STUDIES OF SEMINAL VESICLE EPITHELIUM IN AGING RATS

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BY

VENITA JOYCE (FOSTER) ALLISON, B.S., M.S.

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Texas Woman's University

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We hereby recommend that the dissertation prepared under our supervision by Venita Joyce (Foster) Allison entitled "Studies of Seminal Vesicle Epithelium of Aging Rats"

be accepted as fulfilling this part of the requirements for the Degree of Doctor of Philosophy.

Committee:

Janet Anne
Chairman
M. Aboul-Ela
Richard Rydzek
Alan Tickerine
Kenneth A. Fry

Accepted:

Phyllis Bridges
Dean of The Graduate School

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INTRODUCTION

The seminal vesicle in the rat is a male accessory reproductive organ which secretes the largest component of the seminal plasma. It develops from the Wolffian duct cells during 18 to 20 days gestation (Price, 1936) in response to the secretion of androgen by the fetal testis (Price and Ortiz, 1945). The fetal development and the postnatal differentiation of the seminal vesicle have been intensively studied in rodents (Deane and Wurzelman, 1965; Flickinger, 1970).

The trophic dependence of the seminal vesicle on the testis was recognized by Moore, Hughes and Gallagher (1930) who reported that the epithelium underwent atrophy following castration of the adult rat and that exogenous injections of androgens (testis extract) resulted in the regeneration of the cells. The effects of androgens on the fine structure of the seminal vesicle were examined by Szirmai and Van der Linde (1962, 1965), Zebrun and Allison (1962) and Allison (1964). These studies indicate that the testis hormone, testosterone, influences the development as well as the continuous maintenance of the seminal vesicle epithelium.

Several metabolites of testosterone are produced in target cells. Heinrichs and Dirscherl (1969) have studied the activity of Δ^4-3-ketosteroid 5α-reductase, 3α-hydroxy-

steroid oxidoreductase and 17β-hydroxy-C19-steroid oxidoreductase in the rat seminal vesicle. Although other reported modes of androgen transformation exist, such as hydroxylation, 10-demethylation, aromatization and conjugation reactions, the formation of significant amounts of reaction products has not been satisfactorily established. The predominant steroid in the nuclei of ventral prostate (Tveter and Aakvaag, 1970) and the seminal vesicle (Bruchovsky and Wilson, 1968; Stern and Eisenfeld, 1969; Tveter and Aakvaag, 1970) is 5α-dihydrotestosterone (DHT) with 5α-androstane-3α-17β-diol and androsterone the major additional transformation products in the seminal vesicle (Anderson and Liao, 1968; Bruchovsky and Wilson, 1968).

Studies dealing with the metabolism of androgen indicate that DHT promotes cell division (Saunders, 1963; Gloyna and Wilson, 1969). DHT induces cell proliferation in organ cultures of ventral prostate whereas other metabolites of testosterone may affect the secretory activity of the epithelial cell (Baulieu et al., 1968a, 1968b). In contrast to these reports, no significant differences in the in vivo mitogenic activity of DHT and testosterone were observed by Jeffcoate and Short (1970). Unlike the rhythmic mitotic activity observed in various proliferating tissues the epithelium in the seminal vesicle does not follow similar regular cyclic pattern after either DHT or testosterone stim-

ulation, at least until 40 hr or later after androgen administration (Touhimaa and Niemi, 1974). Studies specifically concerned with the effects of testosterone and its metabolites on cell division in the seminal vesicle of aging rats have not as yet been undertaken.

The site of action of testosterone, or rather its metabolites, is believed to be either in the nucleus or the cytoplasm, where it regulates protein synthesis (Williams-Ashman and Liao, 1963; Kochakian, 1963; Liao and Fang, 1969; Tata, 1970; Williams-Ashman and Reddi, 1971). The hormonal effect on protein synthesis is believed to take place at the transcriptional level (Karlson, 1963; Liao and Fang, 1969; O'Malley, 1971; Tata, 1970) or at the translational level (see Tomkins et al., 1974, for review). The precise site of hormone action remains unknown although there seems to be general agreement that the mechanism of action in the seminal vesicle and other target organs involves the regulation of protein synthesis.

It is well known that epithelial hyperplasia (benign hypertrophy) occurs frequently in the prostate of aging dogs (O'Shea, 1962) and man (Mao et al., 1965) whereas abnormal epithelial growth and differentiation in seminal vesicles of aging animals is relatively rare. Both accessory reproductive organs, i.e. the seminal vesicle and the prostate depend upon circulating androgen for the maintenance of normal form

and function. The enlargement of the seminal vesicles reported by Finch and Fakhry (1974) in senescent mice was apparently due to increased storage of secretory fluid in the lumen and was accompanied by epithelial hypoplasia. The seminal vesicles do not seem to exhibit an age-associated tendency for hyperplasia.

The epithelium of the crypts of the seminal vesicle is pseudostratified consisting of columnar secretory cells and basal cells. The columnar cells extend from the basement membrane to the lumen while the ovoid to elongate basal cells lie close to the basement membrane and do not extend up to the lumen. The functional role played by the basal cell in the secretory physiology of the seminal vesicle remains obscure. Some investigators suggest that the basal cells should be considered as reserve cells capable of differentiating into columnar cells or of undergoing mitosis to replenish the epithelial cell layer (Macklin and Macklin, 1932; Bern, 1963). However, developmental studies tend to contradict this interpretation. For example, the basal cells in the seminal vesicle of the mouse are first detectable around 10 days of age and seem to arise from the dedifferentiation of columnar cells (Deane and Wurzelman, 1965). The growth of the epithelium thereafter occurs by mitosis of columnar cells. The absence of basal cells at birth and their presence at 5 days of age has been reported in the epididymis of the mouse

by Benoit (1926) who commented that the basal cells were formed by "retraction of columnar cells from the lumen". Mitotic figures among the columnar and basal cells are rarely reported in the normal adult seminal vesicle (Price, 1936; Allison, 1965; Tuohimaa and Niemi, 1974). These observations suggest that the columnar cells in the adult rat seminal vesicle may not arise by the mitosis and differentiation of the basal cells as previously assumed.

Previous studies on age-associated changes in the seminal vesicle include those of Mainwaring and Brandes (1974) who reported the presence of secondary lysosomes in the seminal vesicle epithelium of 8- and 12-month-old rats. Otherwise, the electron microscopic investigations of the changes in the accessory sex organs of aging animals are largely concerned with the study of the prostatic complex in rodents and man (Harkin, 1961; Stewart and Brandes, 1961; Brandes, 1963; Mao et al., 1965; Brandes, 1966a, 1966b; Rowlatt, 1970; Mainwaring and Brandes, 1974).

Although the cytology of the prenatal, postnatal and adult rat seminal vesicle epithelium has been investigated, little is known concerning age-associated cellular changes in this tissue. The present study is concerned with light and electron microscopic examination of the epithelium of the seminal vesicle in normal, castrated and testosterone-injected rats of different ages. Additionally, autoradiography employ-

ing [^{3}H]thymidine is used to investigate the mitotic pattern of epithelial cells. Finally, measurements of cell height and counts of secretion granules are taken from light and electron micrographs, respectively, to determine a sensitive bioassay of cellular activity associated with circulating androgen levels and/or changes occurring with aging. The purpose of this study is to elucidate the normal aging changes in the epithelium and the effect of androgen on epithelial cells in aging animals.

The results of a preliminary study investigating the fine structure of seminal vesicle epithelial cells in intact, castrated and injected-castrated rats at 12 months of age have been communicated previously (Allison, 1975).

MATERIALS AND METHODS

Maintenance of animals

Sprague-Dawley outbred male rats from Texas Inbred Mice Co. were maintained under 12 hr light, 12 hr dark conditions. They were fed Purina Rat Chow and provided with water ad libitum.

Six groups of rats were studied (See Table 1).

Microscopic studies

Seminal vesicles dissected from etherized rats were fixed in cold phosphate-buffered 4% gluteraldehyde (Gomori, 1955), pH 7.3 for 2 1/2 hr, washed in several changes of cold buffer solution for 2-3 hr, post-fixed in cold phosphate buffered 1% OsO_4, pH 7.3, for 1 1/2 hr, rinsed in cold buffer wash, dehydrated through an ascending series of ethanol solutions and embedded in Maraglas (Spurlock, Kattine and Freeman, 1963). The fixatives contained 5% sucrose (w/v) while the buffer wash contained approximately 8% sucrose (w/v).

Thick (1-3 μm) and thin (50-60 nm.) sections were cut with an LKB 8801A ultramicrotome using glass and diamond knives, respectively. Thick sections were stained with Paragon 1301 (Spurlock, Skinner and Kattine, 1966) or toluidine blue (Trump et al., 1961). Thin sections were stained with saturated uranyl acetate and lead citrate (Reynolds, 1963).

Light microscopic observations were made with the

TABLE 1.

SUMMARY OF ANIMALS STUDIED

ANIMAL AGE (months)	NUMBER EMPLOYED	TREATMENT	PREPARATION
3	10	none	two controls each for LM-TEM of the 6-, 12-, 18-, 22-, 24-month-old rats
	6	[^{3}H]thymidine	autoradiography**
	6	none	two controls each for 1 hr, 24 hr, 72 hr-pulse-labelled ^{3}H-thymidine autoradiography groups
6-8	2	castrated	LM-TEM
	5	castrated and testosterone injections*	LM-TEM
	3	none	LM-TEM
	6	[^{3}H]thymidine	autoradiography**
	3	none	controls for autoradiography
11-12	3	castrated	LM-TEM

TABLE 1. (CONTINUED)

ANIMAL AGE (months)	NUMBER EMPLOYED	TREATMENT	PREPARATION
	3	castrated and testosterone injections	LM-TEM
	3	none	LM-TEM
	3	intact and testosterone	LM-TEM
	6	[^{3}H]thymidine	autoradiography**
	3	none	controls for autoradiography
18	3	castrated	LM-TEM
	4	castrated and testosterone	LM-TEM
	4	none	LM-TEM
	3	intact and testosterone	LM-TEM
21-22	2	castrated	LM-TEM
	3	castrated and testosterone	LM-TEM
	2	none	LM-TEM
24	2	castrated	LM-TEM
	1	castrated and testosterone	LM-TEM

TABLE 1. (CONTINUED)

ANIMAL AGE (months)	NUMBER EMPLOYED	TREATMENT	PREPARATION
	3	none	LM-TEM

*2 animals were injected with testosterone 4 months after castration.

**pulse labelled, 2 animals sacrificed at intervals of 1 hr, 24 hr, 72 hr
LM-TEM: Routine preparation for thick and thin sections (see Materials and Methods, page 7).

American Optical Series 20 Microstar, Olympus EH, and Reichert light microscopes. Electron microscopic observations were made with the Hitachi HU11-B and the Philips 300 transmission electron microscopes.

Autoradiography

Rats were injected with either 100 or 200 uCi [methyl-^{3}H]thymidine, New England Nuclear product NET-027A, M.W. 242.2, specific activity 2 Ci/mmole, in sterile aqueous solution. This product was delivered in 0.1 ml or 0.2 ml quantities into the femoral vein of etherized rats.

Tissues were processed according to the procedure outlined above except that they were embedded in an Epon-Araldite mixture (Hayat, 1970) cut at a thickness of 1.5 mμ and stained only with the monochromatic toluidine blue so as not to obscure developed silver grains. Slides were coated with Kodak NTB, diluted 1:1 (NTB:H_2O). Exposure times were determined by a pilot study to be around eight weeks for both quantities of thymidine.

Labelling index was determined by calculating the ratio of the counts of labelled nuclei with more than 5 reduced silver grains within the epithelial layer to the total number of cells counted.

Administration of androgen

Testosterone propionate (U.S.P.) was administered intramuscularly (for specific groups of animals, see Table 1). The Schering product, Oreton (50 mg. testosterone

propionate/cc) was selected. This androgen in sesame oil remains active if stored in the dark and may be used with or without refrigeration within a few days after opening. Sesame oil permits a suitable rate of diffusion and is, by itself, relatively inactive (Tuohimaa and Niemi, 1968). The animals received 6 mg of the androgen daily for 3 days and were sacrificed between 72 and 76 hr after the first injection. Castrated animals were injected beginning on the twenty-second day following castration in the rat according to Moore, Hughes and Gallager, 1930). Some castrated rats were injected with androgen 4-6 months after castration in order to test the effects of long-term castration on cell restoration by androgen.

Bioassay procedures

The measurement of cell heights in the rat seminal vesicle is recognized as a more sensitive bioassay for androgen stimulation than the wet or dry weight measurements of the excised seminal vesicles (Moore, Hughes and Gallager, 1930). It is for this reason that cell height measurements were taken from light photomicrographs of tissues of 3-, 6-, 12-, 18-, and 24-month-old animals. Additionally, cell height was measured in tissue from intact-injected 12-month-old animals.

The number of secretion granules present in the Golgi zones were counted in electron micrographs of tissues of 6-,

12-, 18-, and 24-month-old intact animals. Additionally, similar counts were also made from 12- and 18-month-old castrated injected rats. Finally, counts were made from 12-month-old injected intact rats.

The measurement and enumeration data described above were subjected to the t-test and to linear regression analysis. Analysis of variance was employed regarding the counts of secretion granules.

RESULTS

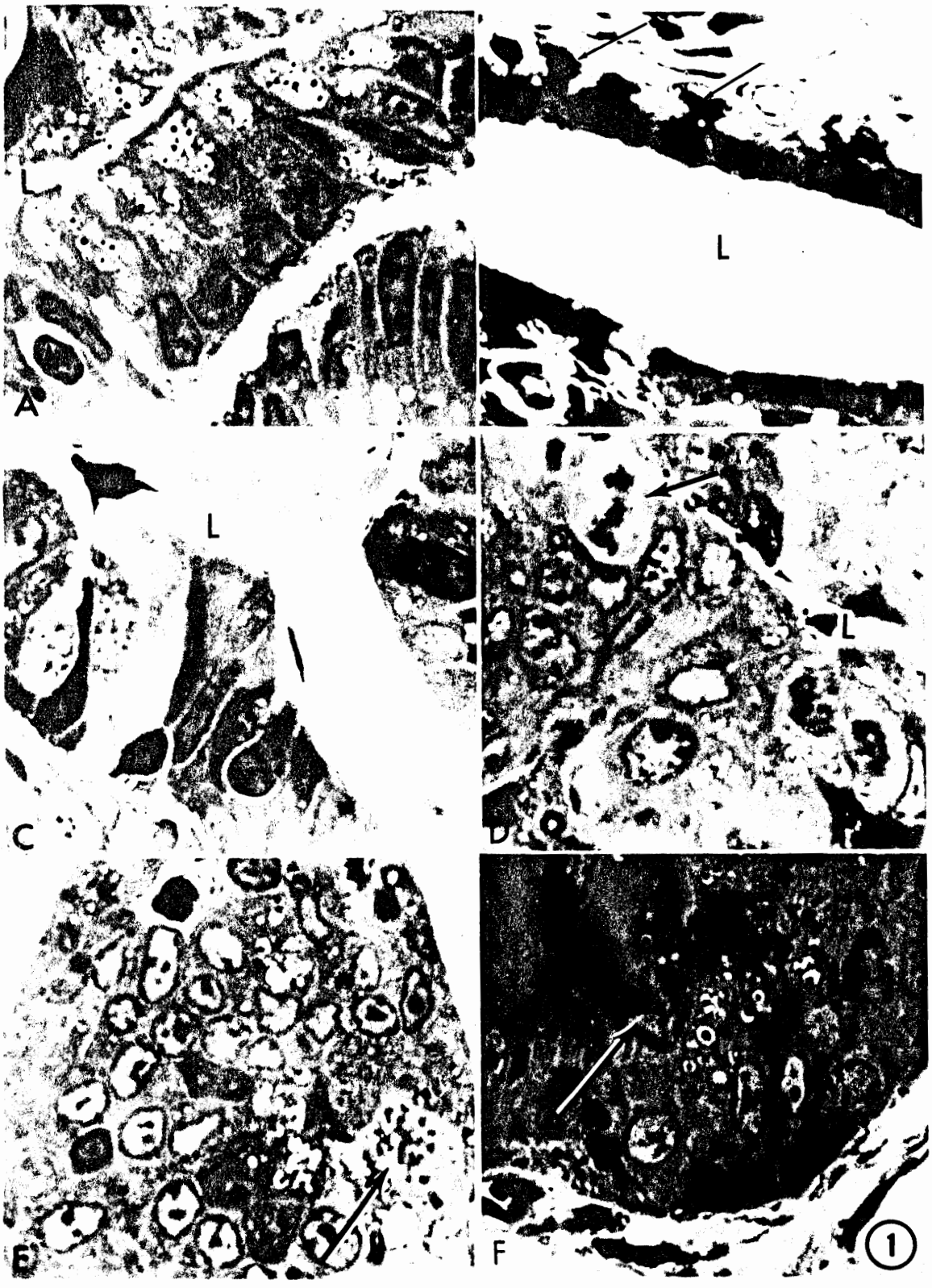
Light microscopy

The seminal vesicle is located in the posteromedial region of the abdomen. It is invested on the outside by a sheath of connective tissue which surrounds a mass of smooth muscle cells. The epithelium follows the many luminal folds and it separates the lamina propria from the lumen. The epithelium is formed by columnar cells and oblong basal cells which are arranged together in a pseudostratified configuration. In the intact rat the columnar cells contain granules often referred to as "haloed" secretory granules, so-called because they partially fill the Golgi vesicles (Fig. 1A). After castration, the columnar cells undergo involution so that the normally tall cells become quite dense and low-cuboidal in shape. The basal cells also undergo nuclear and cytoplasmic condensation and can be easily identified (Fig. 1B). Following androgen replacement the cells are rapidly restored (Fig. 1C). Androgen induces cell division in the injected-castrated animal (Fig. 1D) as well as in the injected intact rat (Figs. 1E and F).

