy. A cancer cell with many small lysosomes is situated in the stroma with smooth muscle cells and cytoplasmic pockets (CP) around it. The cytoplasmic pockets contain an electron clear material, without organelles, except for the lysosomes. 5,660 X.



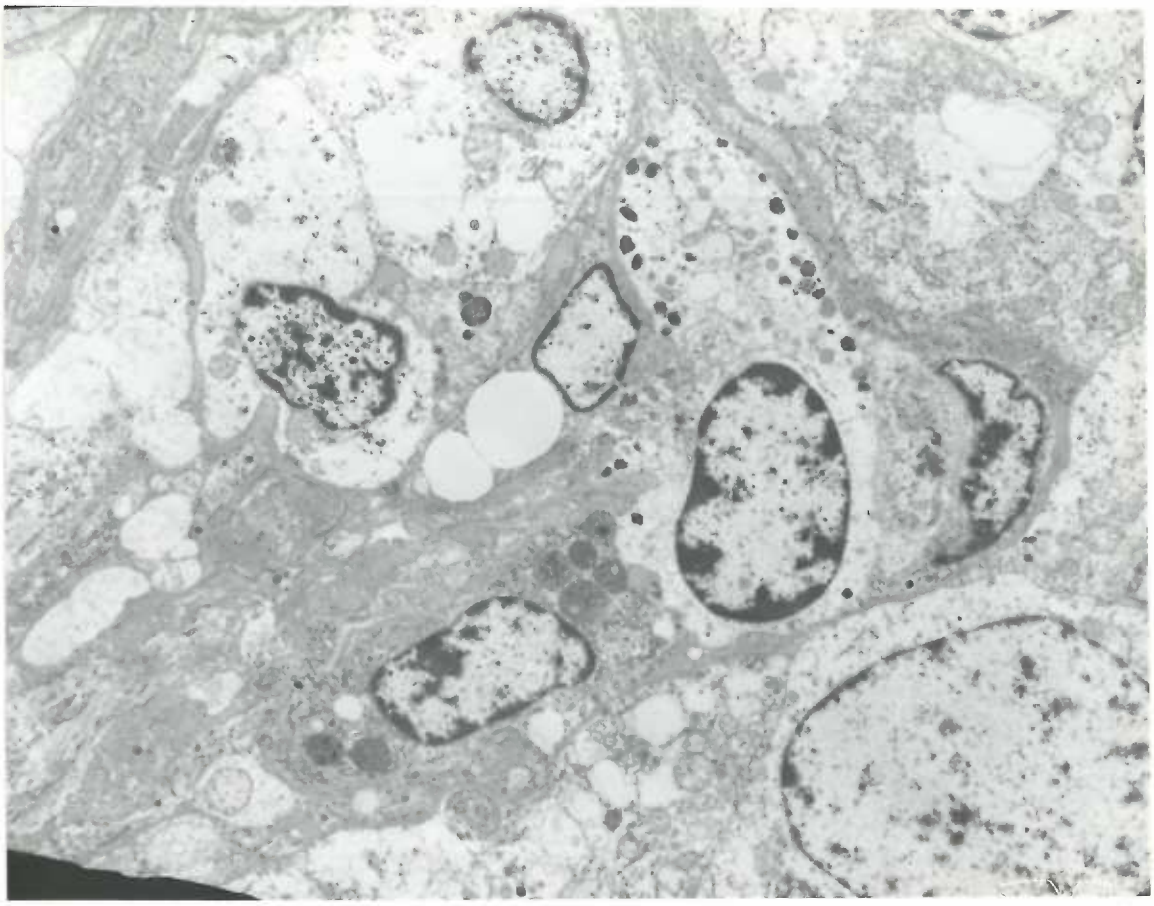
71

Figure 72. A Grade III cancer specimen, represented in the light micrograph in Fig. 69, stained with uranyl acetate and lead citrate for electron microscopy. Dark and light cancer cells are associated with several cytoplasmic pockets and various stromal elements (collagen, a small capillary and a fibroblast). The lysosomes are situated in the cytoplasm of a cell of unknown origin. 4,470 X.



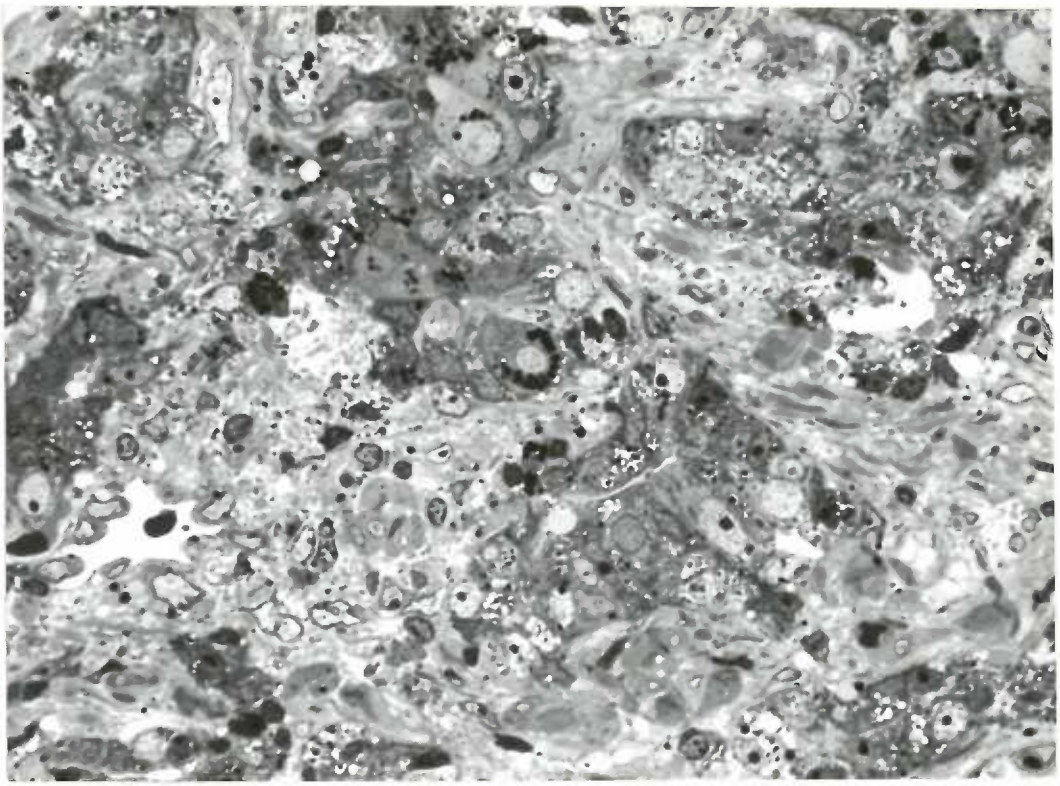
72

Figure 73. A Grade III cancer specimen prepared the same as the tissue in Fig. 69. The lysosomes are associated with a cell that has a nucleus similar to the cell nearby with dense bodies. The cytoplasm contains more organelles and is denser than that usually seen in the light cancer cells. The large cell at the bottom right is a cancer cell, the other two cells containing lysosomes and dense bodies are probably white blood cells. The dense bodies appear morphologically like lysosomes, but they lack esterase. 4,770 X.