Fine structure of epithelium of 3- to 6-month-old rats

The columnar cells contain an abundance of granular endoplasmic reticulum (GER) and an extensive supranuclear Golgi

FIGURE 1 Epithelium of the rat seminal vesicle. A. Before castration. (Notice secretory granules). B. After castration. C. Cell restoration following castration and androgen replacement. D. Androgen treated castrated animal. Lumen (L); Macrophage (M); mitotic figure (large arrows); Basal Cells after castration (small arrows). (Figures 1B and 5A represent adjacent thick and thin sections). X1,500.


L
A
L
C
L
D
L
E
F
1

complex with associated secretory granules (Figs. 2 and 3A). The GER cisternae are especially well developed in the basal region surrounding the nuclei (Fig. 2). Secretory granules are released by a merocrine mechanism (Fig. 3A) in which the membrane of the secretory granule fuses with the plasma membrane and in some instances by an apocrine mode in which a portion of the apical cytoplasm is lost with the secretory products into the lumen. A few dense bodies resembling lipofuscin granules, or age-pigment, are seen preferentially in the basal region of the cytoplasm. They are identical to the yellowish-brown pigment observed by light microscopy but have a variable morphology composed of lipid droplets, whorls of parallel membranes, remnants of cytoplasmic structures and lysosome-like granules albeit arranged in a pleomorphic manner (Fig. 3B).

Fine structure of epithelium in 12-month-old rats

As the animal ages, certain changes become readily apparent. Some of the cells in the pseudostratified epithelium exhibit degenerative changes such as the erosion of the cytoplasm and disintegration of the organelles. The relative volume of the lipofuscin in the cytoplasm increases. Both the size and the number of lipofuscin granules show gains over the younger rats. Secretory granules present in the Golgi zone of the cells of 12-month-old rats appear to be fewer in number than in the 4- to 6-month-old rats (compare Figs. 2 and 4). Another notable change is the infiltration of epithelium by

FIGURE 2 Epithelium of the rat seminal vesicle. Animal age, 6-7 months. Basement membrane (B), capillary (C), fibroblast (F), granular endoplasmic reticulum (ER), Golgi (G), lumen (L), pigment (P). X13,500.

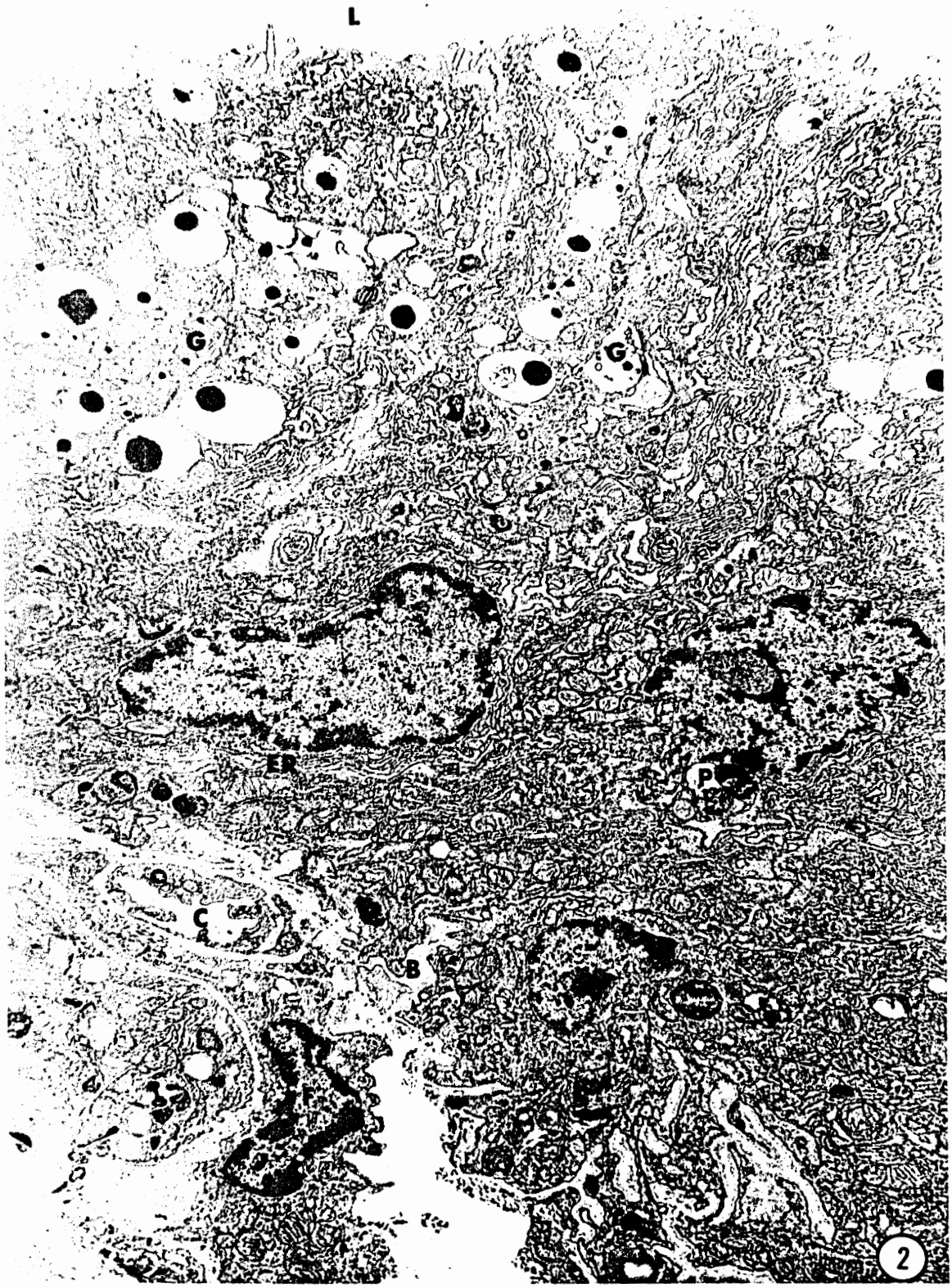
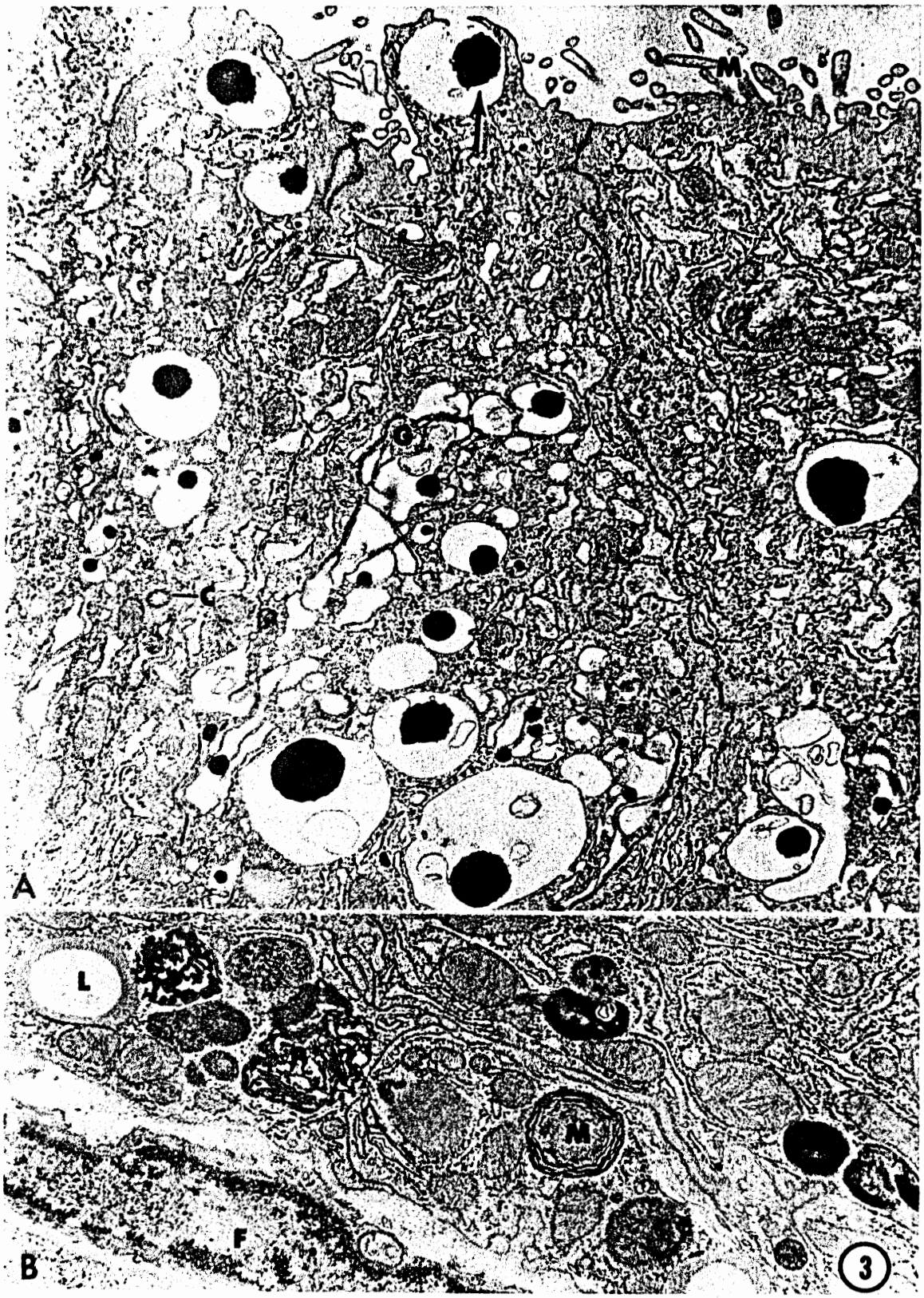

L
G
G
ER
P
C
B
2

FIGURE 3 A. Closer view of apical cytoplasm with Golgi zone. Animal age, 6 months. Notice secretory granule escaping into lumen (large arrow). Desmosome (small arrow), centriole (C), Golgi complex (G), microvilli (M). X33,600. B. Basal region of epithelial cell. Animal age, 6 months. Fibroblast in subjacent connective tissue (F), lipid (L), myelin figure (M) lysosome-like dense bodies (DB), pigment (P). X31,000.


M
C
C
D
D
A
L
M
F
B
3

macrophages which are seen amidst the basal and columnar cells (Fig. 4).

After castration, cell height is greatly reduced. The cytoplasm of the columnar cells is greatly reduced in volume forming a thin layer around the nucleus (Fig. 5A). In relation to these cells, the basal cells appear to be lying within the collagenous lamina propria and are partially surrounded by the intact epithelial basement membrane (Fig. 5A). The nuclei and cytoplasm of most of the basal cells are very dense (see Figs. 1B and 5A) probably due in part to the abundance of heterochromatin in the nuclei and the packing of the remaining cytoplasmic organelles after castration (Figs. 5A and 5B). Large accumulation of the lipofuscin and lipid droplets appear in the cytoplasm (Fig. 5B). GER is quite sparse as are the poorly developed Golgi bodies. Secretion granules are not visible. Some free ribosomes are observed and glycogen-like particles are abundant. Mitochondria are quite elongated and are often coiled (Fig. 5B). Intercellular spaces between epithelial cells become quite widened in castrated animals (compare Figs. 2, 3A and 4 with 5B and 6).

The lamina propria underlying the epithelium contains numerous capillaries which occur in the close vicinity of the epithelial cells (Figs. 2, 4, 6, 7A and 7B). After castration the fibroblasts become exceedingly active and the amount of collagen produced is abundant (Figs. 5A, 6, and 7B). In

FIGURE 4 Epithelium of the seminal vesicle of the rat. Animal age, 12 months. Capillary with red blood cells (C), degenerating cells (D), Golgi zone (G), macrophage (M), lumen (L), pigment (P). X10,800.

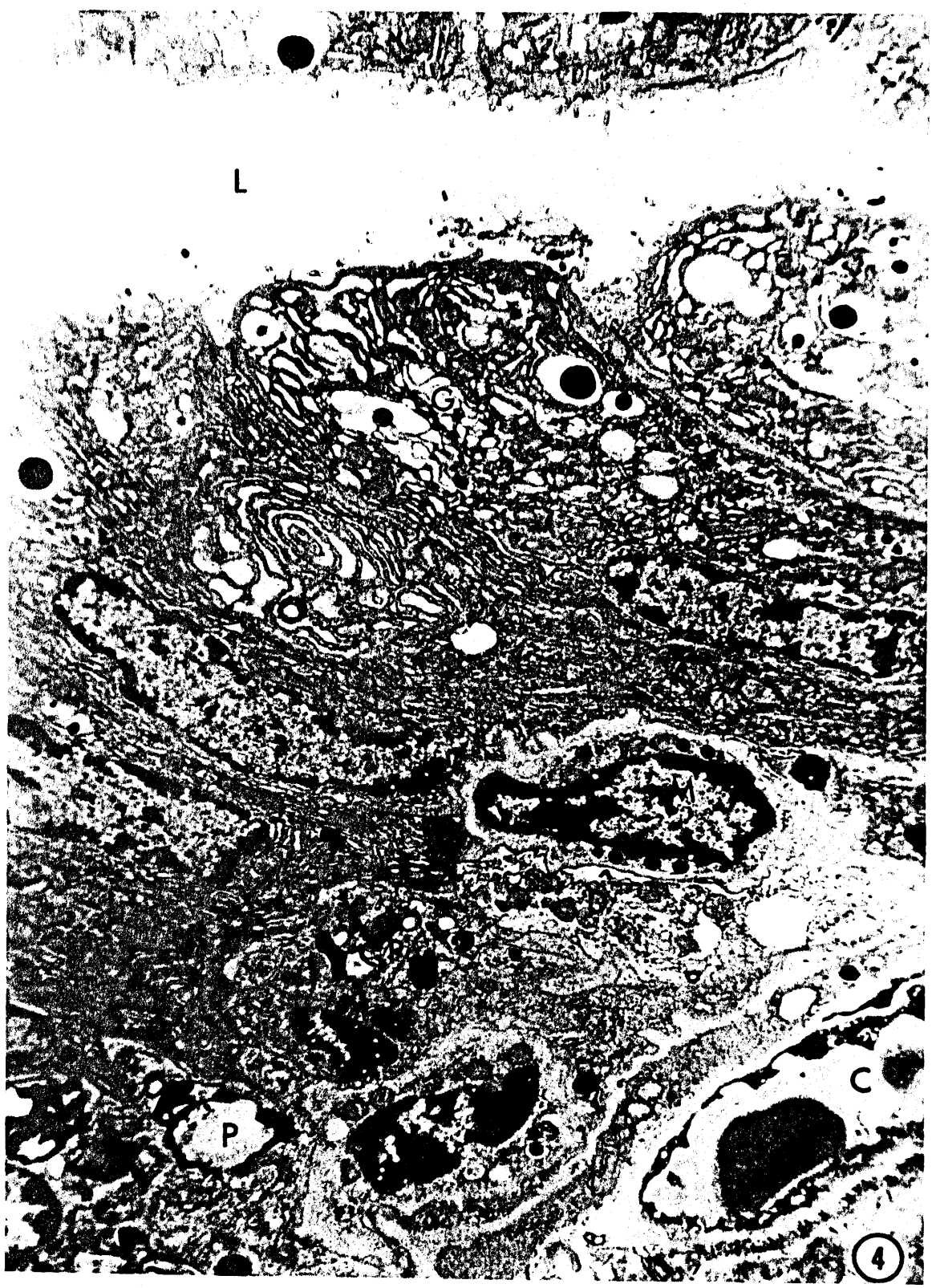

L
G
P
C
4

FIGURE 5 Epithelium of the rat seminal vesicle after castration. Animal age, 12 months. Basal cell (B), Golgi (G), lumen (L), lipid (l), pigment (P), intercellular-space (small arrows), coiled mitochondrion (large arrow). A. X8,500. B. X13,200.