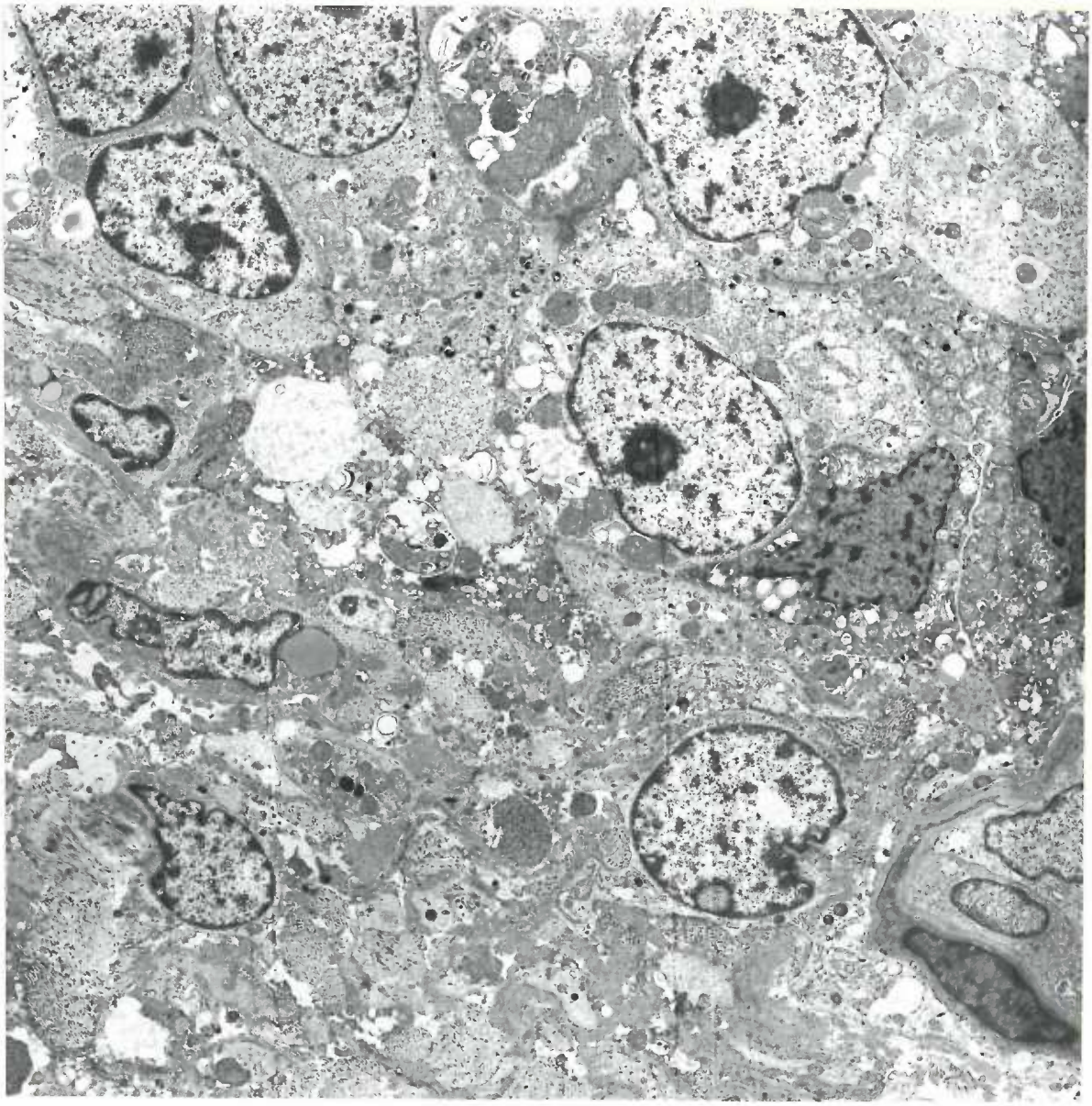
73

Figure 74. A light micrograph of a Grade III cancer specimen prepared for enzyme localization by fixing for 2 hours in 5% glutaraldehyde, washing 11 hours in buffer and incubating for 9 minutes in the thiolacetic acid substrate medium. Postfixation was carried out in 1% osmium-S-Collidine for 1 hour. Two groups of cancer cells are situated in the stroma partly enclosed by a basement membrane (BM). The arrows point out two large cancer cells with conspicuous dense bodies surrounding the nucleus. There are numerous small white blood cells mixed in the stroma around the cluster of tumor cells. 615 X.



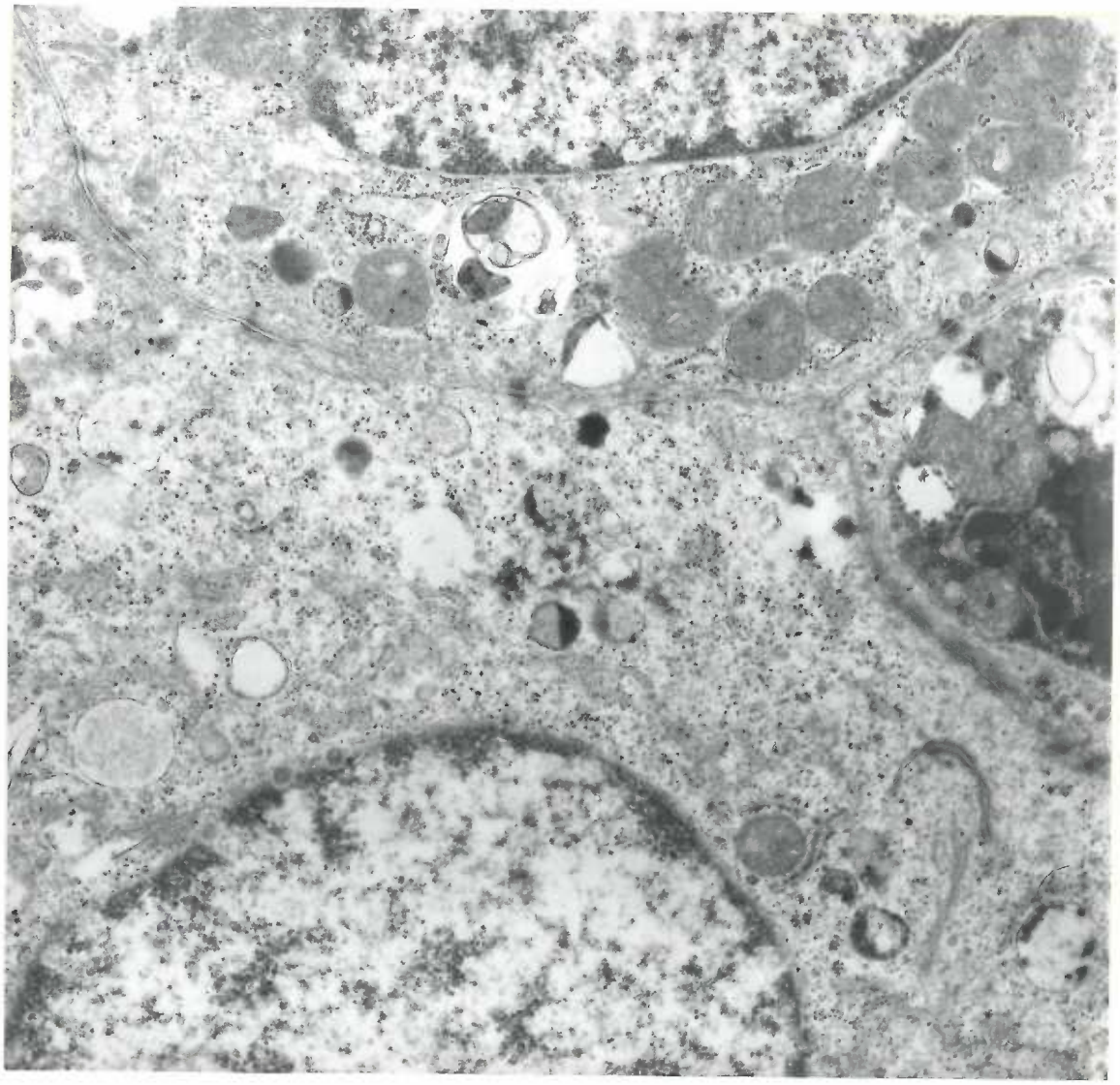
74

Figure 75. An electron micrograph of Grade III cancer cells corresponding to the area represented by the light micrograph in Fig. 74; stained with uranyl acetate and lead citrate for electron microscopy. Several dark and light cancer cells are mixed with stromal collagen and fibroblasts. The light cancer cells contain very small lysosomes with only a slight amount of enzyme reaction product. The dark cells also contain the very small lysosomes but they are hidden in the dense cytoplasmic matrix and not as conspicuous as those in the light cells (arrow). 7,000 X.



75

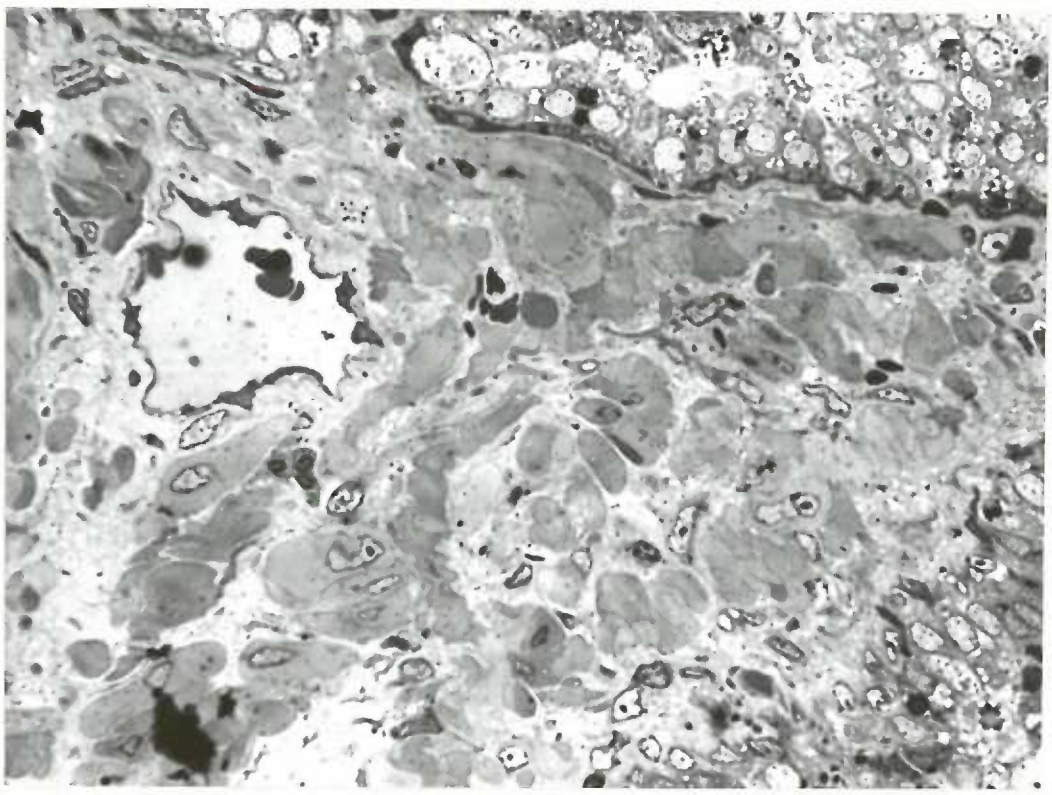
Figure 76. Tissue prepared the same as that in Fig. 74. Very small lysosomes similar to those in Fig. 75 are shown at a higher magnification. 30,400 X.



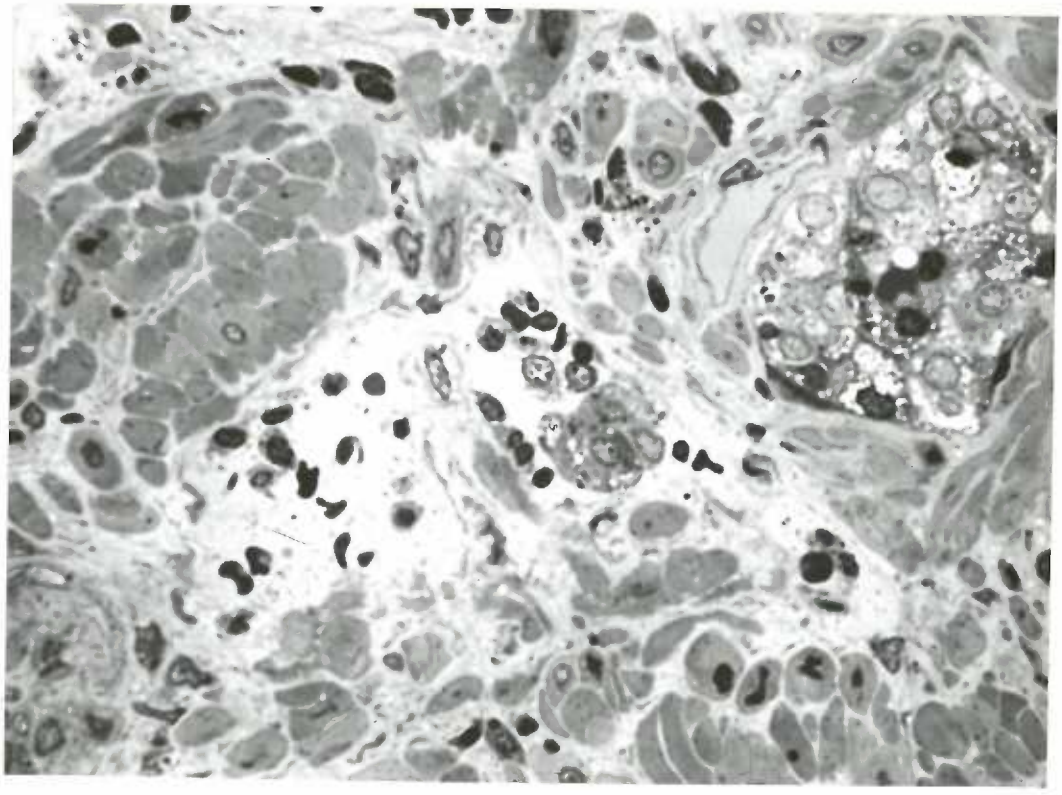
76

Figure 77. Stroma and glands from a Grade II cancer specimen prepared by fixing in 6.3% glutaraldehyde for 2 hours, washing overnight in buffer and incubating for 9 minutes for esterase activity. Electron micrographs shown in figures 80 and 81 are taken from the area represented in this figure.
615 X.

Figure 78. A cancer specimen prepared by the same method as for Fig. 77, except the tissue was not incubated for enzyme activity. The group of cancer cells (CC) appear morphologically similar to those in the above figure.
615 X.