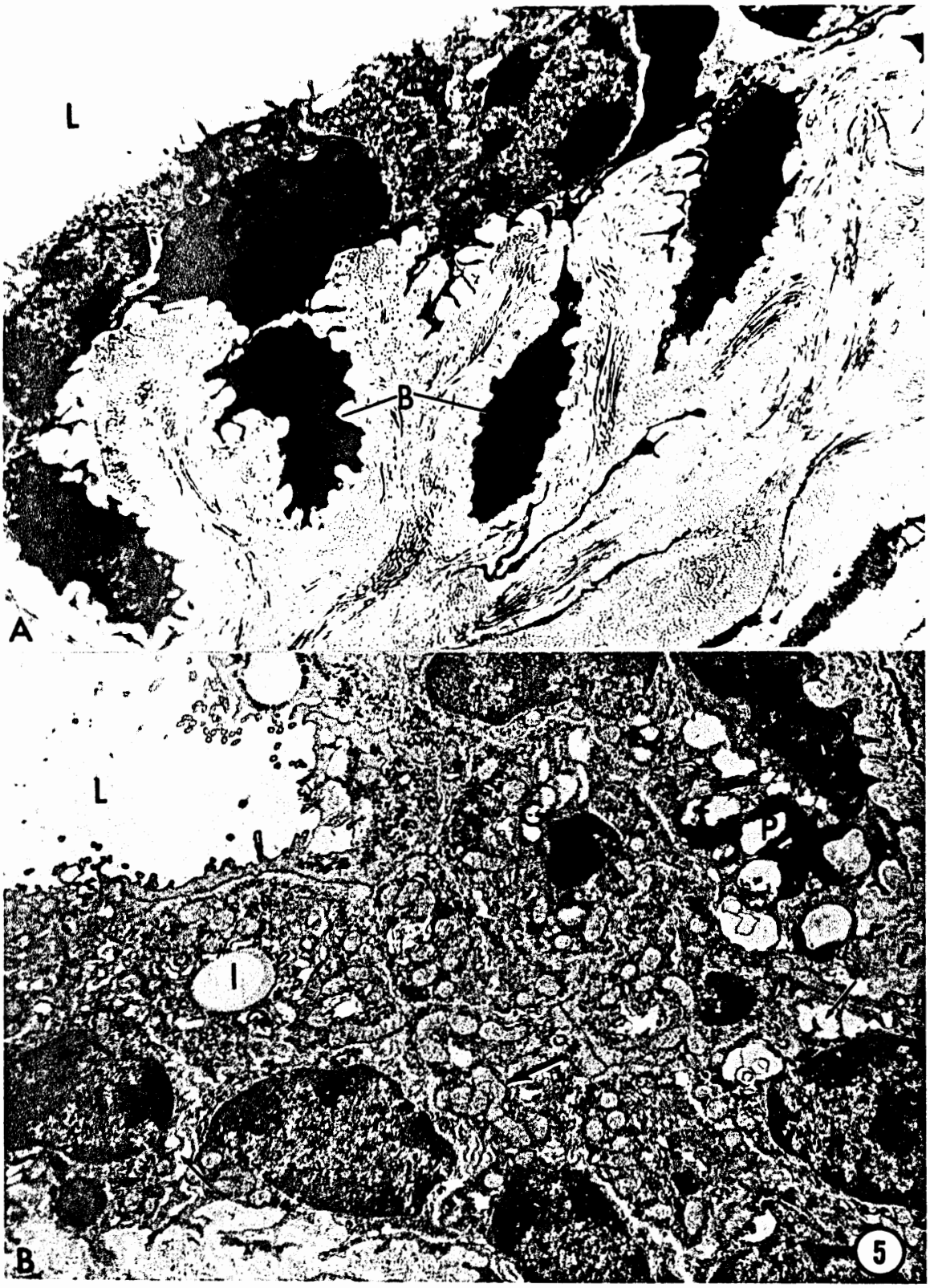

L
B
A
L
P
I
B
5

FIGURE 6 Capillary in lamina propria of seminal vesicle. Castrated animal 12 months of age. Notice basement-membrane-like material (B) around endothelium. Fibroblast processes (arrows), collagen (C), epithelium (E), lumen (L). X16,400.

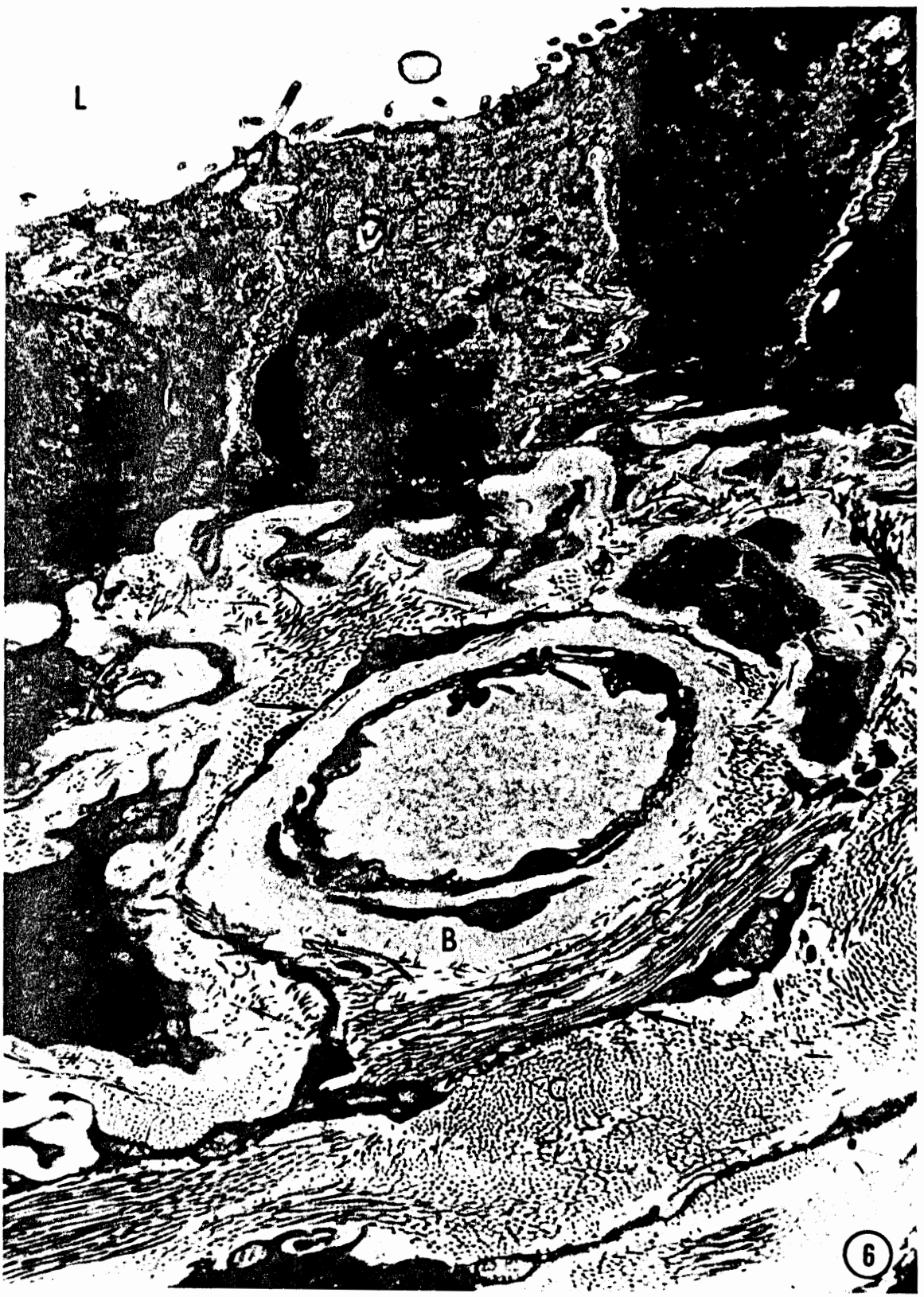
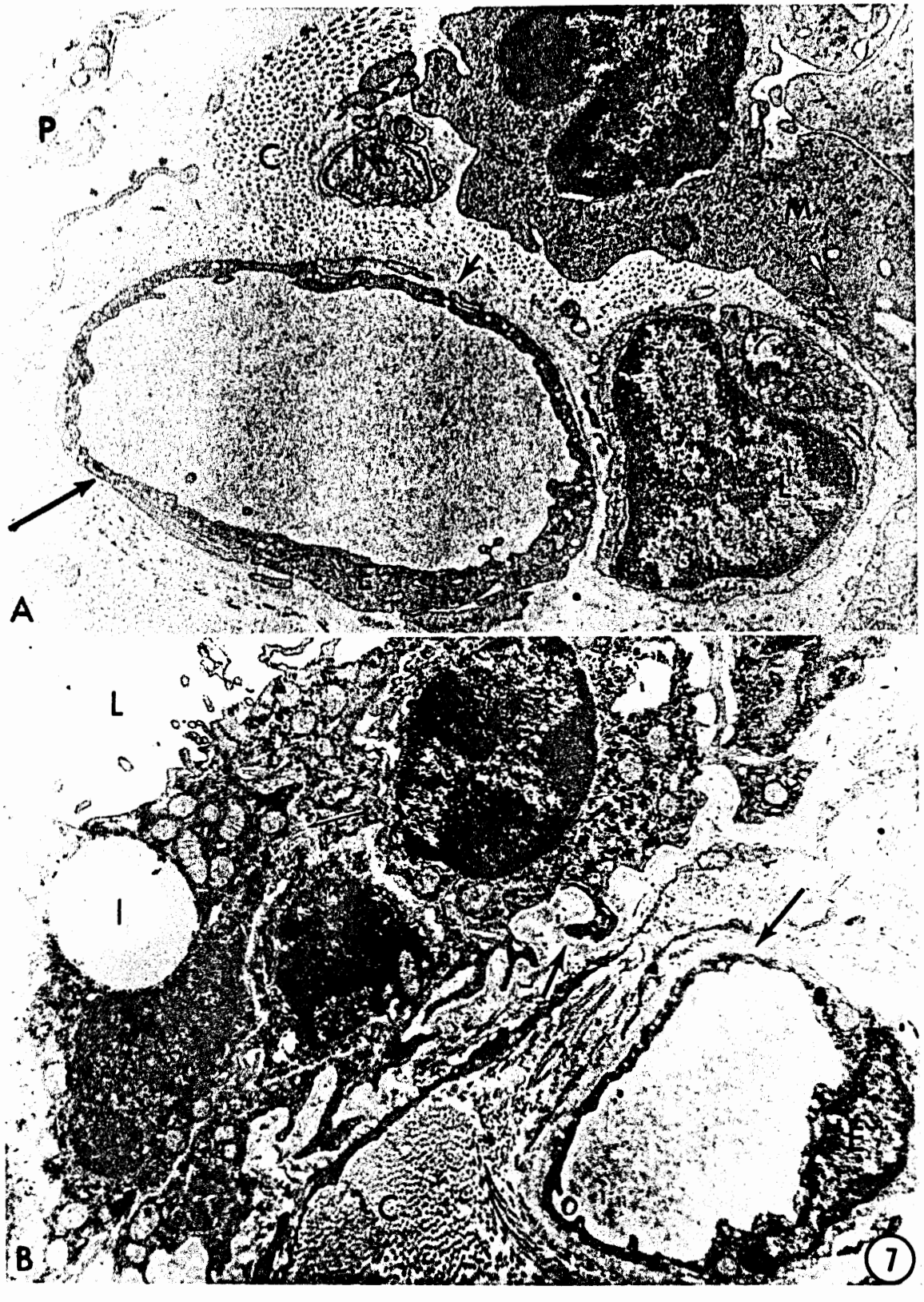

L
B
6

FIGURE 7 A. Lamina propria of rat seminal vesicle. Animal age, 8 months. Collagen (C), endothelium of capillary (E), lymphocyte (L), macrophage (M), nerve (N), fibroblast cytoplasmic process (P), basement membrane of capillary (arrow). X 15,600. B. Epithelium and subjacent lamina propria of seminal vesicle of castrated rat. Animal age, 12 months. Collagen (C), endothelium (E), lipid (l), lumen (L), basement membrane (large arrow). intercellular spaces (small arrow). X 16,000.


P
C
M
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L
I
B
C
O
7

the castrated animals the endothelial cells of the capillaries are surrounded by a relatively thick layer of basement membrane-like material, collagen, and fibroblast cytoplasmic processes (Fig. 6). Some capillaries appear to retain the basement membranes of normal dimensions (compare Figs. 7A and 7B).

Epithelium in the castrated animal is restored to an approximately normal height after 3 injections of 6mg/day androgen. Following hormonal replacement, the columnar cells reacquire a relative abundance of GER and well developed Golgi bodies. Some secretion granules are formed and small pigment granules can be seen in the basal cytoplasm. Large macrophage-like cells occasionally exhibiting mitotic division are frequently observed in the epithelium. It should be noted that the macrophages in the basal region of the epithelium can be confused with the basal epithelial cells, especially at the light microscopic level, and their mitotic activity erroneously ascribed to the epithelial cells (Fig. 8).

Waiting four months after castration does not prevent cellular restoration by androgen replacement therapy; however, many cells still exhibit age-associated degenerative changes in the Golgi regions and intercellular spaces similar to those in the cells of the castrated animal of the same age (Fig. 9). Fine structure of the epithelium in the 18-month-old rats shows some secretory activity; however degradative changes at this

FIGURE 8 Epithelium of seminal vesicle which has been restored after castration by androgen therapy. Animal age, 12 months. Macrophage (M), mitotic figure of non-epithelial cell (MF), pigment (P), secretory granules in Golgi zone (arrows). X12,800.

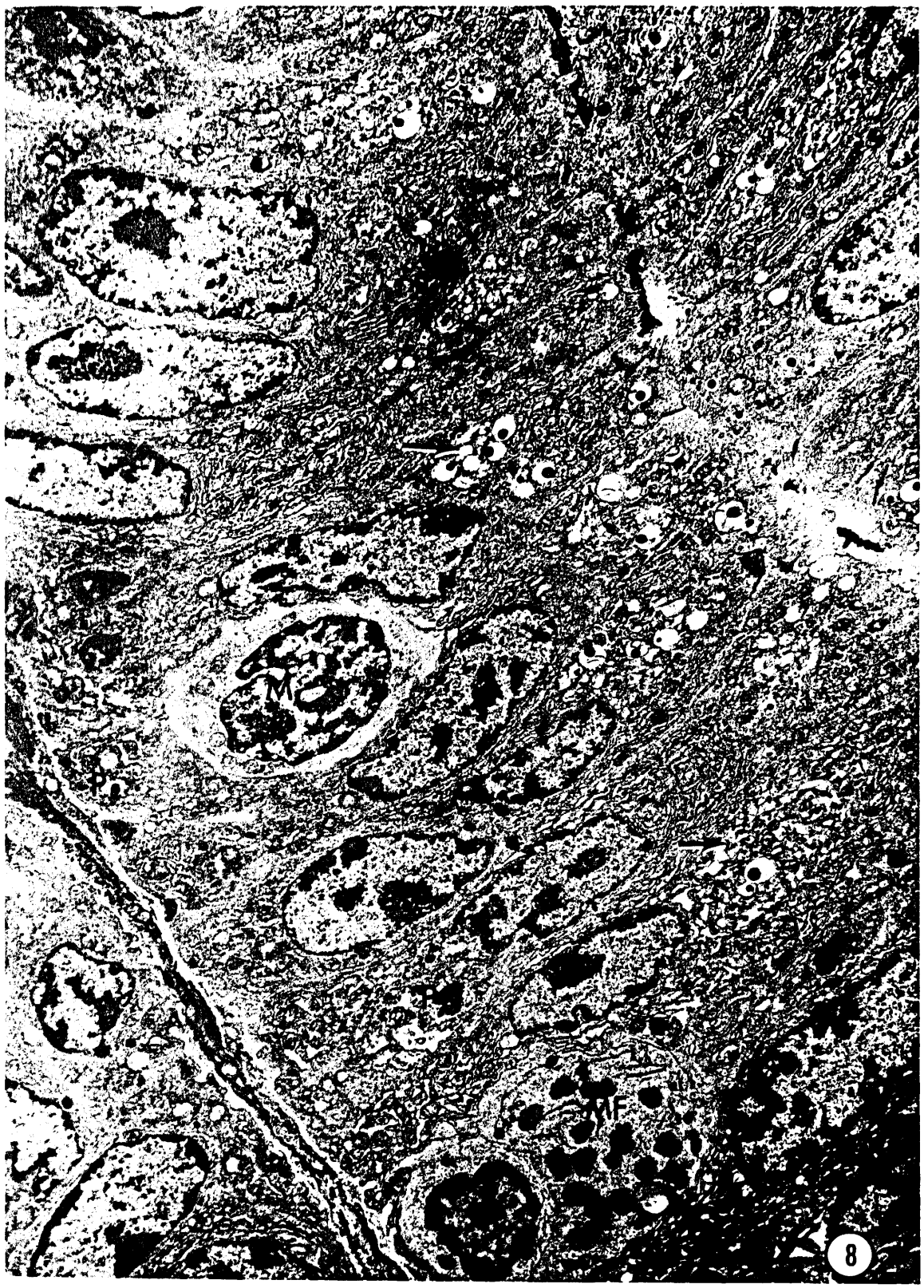
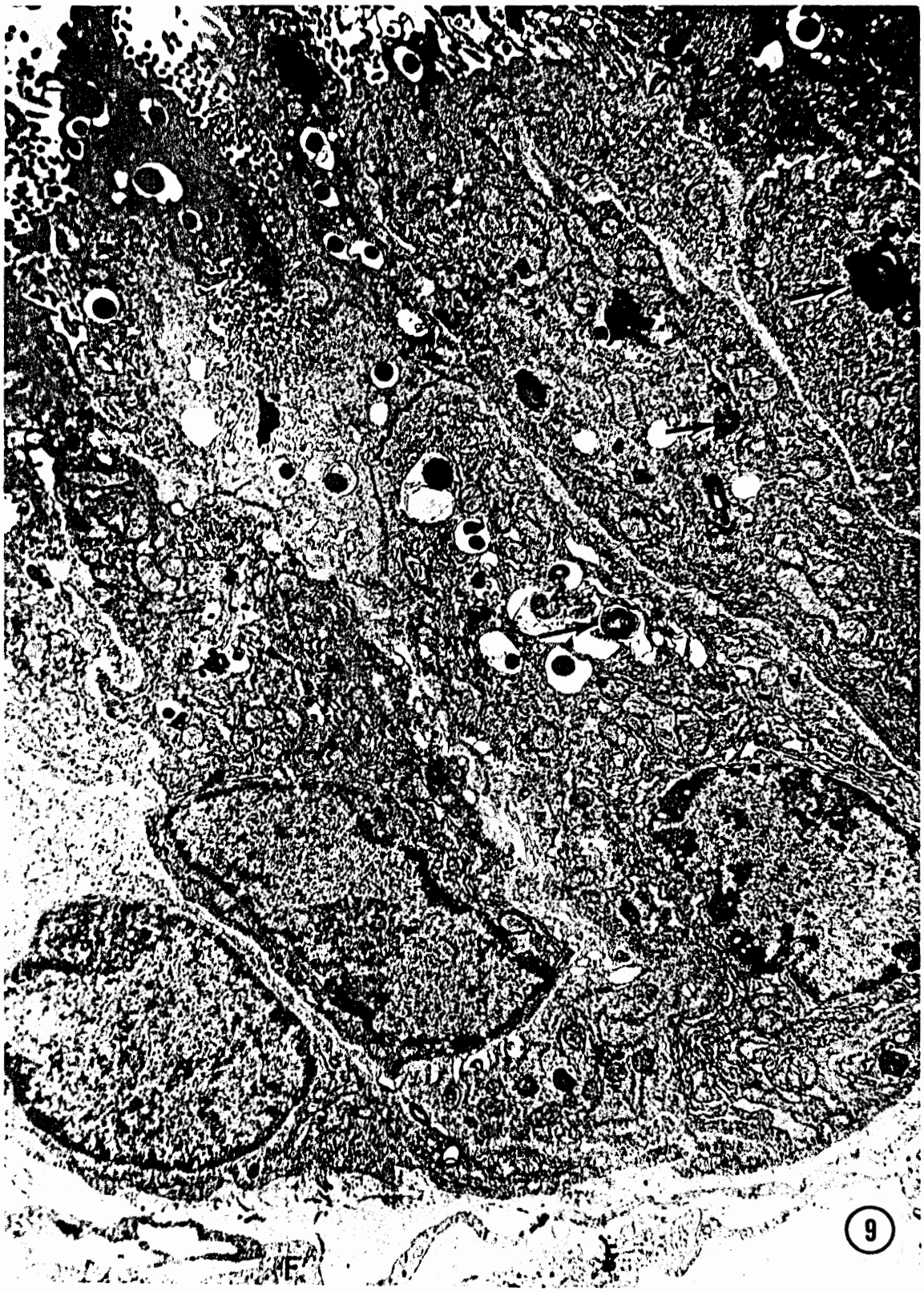

M
Pn
Pg
MF
8

FIGURE 9 Epithelium of seminal vesicle of rat to which androgen was administered, four months after castration. Animal age, 12 months. Notice many myelin figures (arrows) associated with Golgi zone. Fibroblast of connective tissue (F). X20,000.


9

age become quite pervasive and severe. Degenerative cells are frequent; cellular debris, including distorted GER cisternae as well as other organelles, is often observed within the lumen (Fig. 10A), Golgi vesicles are frequently associated with membranous whorls which is indicative of membranous disorganization (Fig. 10B). Many macrophages are present and often these cells extend several cytoplasmic processes into the widened intercellular spaces (Fig. 10B). Some of the epithelial cells in 18-month-old rats have an overall electron density greater than the other cells. Such differences in density are observed in younger rats with lesser frequency. The physiological significance of this observation is unclear.

Effects of castration are quite pronounced in the 18-month-old rat and are reflected by the presence of lipid droplets and lipofuscin bodies within the low cuboidal cells. The lipofuscin granules in 18-month-old rats are structurally similar to those in 12-month-old intact and castrated rats but they are larger and more numerous (Fig. 11).

Androgen replacement therapy results in the reestablishment of the pseudostratified appearance of the epithelium. Membranous whorls are associated with Golgi vesicles and with mitochondria. The epithelial cells exhibit distinct intercellular spaces of low electron density which extend to the apical terminal bars (Fig. 12). The intensity of the altera-

FIGURE 10 Epithelium of the rat seminal vesicle. Animal age, 18 months. Cytoplasmic debris in lumen (D), degenerating cell (DG), myelin figures (M), pigment (P), macrophage cytoplasmic processes (large arrows). A. X10,000. B. X8,700.

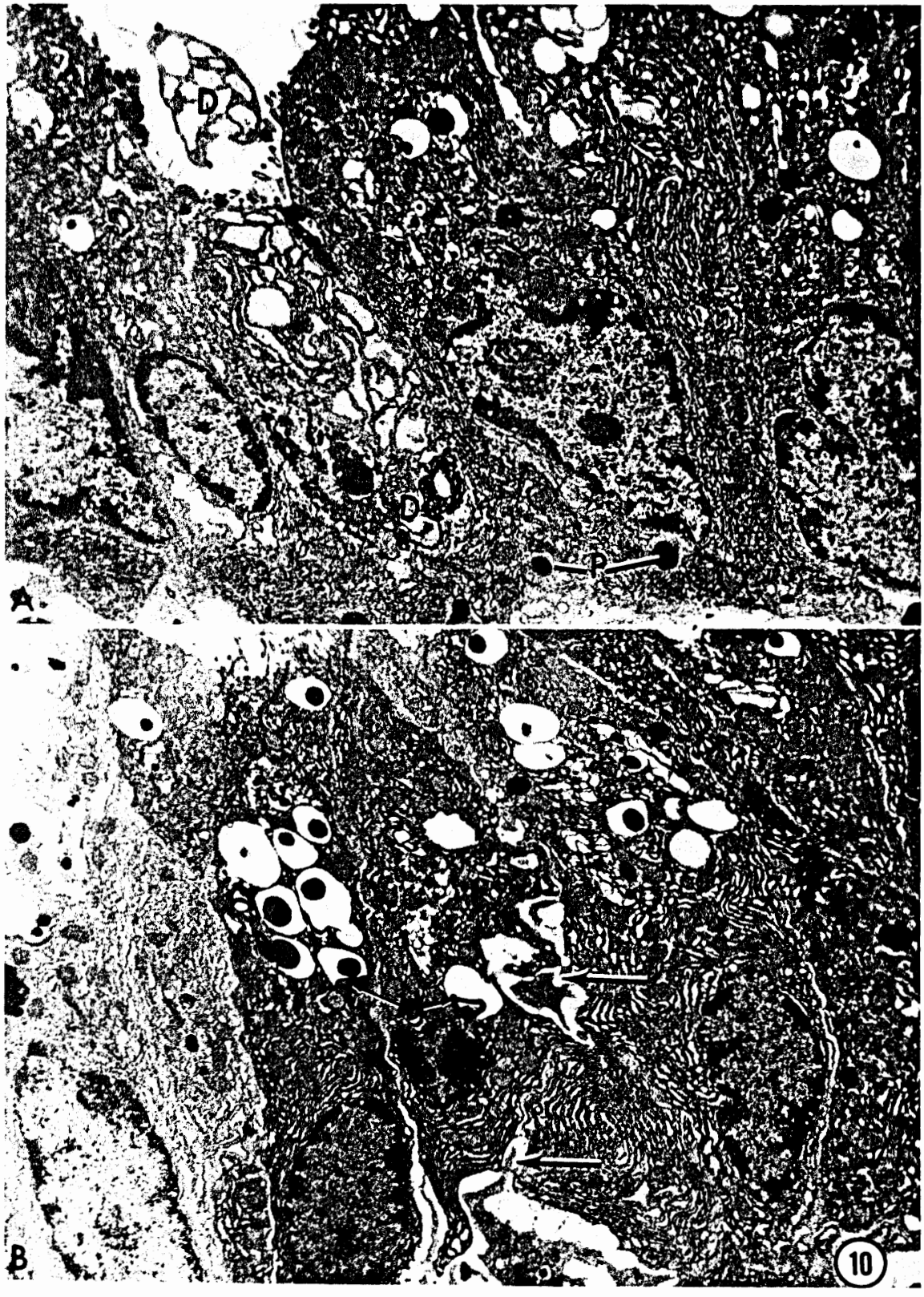
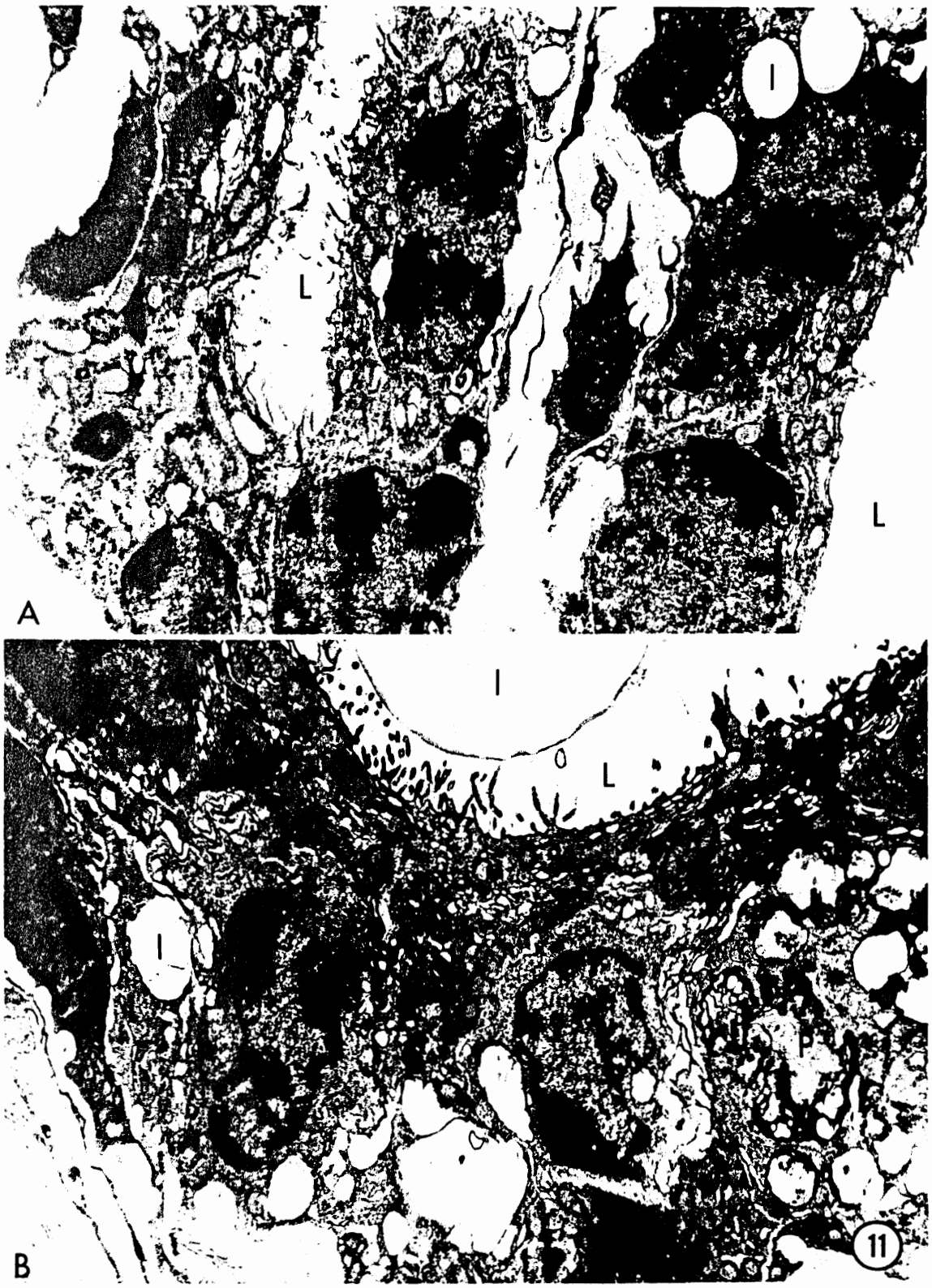

D
D
P
A
B
10

FIGURE 11 Seminal vesicle epithelium after castration. Animal age, 18 months. Lumen (L), lipid (l), pigment (P). A. X17,600. B. X17,200.


I
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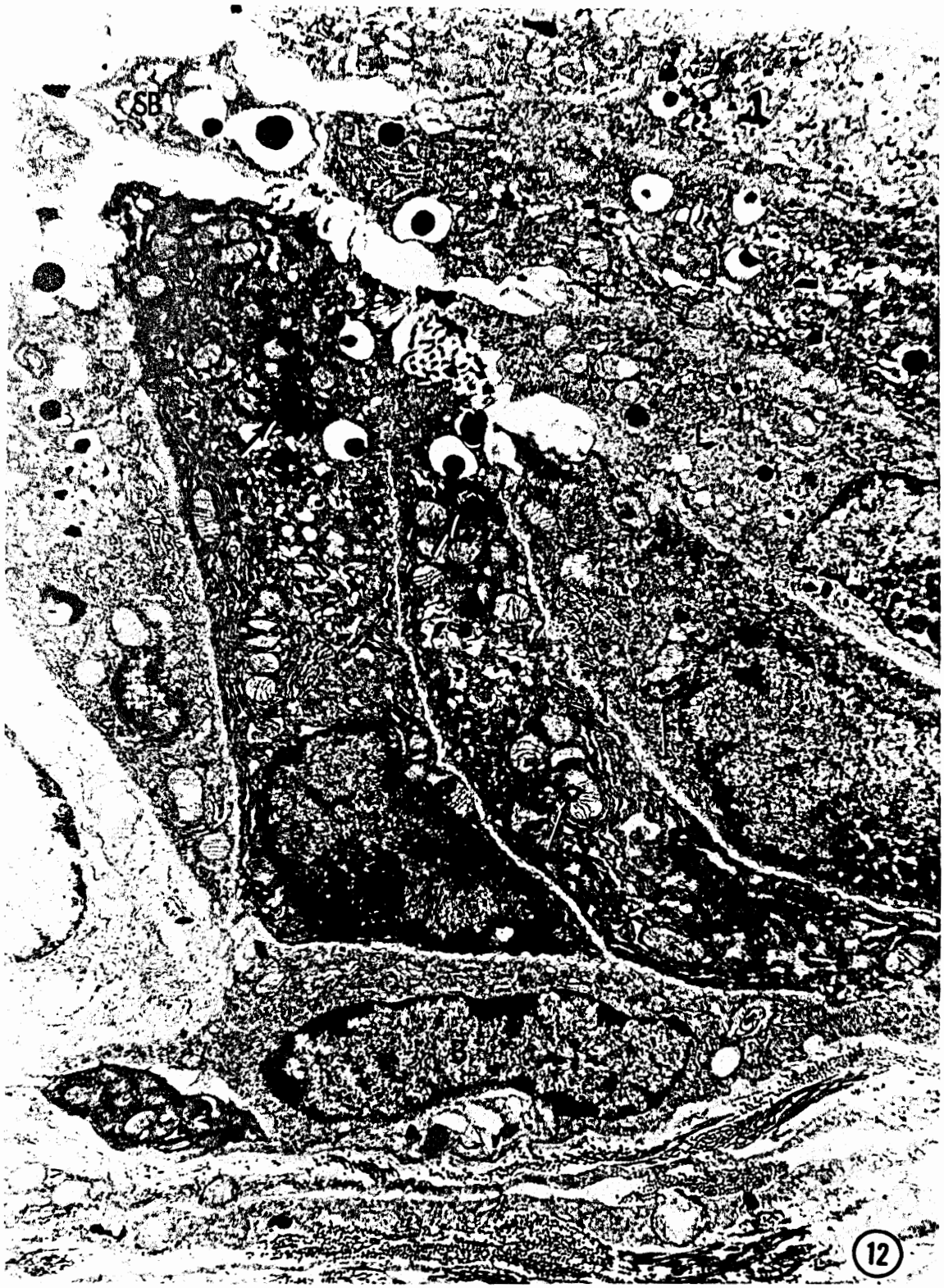
L
A
I
L
I
P
B
11

FIGURE 12 Epithelium of seminal vesicle of rat after castration and androgen replacement therapy. Animal age 18 months. Myelin figures (large arrows), myelin figures in association with mitochondria (little arrows), basal cell (B), columnar cell (C), apocrine secretory "blebs" in lumen (SB), dark cell (D), intercellular space (I), light cell (L), terminal bars (T). X15,700.