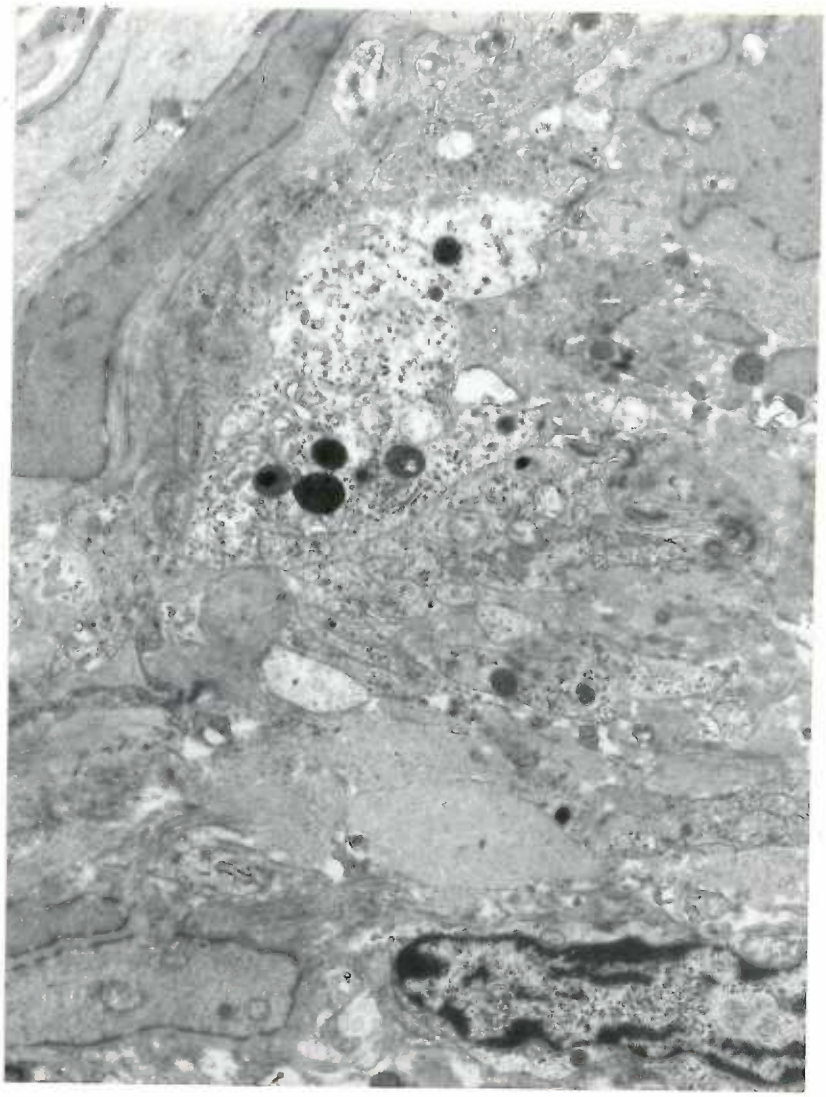


77



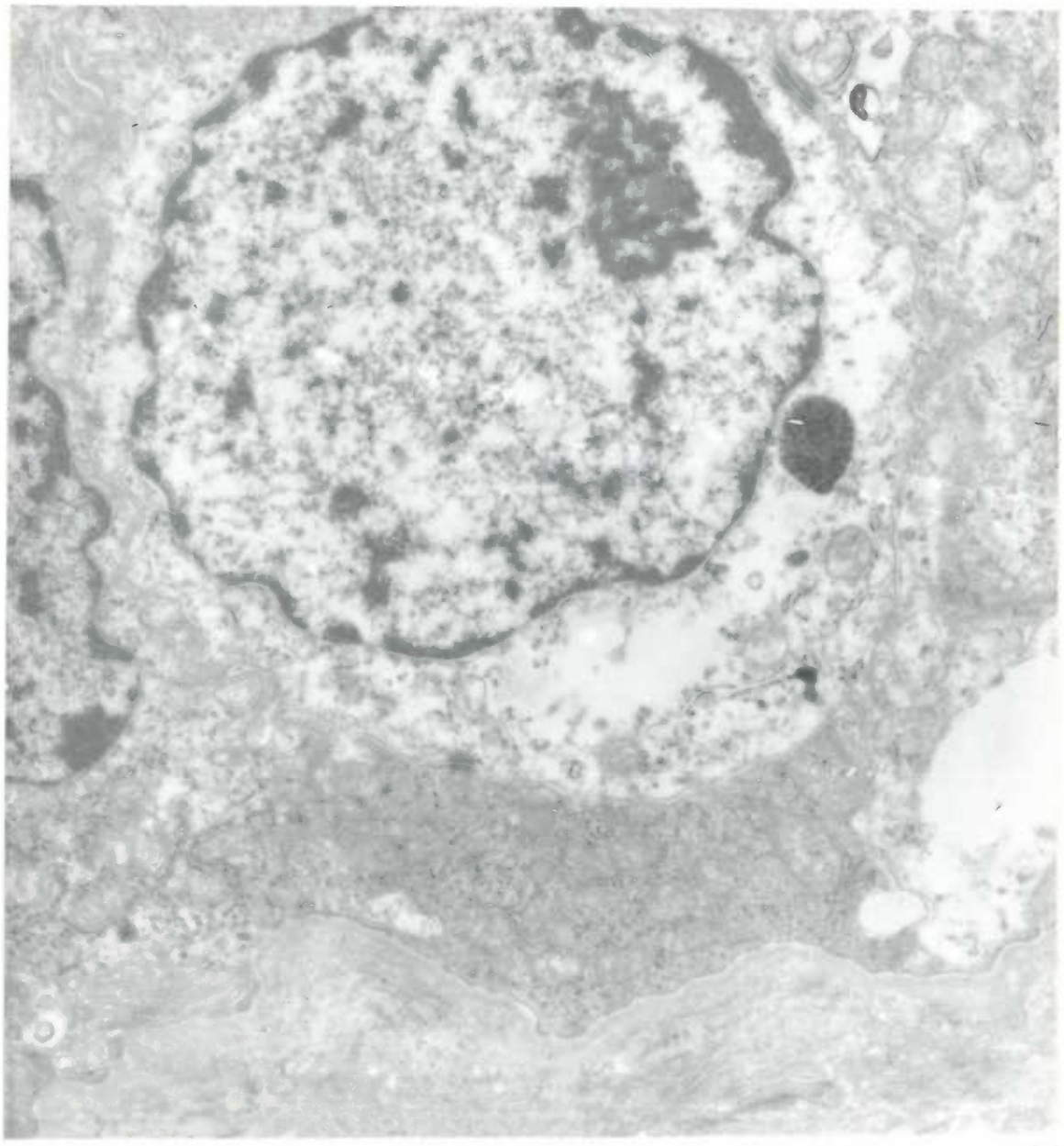
78

Figure 79. An electron micrograph from the section represented in the light micrograph in Fig. 77, and prepared the same as Fig. 77 except for the uranyl acetate and lead citrate stain used for electron microscopy. The moderate sized lysosomes are in the cytoplasmic extension of a cell which forms desmosomal attachments to neighborning cells. Collagen bundles and smooth muscle cells surround the cytoplasmic pocket. 7,480 X.