SB
1
L
B
12

tions remaining after androgen administration in the 18-month-old rats varies in different cells with some cells showing lateral cell contacts well reestablished by the formation of desmosomes, cell interdigitations and terminal bars (Fig. 13). In general, there appears to be a decline in the number of secretion granules and volume of Golgi components as compared to young animals. The GER appears relatively well developed (Figs. 12 and 13).

Fine structure of epithelium in 22- to 24-month-old rats

The most striking modification in the epithelial layer of the seminal vesicle of the 22- to 24-month-old rat observed by light microscopy is the accumulation of huge yellowish-brown pigment masses (Figs. 14A, B). Electron micrographs reveal that most of the cytoplasm in pigmented cells is occupied by lipofuscin granules composed of myelin figures in association with lipid droplets and an abundance of dense beaded granular material (Fig. 14C).

Although a proposed sequence of events of a dynamic process based on static images remains tentative, a scheme of events leading to the formation of lipofuscin can be suggested. An early event in cytoplasmic degradation appears to be the degranulation of GER membranes especially in the lateral and basal portions of the perinuclear cytoplasm (Fig. 15A). Areas of the cytoplasm become enclosed within an envelope formed by the cisternae of the endoplasmic reticulum. The inner membrane

FIGURE 13 Seminal vesicle epithelium after castration and androgen replacement. Animal age, 18 months. Desmosomes (small arrows), Golgi zone (G), interdigitation of lateral cell boundaries (I), terminal bars (T). X19,600.

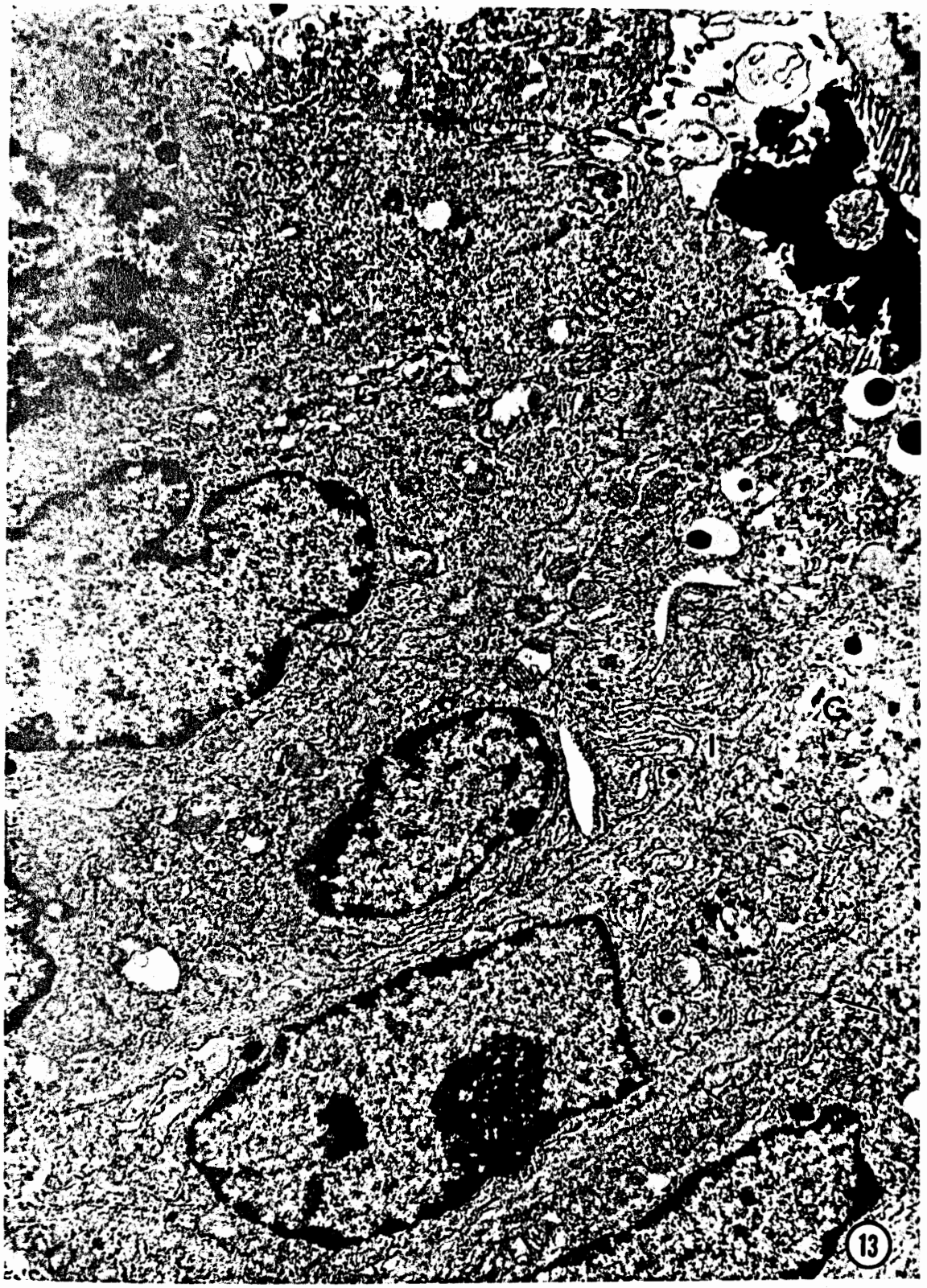
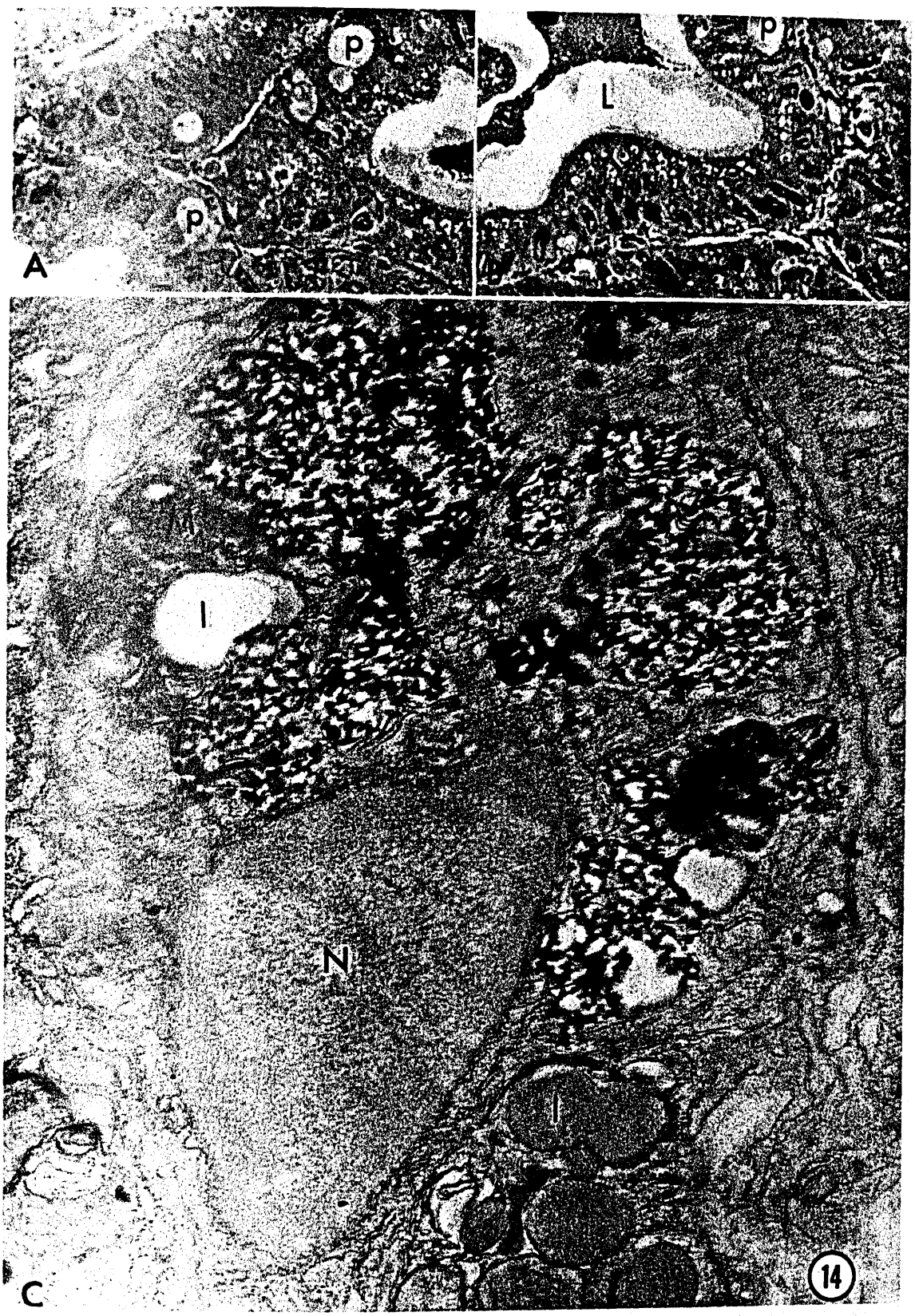

G
I
13

FIGURE 14 A. and B. Light microscopy of rat seminal vesicle epithelium. Animal age, 24 months. Lumen (L). Notice large masses of pigment (P). X750. C. Electron microscopy of pigmented cell. (Adjacent thin section to cell labelled P in upper right corner of 14A). Granular material (G), lipid (l), myelin figures (M), nucleus (N). X33,000.


P
P
A
P
L
I
N
I
C
14

of the cisternae and the enclosed cytoplasm undergo degeneration resulting in the formation of membranous whorls and lipid-like structures (Fig. 15B). The larger lipofuscin may be formed by the fusion of smaller structures. In some large lipofuscin granules dense granular and flocculent material is more predominant than the membranous components (compare Figs. 15C and 15D). It appears that degenerating cytoplasmic components in the Golgi zones and the supra-nuclear cytoplasm may be cast out into the lumen along with the secretory granules whereas degenerating components around the nucleus and in the basal cytoplasm give rise to lipofuscin. The lipofuscin either accumulates in the cytoplasm (Fig. 14C) or it may be cast off into the intercellular space from where it can be removed by the numerous macrophages present in old tissue (Fig. 16).

Large pigment accumulations are prominent after castration (Fig. 17A) and remain relatively unaffected by androgen restoration (Fig. 17B). However, the secretory activity of the epithelium is somewhat restored by androgen administration (Fig. 18).

Non-epithelial cells

The non-epithelial cells that appear within the epithelial layer are of two types: macrophages and lymphocytes. These cells are often seen in the lamina propria near the capillaries from which they probably escape (Fig. 7A). The

FIGURE 15 Images depicting stages in the genesis of lipofuscin. Animal age, 24 months. A. Early stage of focal degeneration of granular endoplasmic reticulum. Notice limiting membranous envelope (arrow). Nucleus (N), pigment (P), lysosome-like dense body (L). X27,000. B. Later stage illustrating enlarged area of degeneration with myelin figures forming (arrows). Nucleus (N). X27,000. C., D. Residual structures. Myelin figures (M), nucleus (N), lipid (l), granular material (arrows). C. X16,600. D. X17,000.

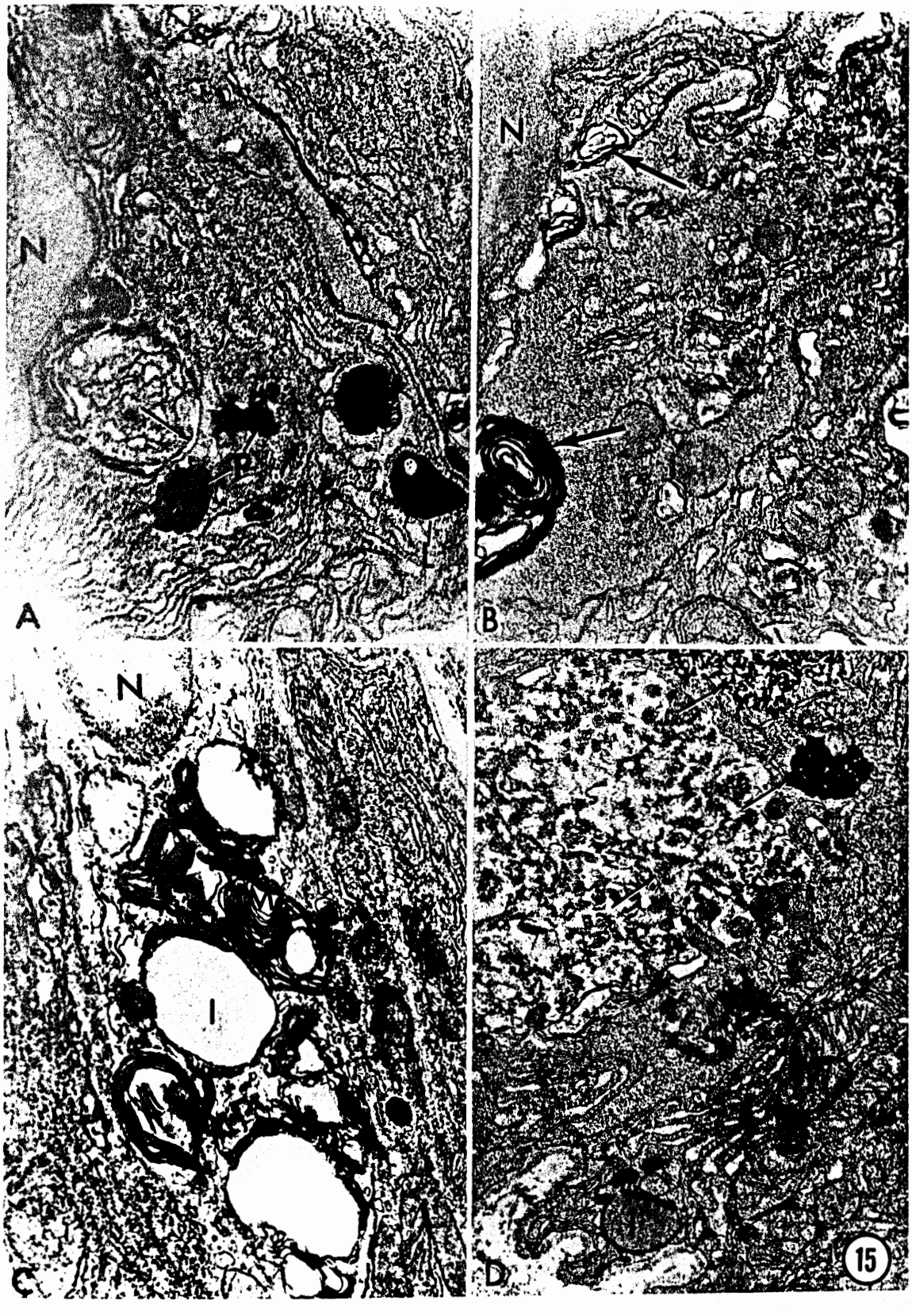

N
A
N
B
N
I
C
D
15

FIGURE 16 Epithelium of rat seminal vesicle. Animal age, 24 months. Notice macrophages (M) and their many cytoplasmic processes (P). X9,200.