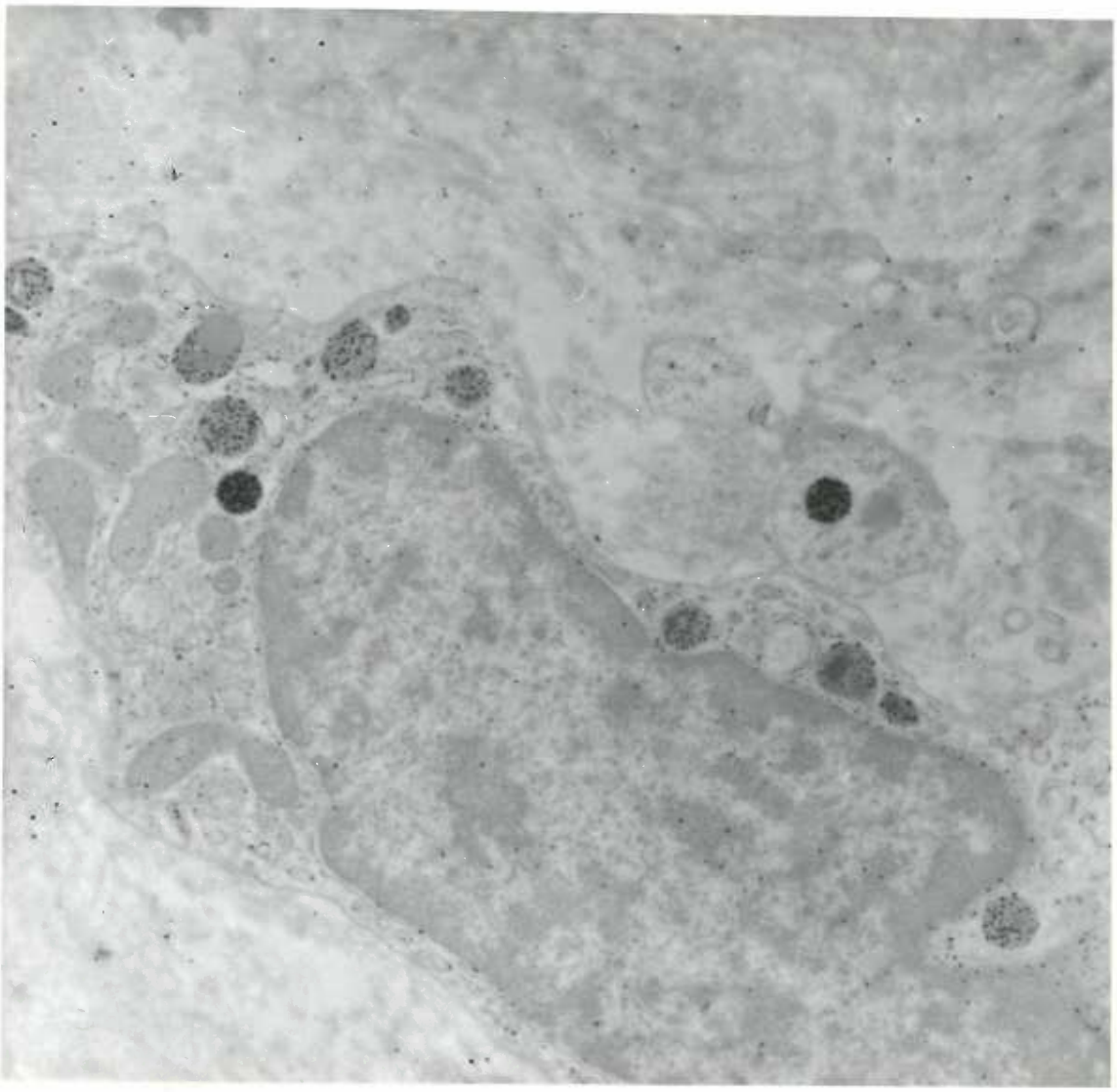
79

Figure 80. A light and dark cancer cell from a Grade II cancer specimen shown in Fig. 77. The tissue was fixed in 6.3% glutaraldehyde for 2 hours, and prepared for enzyme localization as described in Fig. 77. The light cancer cell (LC) contains one moderate sized lysosome with enzyme reaction product; the dark cell (DC) is filled with mitochondria and a dense cytoplasmic matrix and lacks evidence of enzyme activity. There is a small amount of granular endoplasmic reticulum in the light cell, but much more in the dark cell, especially around the mitochondria. The mitochondria in the dark cell are small and similar to those found in normal cells. Those in light cells are larger and contain only a few cristae. 16,600 X.



80

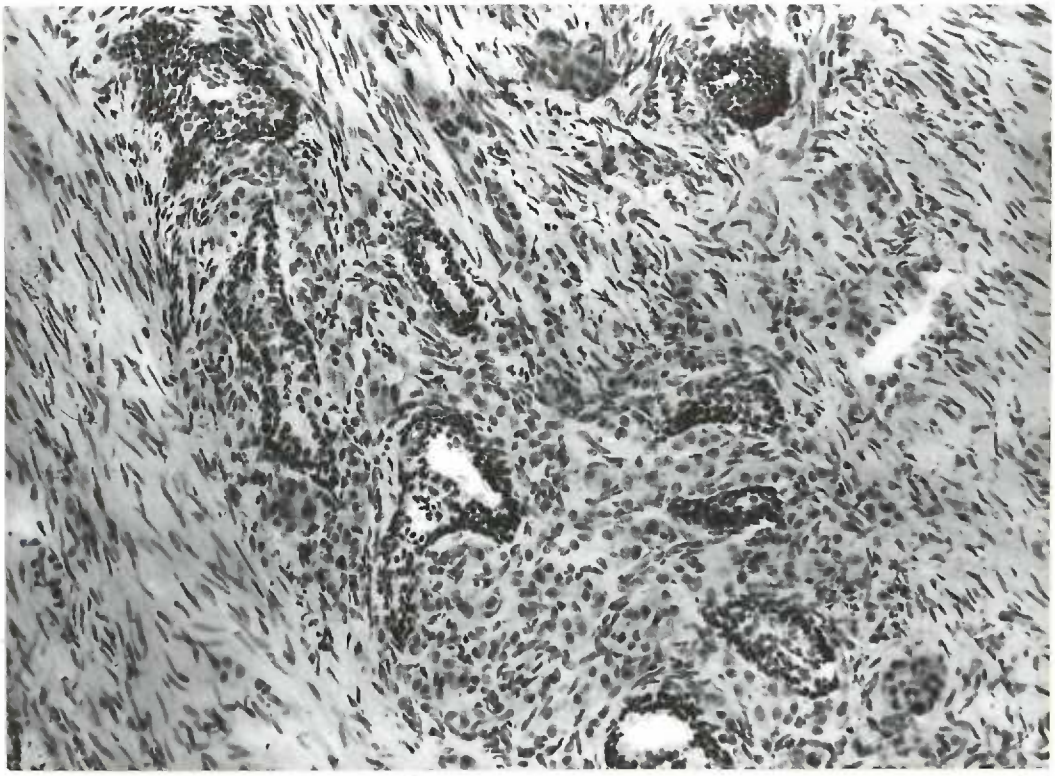
Figure 81. A fibroblast from a Grade II cancer specimen prepared for enzymatic study by the method described for Fig. 77. The sections were not stained with lead citrate or uranyl acetate for electron microscopy. The moderately sized lysosomes contain abundant enzymatic reaction product; the mitochondria, nucleus, collagen bundles, and nuclear membrane also contain lead sulfide granules.
14,000 X.



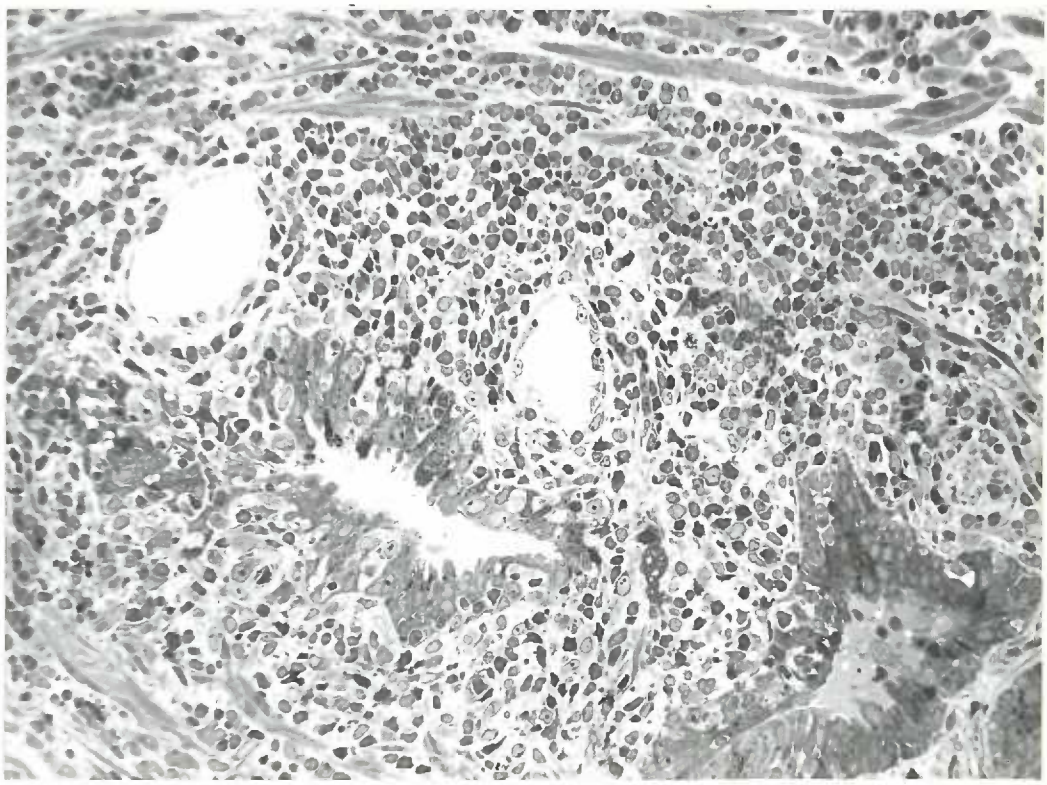
81

Figure 82. Small glands from a Grade II carcinoma specimen embedded in paraffin and stained with hematoxylin and eosin for light microscopy. The small glands are surrounded by white blood cells and stromal cells. An area similar to that outlined is shown in tissue prepared for electron microscopy in the figure below.
185 X.