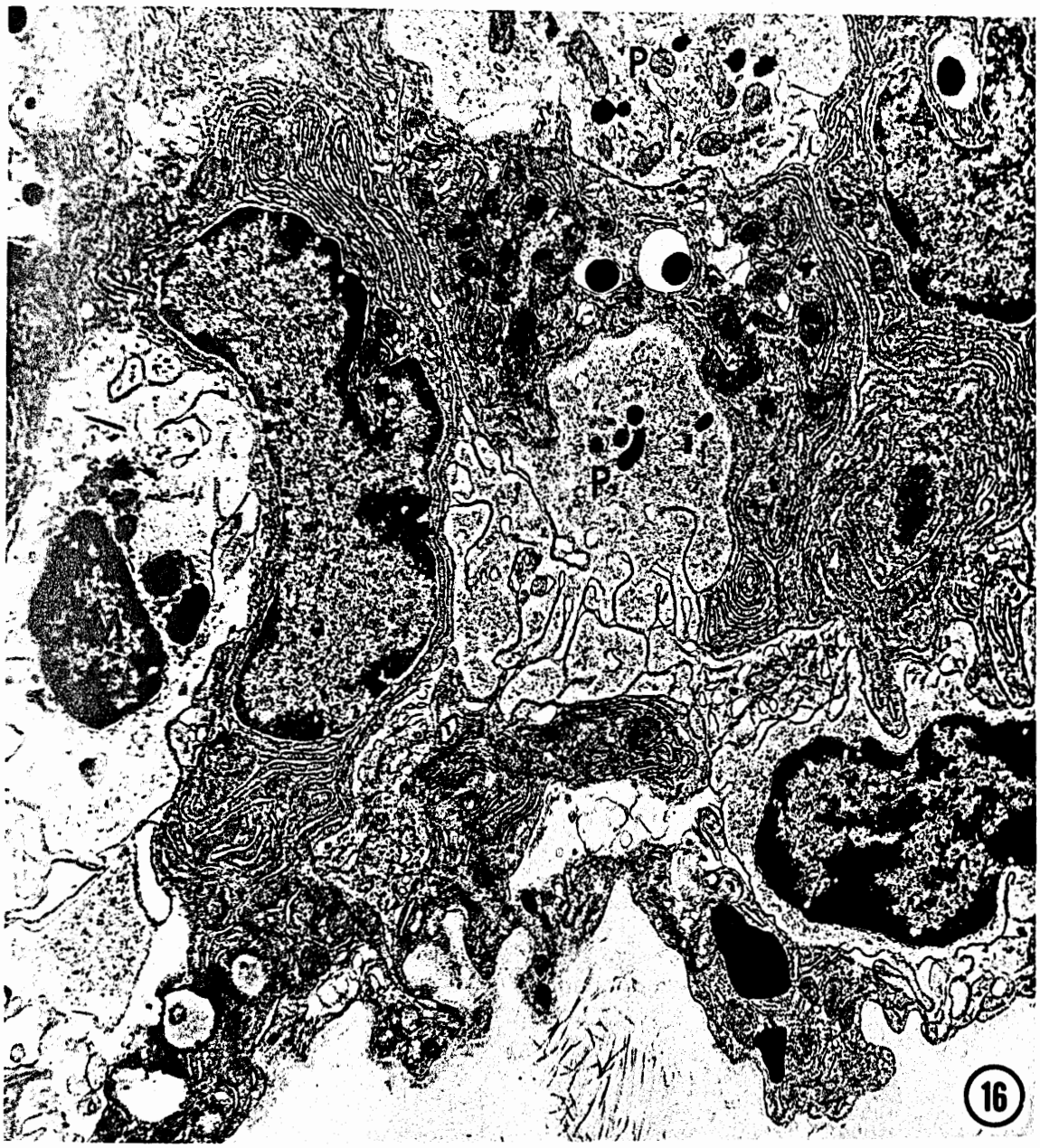

P
P
16

FIGURE 17 A. Epithelium of seminal vesicle of castrated rat. Animal age, 24 months. Plane of section does not pass through lumen. Pigment (P). X9,900. B. Portion of cytoplasm containing lipofuscin in epithelial cell after castration and androgen administration. Animal age, 24 months. X32,500.

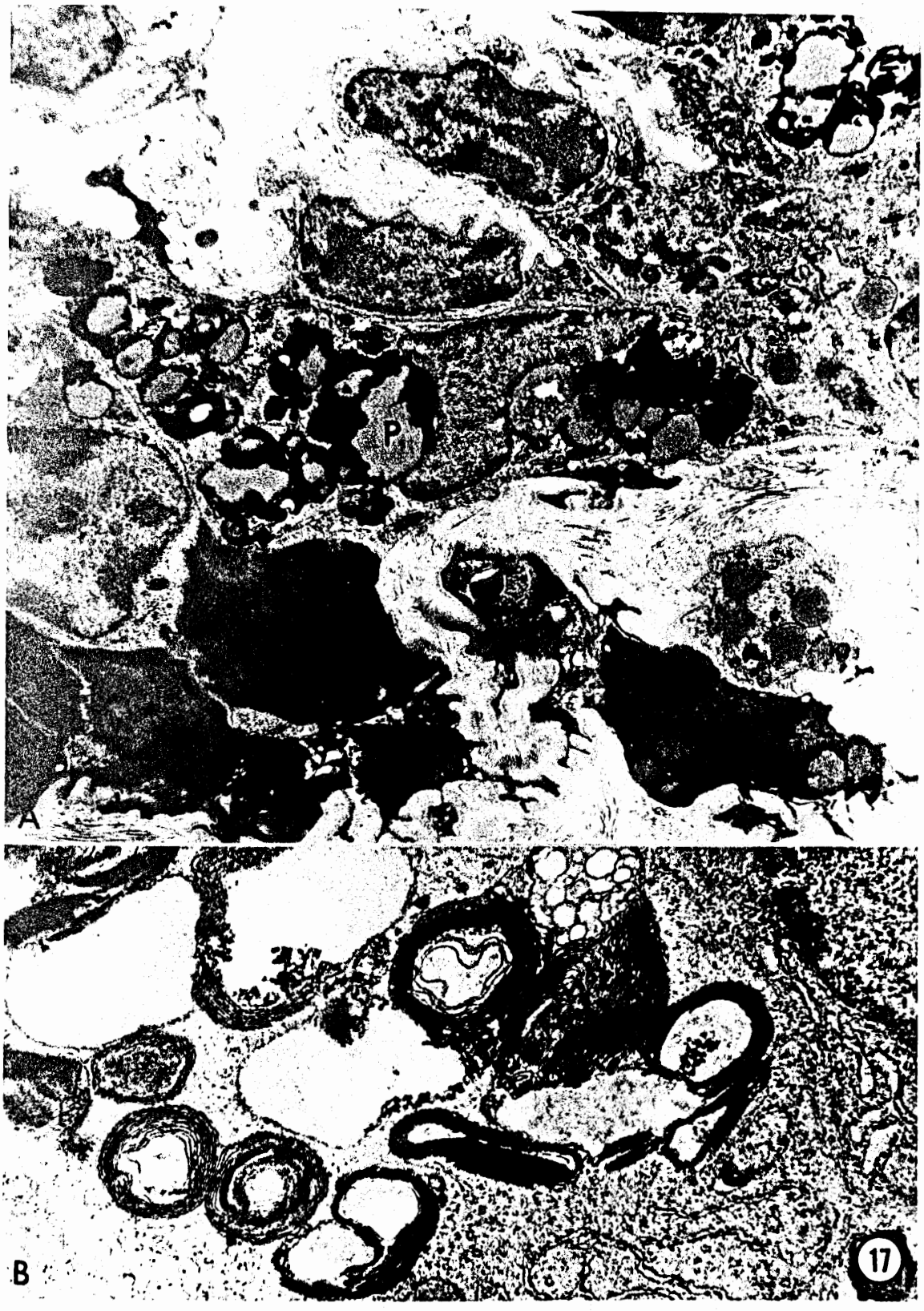
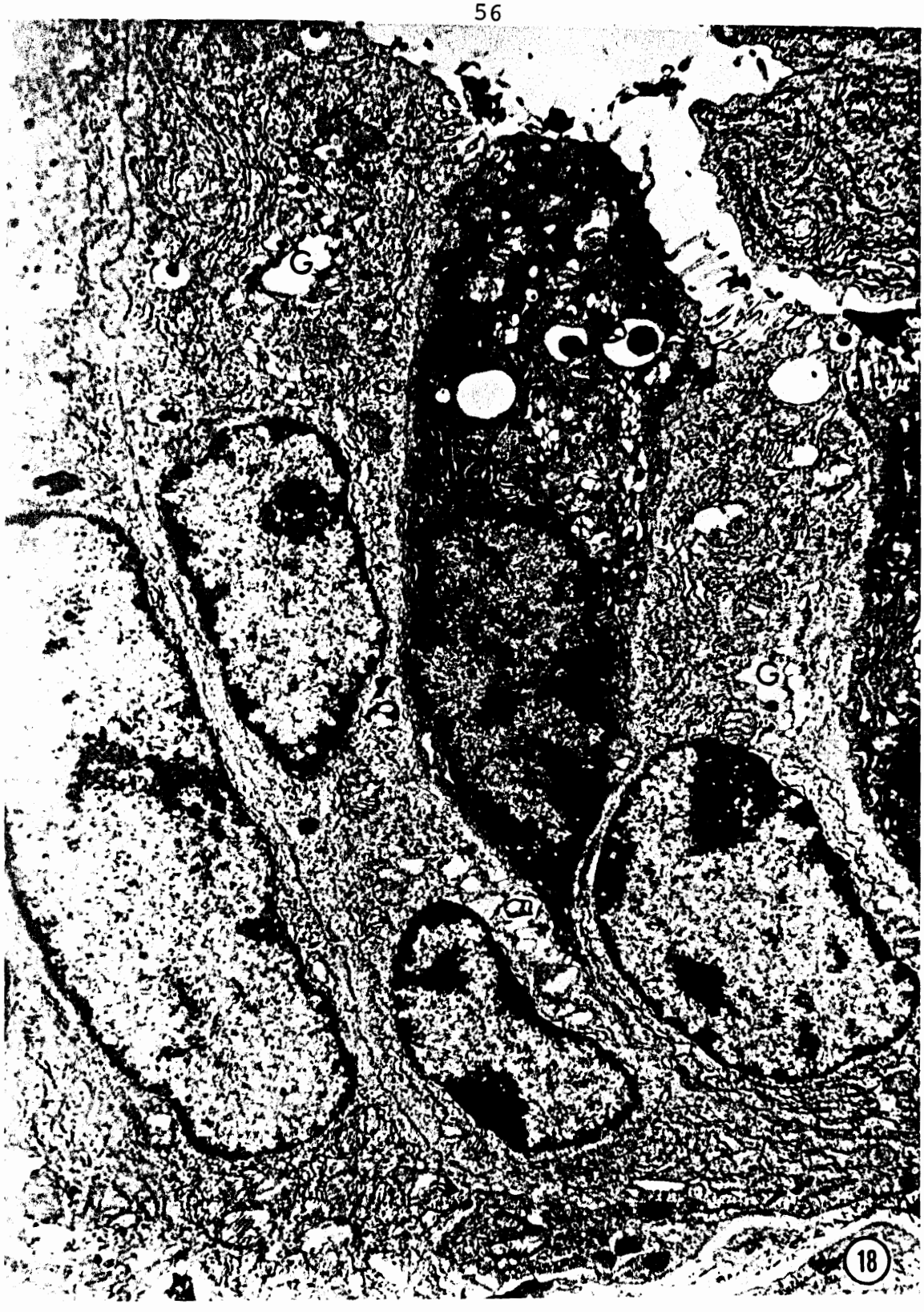

P
A
B
17

FIGURE 18 Epithelium of the rat seminal vesicle after castration and androgen administration. Animal age, 22 months. Dark cells (D), Golgi (G), light cells (L). X11,400.


G
G
A
A
18

macrophage is characterized by an abundance of cytoplasm and attenuated cytoplasmic processes (Figs. 7A, 10B, 16, 19B, 20A-C). The cytoplasm in active macrophages contains dense bodies that represent lysosomes and ingested debris (Figs. 19B, 20C, D). These cells are found above the basement membrane and among the epithelial cells (19C). They apparently actively remove cellular debris. The lymphocyte is characterized by a nucleus indented on one side and the presence of a narrow rim of cytoplasm (Figs. 7A, 19A, 20E, F). Occasionally, a cell resembling a lymphocyte also appears engaged in phagocytic activity (Fig. 20F). Apparently, the epithelial cells of the seminal vesicle can release cellular debris on their lateral surfaces into distended intercellular spaces where the non-epithelial cells are present. Cytoplasmic debris and the remnants of necrotic cells may be removed by macrophage activity in this manner. The macrophage activity progressively increased with age.

Injected intact aging animals

Morphologic data suggest that the number of secretion granules formed in cells of young adult male rats (3-6 months of age) is somewhat reduced by twelve months of age. This could result from either a decrease in circulating androgen level (see Mainwaring and Brandes, 1974, for review) or reduced tissue responsiveness to androgen. The epithelium of intact 12-month-old rats receiving three injections of testosterone

FIGURE 19 Epithelium of the rat seminal vesicle. Animal age, 24 months. A. Lymphocyte. X9,000. B. Epithelium containing macrophages (M) and pigment (P). X3,000. C. Light microscopy. Notice non-epithelial cells in epithelial layer (arrows). X470.

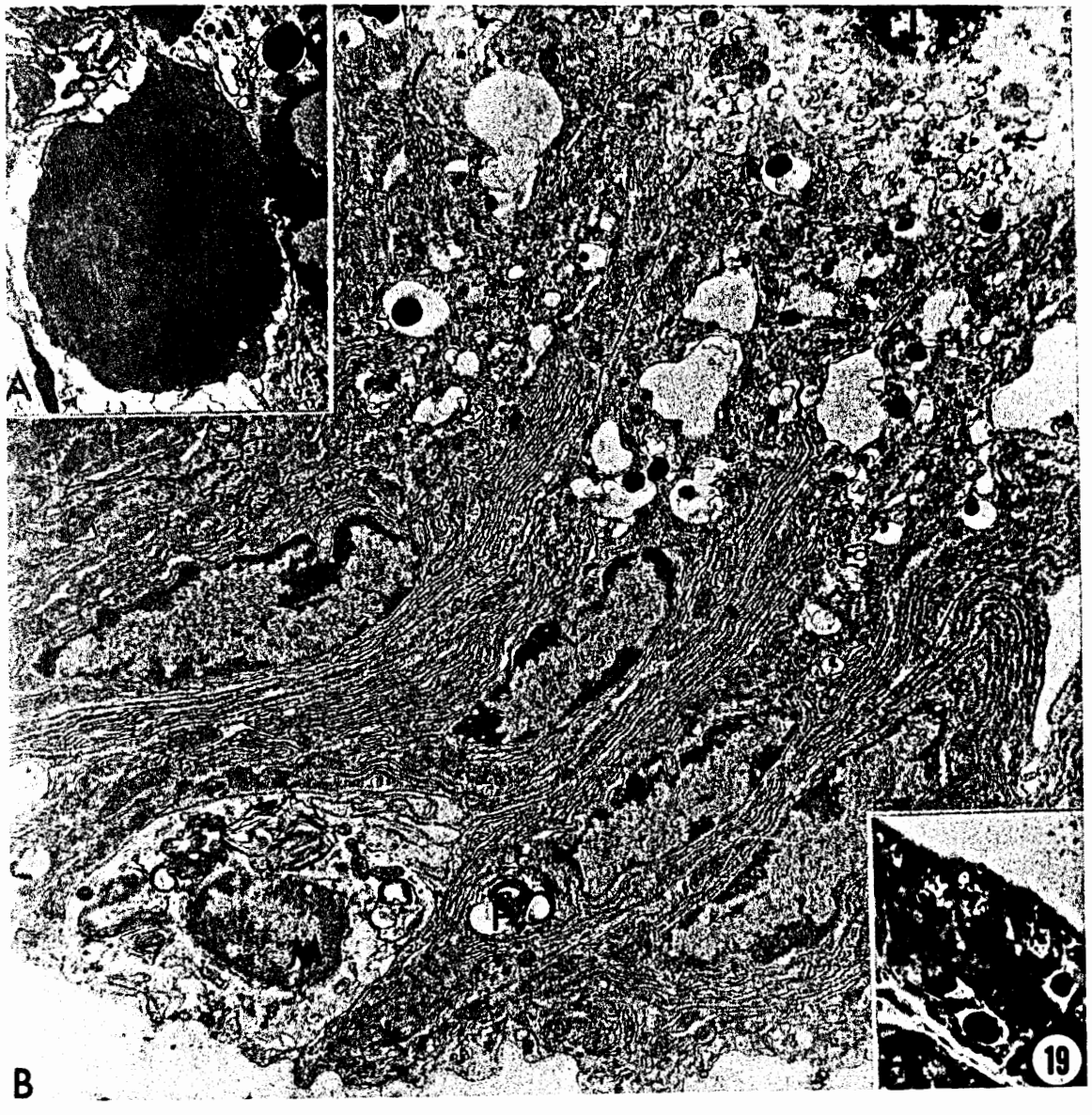
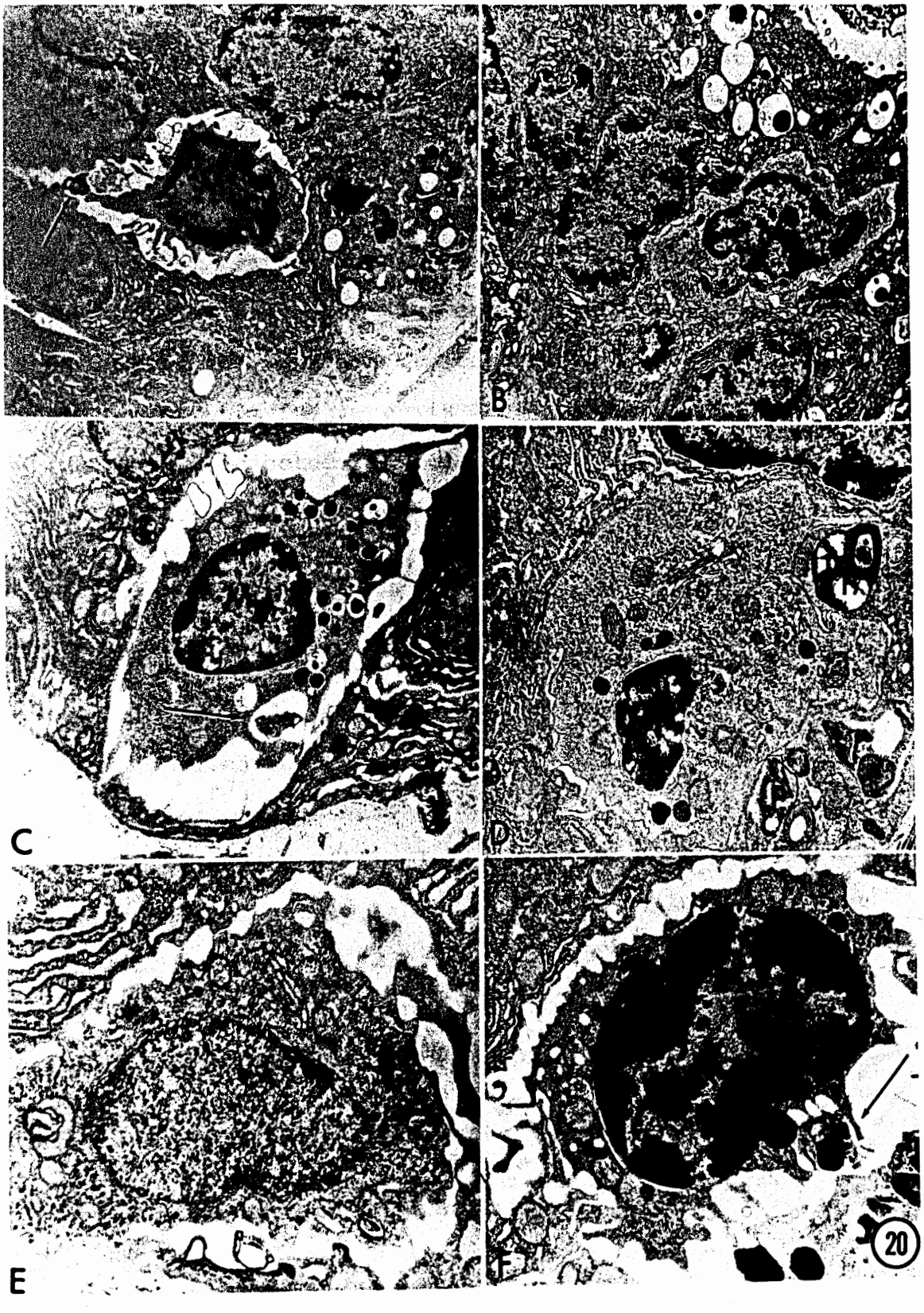

A
B
19

FIGURE 20 Non-epithelial cells within the epithelium of the rat seminal vesicle. A. Macrophage (M), with many cytoplasmic processes below nuclei of epithelial cells. Animal age, 8 months. Cellular debris in intercellular space (arrow); pigment (P). X6,700. B. Macrophage (M) above nuclei of epithelial cells. Animal age, 8 months. X5,800. C. Macrophage (M), phagocytosing cellular debris (arrow). Animal age, 12 months. X8,100. D. Macrophage (M) with cytoplasmic processes partially surrounding pigment (P). Animal age, 8 months. X13,300. E. Lymphocyte next to epithelial basement membrane. Animal age, 12 months. X15,000 F. Lymphocyte phagocytosing cellular debris (arrow). Animal age, 12 months. X12,000.


B
C
D
E
F
20

appears quite similar to that of the injected castrated twelve-month-old animal (compare Figs. 8 and 21). Thus, increasing circulating level of the hormone in the intact animal does not, under the present conditions, result in an increase in cellular function beyond the level of the control animals of the same age. The cells of the intact-injected animal still show degradative changes in the Golgi zones (Fig. 219. The mitotic frequency is increased in the epithelium of the intact-injected rats as compared to controls. Only the columnar-epithelial cells are involved in mitosis (Fig. 22). No basal cell mitoses were observed.

Autoradiography

Control slides for autoradiographic studies showed random clusters of 4-5 reduced silver grains, hence the positively labelled nuclei should exhibit more than five reduced silver grains per nucleus.

Bright field light microscopy of 1 μm sectioned tissue revealed discretely labelled nuclei located within the epithelial layer (Fig. 23A, B). Use of dark-field light microscopic techniques coupled with filters for UV fluorescence produced spectacular clusters of reduced silver grains as presented in Figures 23C, D. The reduced silver grains are very easily identified by dark field microscopy. The results of autoradiographic studies are presented in Table 2 and are expressed as a ratio of labelled nuclei

FIGURE 21 Epithelium of rat seminal vesicle after androgen administration. Animal age, 12 months. Golgi (G), lymphocyte (L), pigment (P). X9,900.

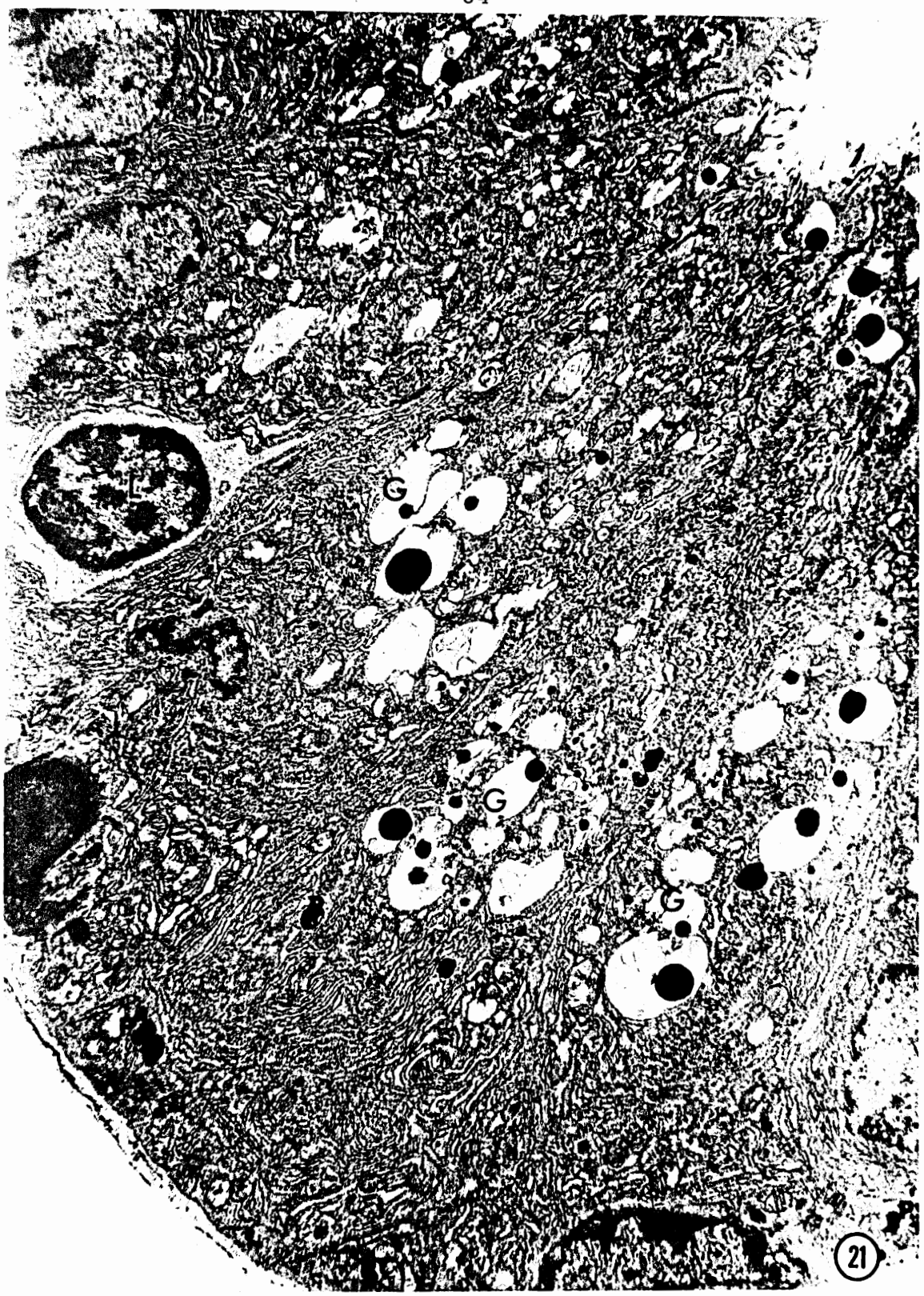

G
G
G
21

FIGURE 22 Epithelial cells of rat seminal vesicle after androgen administration. Animal age, 12 months. Lymphocyte (L), mitotic figure (MF), pigment (P). X10,100.

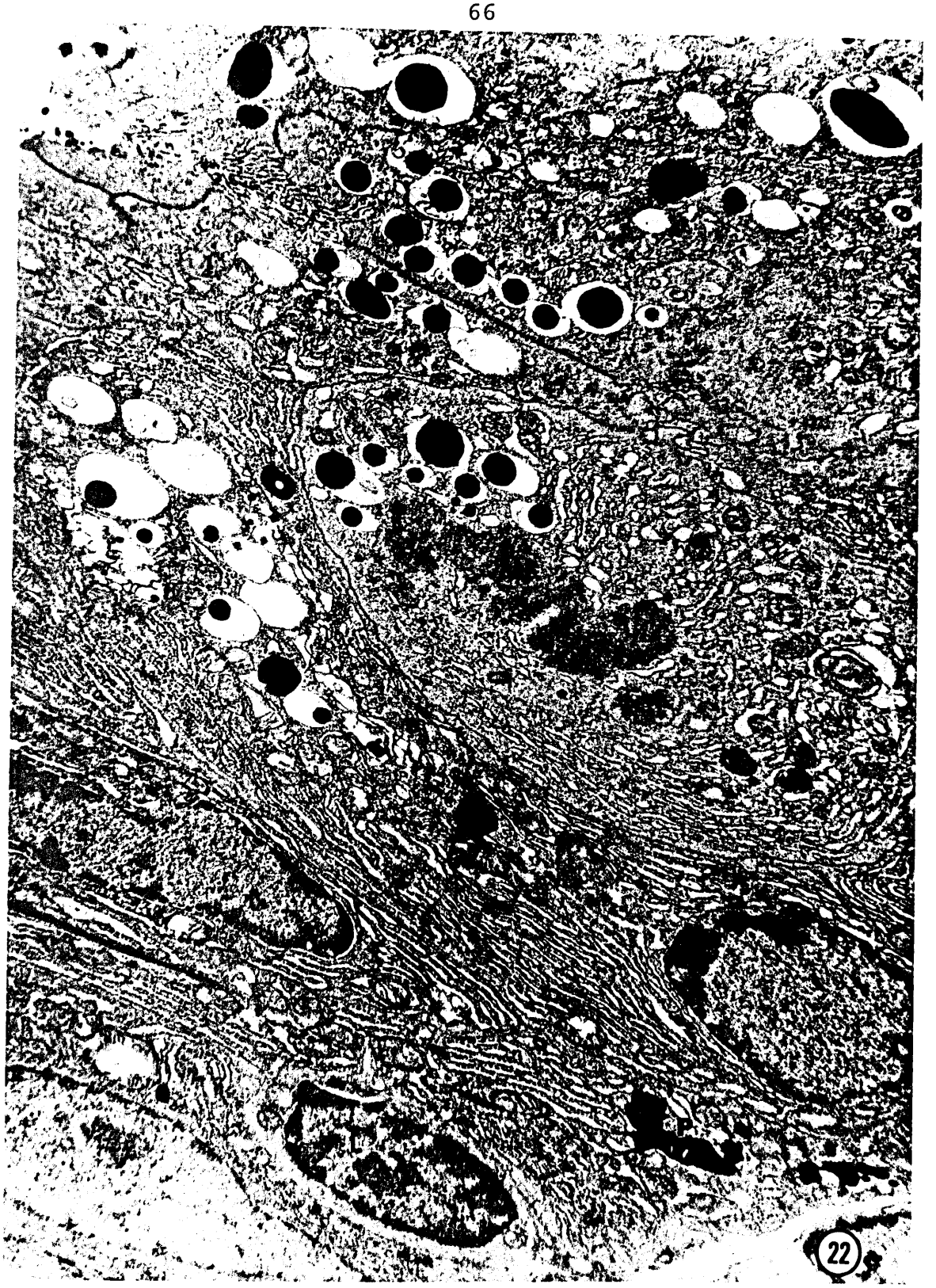

22

FIGURE 23 A., B. Bright field light microscopic image of tolouidine blue stained 1 1/2 µm sections (coated with Kodak NTB for autoradiography). Notice clusters of developed silver grains over nuclei (arrows). X1,500. C., D. Dark field light microscopy with UV illumination of same fields of view as seen in A., B. above. Notice obvious clusters of reduced silver grains (arrows). More than 5 silver grains/ nucleus represents positive labelling. X1,500.

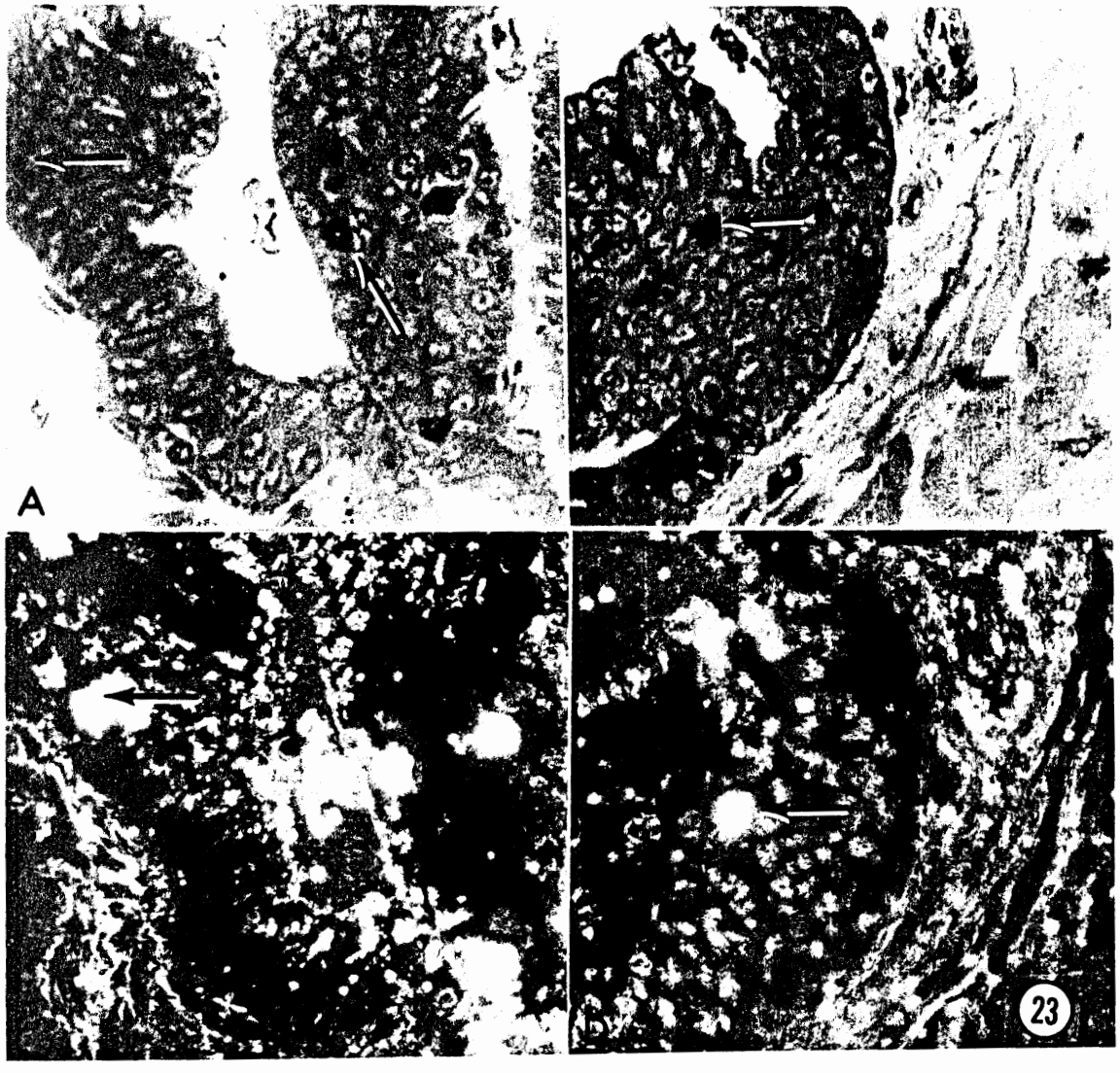

A
23

TABLE 2

COMPARISON OF LABELLING INDICES (LABELLED CELLS/TOTAL CELLS)* IN EPITHELIUM OF SEMINAL VESICLE OF AGING RATS OBTAINED AFTER INTRAVENOUS ^{3}H-THYMIDINE INJECTION