Figure 83. Two cancer glands from a Grade II specimen prepared for enzyme localization and electron microscopy. The glands are formed by dark cancer cells that appear to be invaded by white blood cells. The arrows point out the basement membrane surrounding the two glands. White blood cells appear inside the membrane, mixed with the cancer cells. This point is illustrated by the following electron micrographs in Fig. 84 and 85. 400 X.

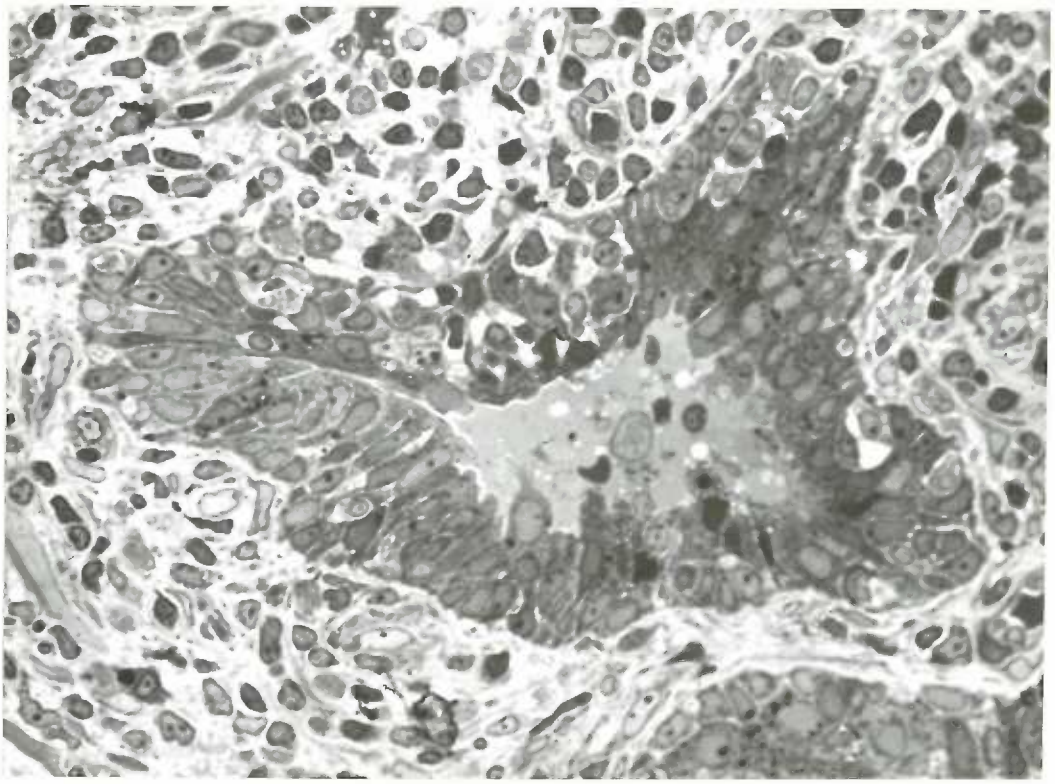


82



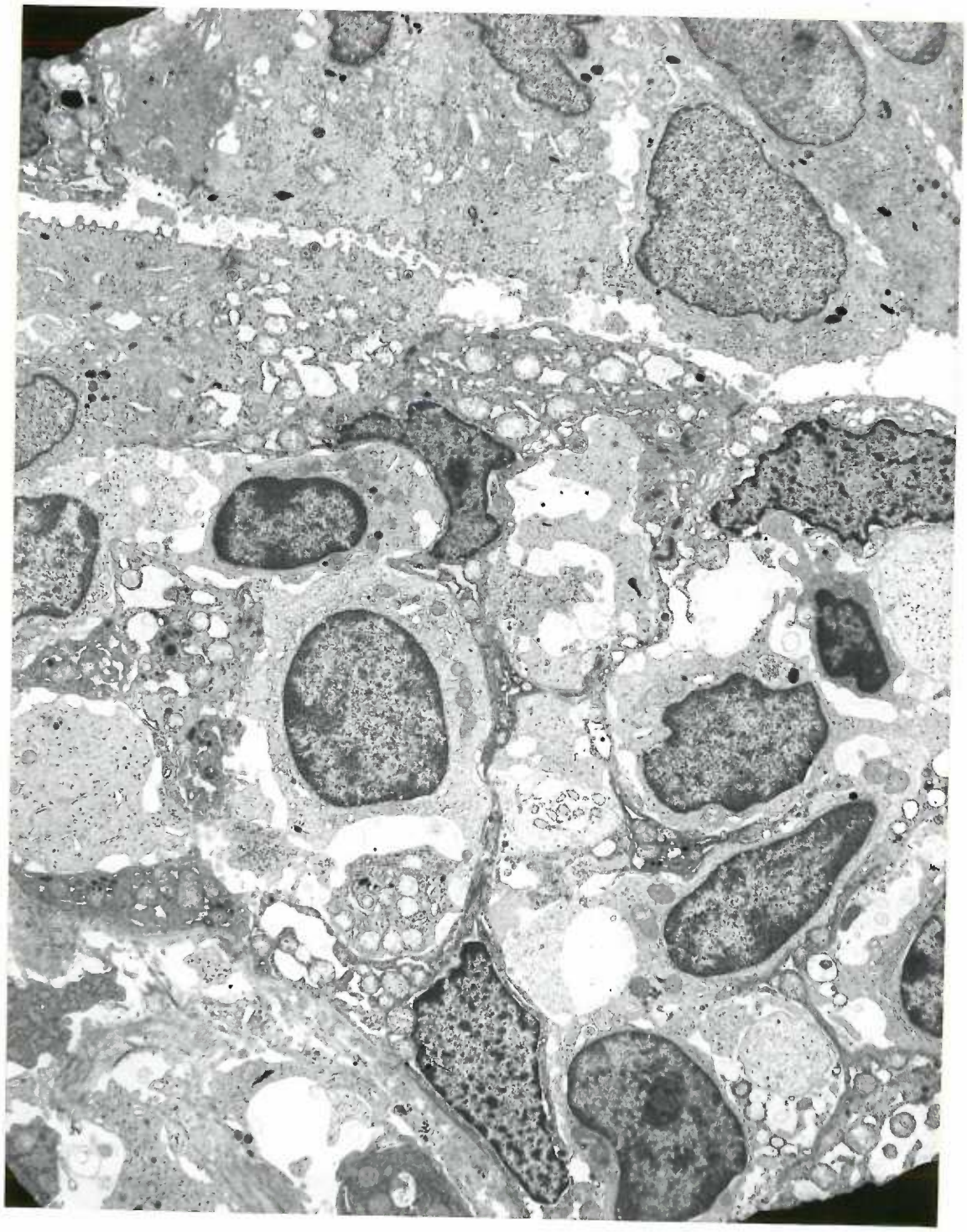
83

Figure 84. A well differentiated gland from a Grade II cancer specimen magnified from the area outlined in Fig. 83. The area outlined here is represented in the electron micrograph that follows. White blood cells are shown mixed in with the tumor cells, and in this gland the border outlined has only a few cancer cells at the luminal border, the rest of the area consists of white blood cells. 720 X.



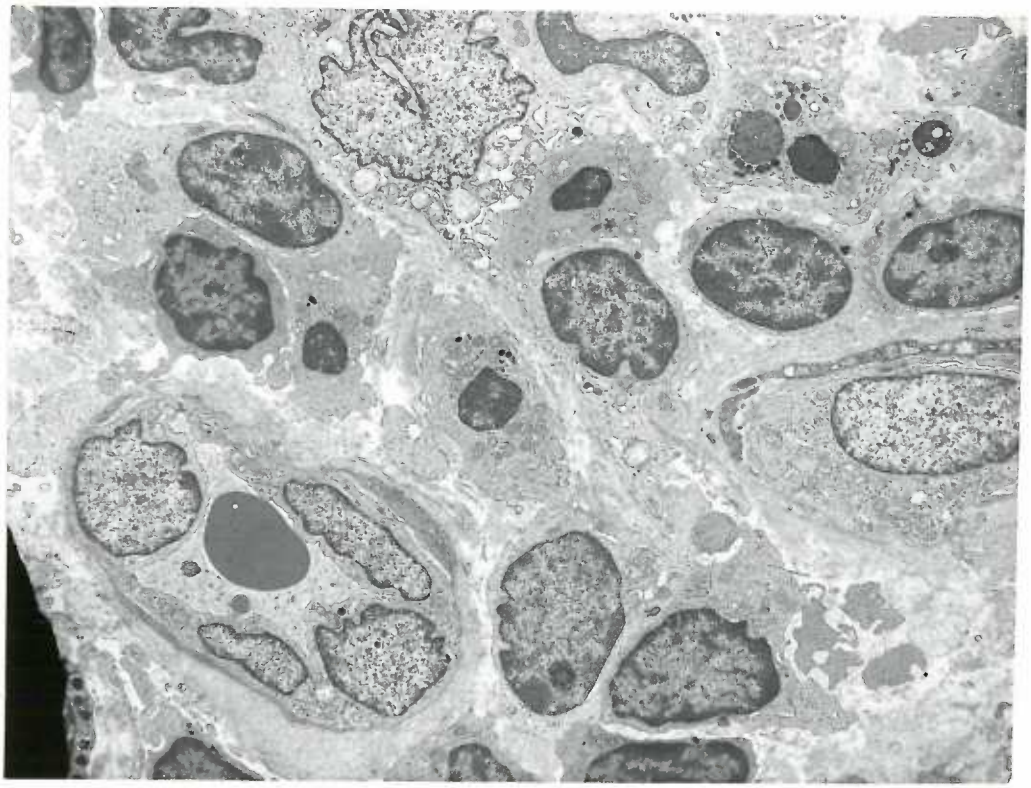
84

Figure 85. An electron micrograph of the tissue shown in Fig. 83 and 84. Prepared for electron microscopy with lead citrate and uranyl acetate stain. Dark cancer cells (CC) are surrounded by white blood cells that are probably lymphocytes. A basal lamina (bl) lies against the cancer cells and remains intact. The area is similar to that outlined in the light micrograph in Fig. 84. Small lysosomes (Ly) are found in some of the cancer cells and a few are located in the invading lymphocytes. 4,500 X.



85

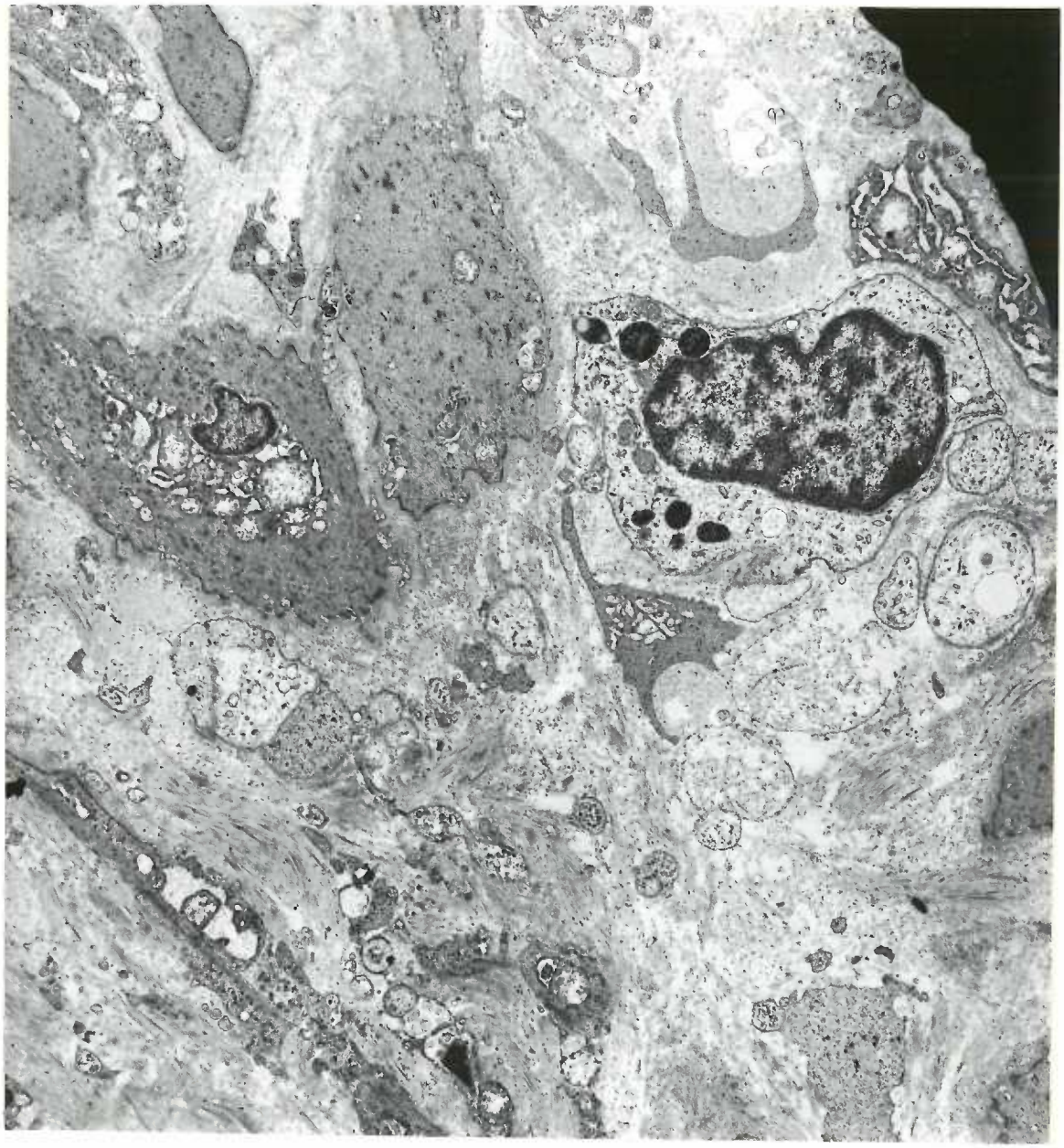
Figure 86. White blood cells in the stroma of a Grade I and II cancer specimen from the area shown in Fig. 83. Most of the white blood cells contain cytoplasmic organelles similar to that of lymphocytes.[148] The large lysosomes shown at the top of the picture are situated in a cell with small, round mitochondria and cristae similar to those in the mitochondria of surrounding cells, but large lysosomes are not found in the other white blood cells. Most of them contain a few small lysosomes. 3,400 X.



86

Figure 87. Stromal cells and a white blood cell from the cancer specimen shown in Fig. 83. The white blood cell contains moderately sized lysosomes with abundant enzyme reaction product. The mitochondria are small, and have well developed cristae; short pieces of rough endoplasmic reticulum are scattered in the cytoplasm. This cell is similar to the macrophage described by Bernhard and Leplus.[148]

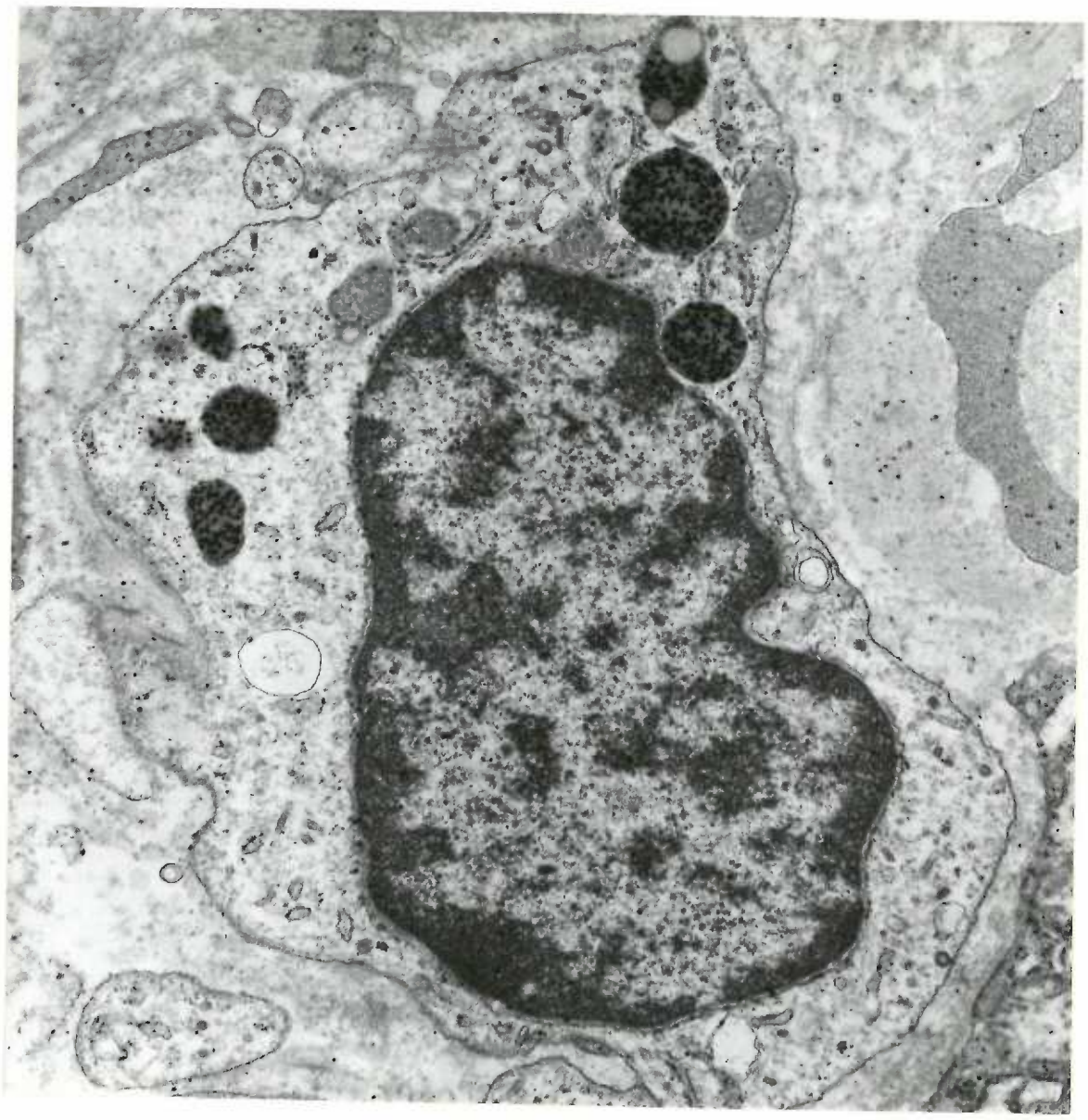
5,900 X.



87

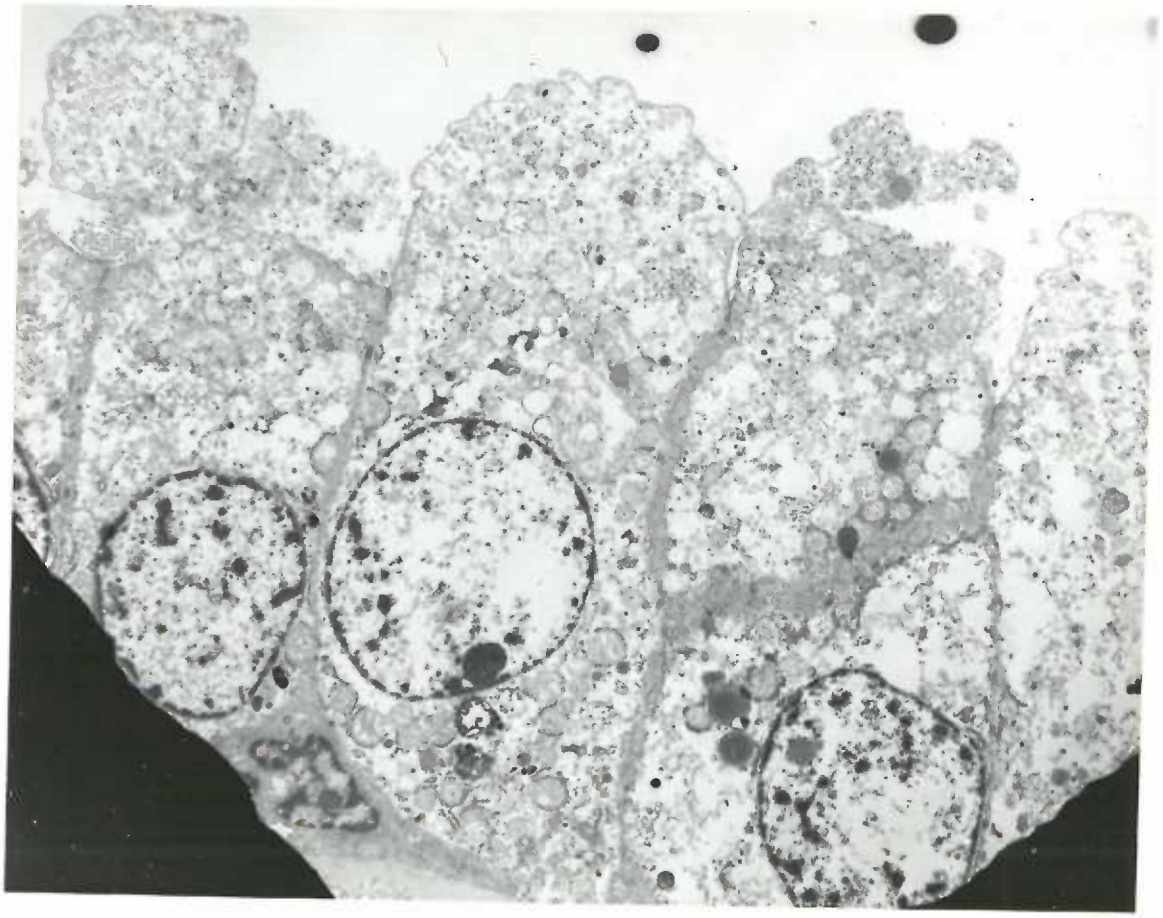
Figure 88. A higher magnification of the white blood cell, probably a macrophage,[148] shown in Fig. 87.

17,600 X.



88

Figure 89. A cloudy control specimen from a Grade II cancer specimen shown in Fig. 70, prepared by the method described for Fig. 70, but stained with uranyl acetate and lead citrate for electron microscopy. The well differentiated secretory cells are free of nonspecific localization of lead chloride granules. 4,470 X.



89