ANIMAL AGE (months)	TIME SACRIFICED AFTER [^{3}H] THY. INJ. (hr)	MEAN ($\bar{x}$) OF LABELLING INDICES $\pm$ STANDARD ERROR ($s_{\bar{x}}$)	RANGE OBTAINED FROM t-TEST ($\mu=\bar{x} \pm t\ (s_{\bar{x}})$)
3	1	$\bar{x}$=0.0086 $\pm$ 0.0008	0.0073-0.0090
	24	$\bar{x}$=0.0092 $\pm$ 0.0010	0.0075-0.0109
	72	$\bar{x}$=0.0082 $\pm$ 0.0007	0.0067-0.0097
6	1	$\bar{x}$=0.0105 $\pm$ 0.0012	0.0085-0.0125
	24	$\bar{x}$=0.0095 $\pm$ 0.0016	0.0068-0.0122
	72	$\bar{x}$=0.0107 $\pm$ 0.0011	0.0088-0.0126
12	1	$\bar{x}$=0.0123 $\pm$ 0.0010	0.0099-0.0147
	24	$\bar{x}$=0.0152 $\pm$ 0.0023	0.0106-0.0140
	72	$\bar{x}$=0.0152 $\pm$ 0.0023	0.0113-0.0191

TABLE 2 (CONTINUED)

ANIMAL AGE (months)	TIME SACRIFICED AFTER ^{3}H THY. INJ.	MEAN ($\bar{x}$) OF LABELLING INDICES $\pm$ STANDARD ERROR ($s_{\bar{x}}$)	RANGE OBTAINED FROM t-TEST ($\mu=\bar{x} \pm t\ (s_{\bar{x}})$)
18	1	$\bar{x}$=0.0177 $\pm$ 0.0008	0.0164-0.0190
	24	$\bar{x}$=0.0182 $\pm$ 0.0018	0.0151-0.0213
	72	$\bar{x}$=0.0180 $\pm$ 0.0016	0.0153-0.0207

*Cell counts were obtained from at least 4 different samples, each containing 500-800 cells. Tissues were obtained from 2 or more different animals in each age group. The labelling index of the control samples from the small intestine was 0.3980 $\pm$ 0.0190 (S.E.)

to the total number of nuclei counted.

The analysis of the labelling indices presented in Table 2 suggests that there is an increased incorporation of [^{3}H]thymidine into the nuclei of cells in the epithelium of 12- and 18-month-old rats as compared to 3-month-old rats. The incorporation of the label into the 18-month-old tissues appears to be significantly greater than in all the other samples except those from the 12-month-72-hr sample. There is no significant difference between the samples studied from 6-month and 12-month-old animals; however, the labelling in all three 12-month-old samples appears significantly greater than the labelling in the 3-month-old samples. When comparing the three samples within each of the four age groups, there are no significant differences observed (Table 2). The results of the t-test suggest that the properties, age and thymidine incorporation, are related. Linear regression analysis reveals a positive correlation between age and thymidine incorporation in that the incorporation of thymidine is significantly greater in older animals as compared to younger ones (Figure 24).

Bioassay procedures

Cell height measurements expressed in μm are given in Table 3. The t-test would suggest that there is considerable overlap among the samples studied. Linear regression analysis reveals that cell height measurements decrease as the animals

FIGURE 24 Labelling index of [^{3}H]thymidine incorporation in epithelium of seminal vesicle of aging rats. Vertical lines indicate 95% confidence limits. Each point is the value determined for the mean of the three age groups presented in Table 2.

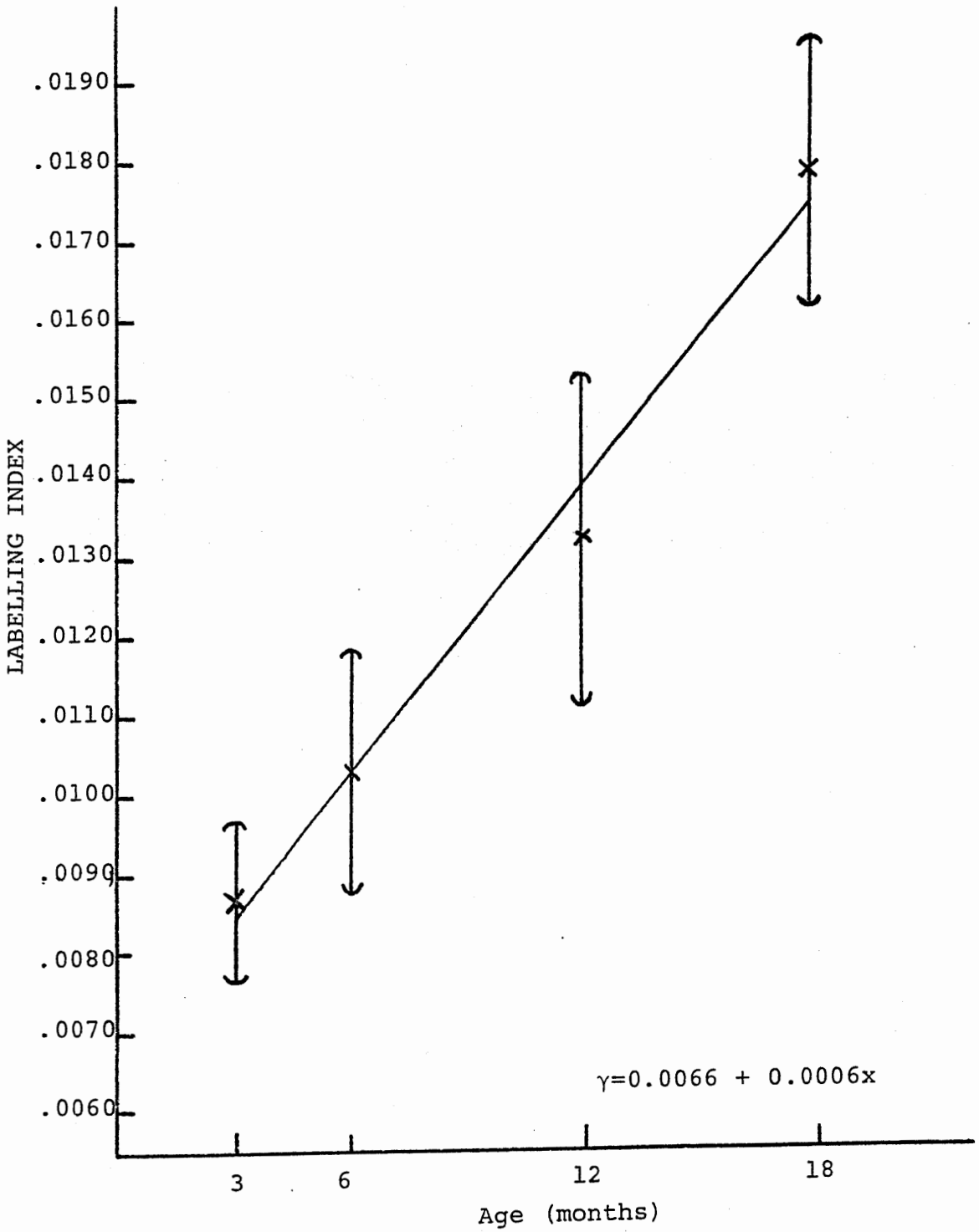

.0190
.0180
.0170
.0160
.0150
.0140
.0130
.0120
.0110
.0100
.0090
.0080
.0070
.0060
LABELLING INDEX
γ=0.0066 + 0.0006x
3
6
12
18
Age (months)

TABLE 3

COMPARISON OF CELL HEIGHT MEASUREMENTS OF EPITHELIUM IN THE SEMINAL VESICLE OF AGING RATS

ANIMAL AGE (months)	REPLICATE	MEAN ($\bar{x}$) OF CELL HEIGHT MEASUREMENTS* $\pm$ STANDARD ERROR (S.E.)	RANGE CALCULATED FROM t-test, $\mu=\bar{x} \pm t\ (s_{\bar{x}})$
3	I	22.29 $\pm$ 0.86 (N=17)	20.54-24.04
	II	22.96 $\pm$ 1.15 (N=30)	21.0-23.92
6	II	20.58 $\pm$ 0.96 (N=30)	18.95-22.21
	I	21.43 $\pm$ 1.00 (N=31)	20.43-22.43
12	II	21.95 $\pm$ 1.13 (N=30)	20.03-23.87
	I	20.02 $\pm$ 1.14 (N=28)	18.08-21.96
11-12 Injected**	I	22.95 $\pm$ 1.35 (N=20)	20.63-25.27
	II	24.62 $\pm$ 1.11 (N=20)	22.71-26.53

TABLE 3 (CONTINUED)

ANIMAL AGE (months)	REPLICATE	MEAN ($\bar{x}$) OF CELL HEIGHT MEASUREMENTS* $\pm$ STANDARD ERROR (S.E.)	RANGE CALCULATED FROM t-TEST, $\mu=\bar{x} \pm t\ (s_{\bar{x}})$
18	I	18.23 $\pm$ 0.99 (N=25)	16.54-19.92
	II	19.32 $\pm$ 0.91 (N=15)	17.73-20.91
22-24	I	19.16 $\pm$ 1.01 (N=20)	17.44-20.88
	II	omit-bad preparation	

*expressed in μm.

**injections of 6.0 mg/daily testosterone proprionate; animals sacrificed 72-76 hr after first injection

age. The correlation between age and reduction in cell height is significant and yet, in a practical sense, could be easily overlooked when studying morphologic data since the reduction in cell height is quite small. A summary of the correlation data is presented in Figure 25. There is a negative correlation between cell height and animal age which suggests that cell height is reduced with age, albeit a small reduction. The correlation coefficient obtained is -0.26480.

In attempting to develop a bioassay more sensitive than the measurements of cell height, counts of the secretion granules in the epithelial cells were made. A comparison of these counts suggests that the number of granules present in the cells of animals up to 7 months of age is significantly greater than the older animals, i.e. 12 months and 18 months of age. It is also noteworthy that despite hormone injection into intact animals, the increased circulating androgen is not reflected in increased granule counts in the 11-12-month injected animals as compared to the 12-month normal animal (Table 4). These results suggest that granule counts are a more sensitive indicator of cellular response to androgen than cell height measurements and that cellular activity reflected in secretion granule production is significantly decreased in the 12- to 18-month-old aging animals.

Analysis of variance was also carried out regarding counts of secretion granules. The results of this analysis

FIGURE 25 Measurements of epithelial cell heights in the seminal vesicles of rats of different ages. Correlation analysis of cell height: age.

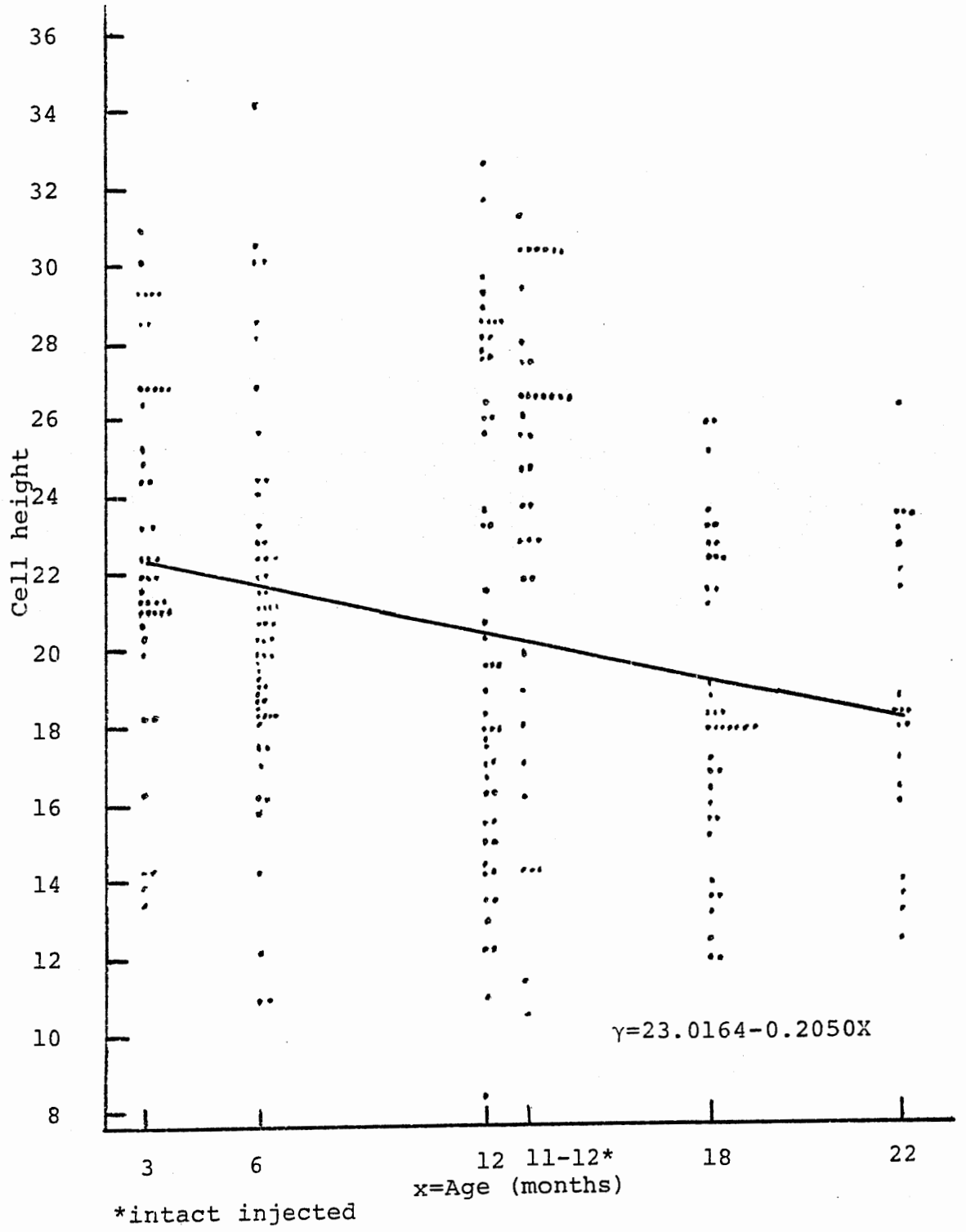

Cell height
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8
3
6
12
11-12*
18
22
x=Age (months)
*intact injected
γ=23.0164-0.2050X

TABLE 4

COMPARISON OF NUMBERS OF SECRETION GRANULES/CELL IN EPITHELIUM OF SEMINAL VESICLES OF AGING RATS

ANIMAL AGE (months)	# OF ANIMALS	CONDITION	MEAN ($\bar{x}$) OF GRANULE COUNT $\pm$ STANDARD ERROR ($s_{\bar{x}}$)	RANGE OBTAINED FROM t-TEST ($\mu = \bar{x} \pm t\ (s_{\bar{x}})$)
6-7**	2	normal	15.27 $\pm$ 1.21 (N=30)	13.21-17.33
12**	2	normal	9.34 $\pm$ 0.79 (N=29)	8.0-10.68
11-12	2	injected intact	10.86 $\pm$ 1.31 (N=26)	8.62-13.10
12	2	castrated injected*	8.41 $\pm$ 1.25 (N=29)	6.29-10.53
18	2	normal	7.53 $\pm$ 0.65 (N=30)	6.42-8.64
18	2	castrated injected*	9.05 $\pm$ 0.90 (N=20)	7.49-10.61

*All injected animals received 6.0 mg/day testosterone proprionate for 3 days and were sacrificed 72-76 hrs after the first injection. All castrated animals were injected beginning on day 22 after castration.

**Reported in Table 5.

are presented in Table 5. Five conditions (Table 4) are analyzed. The injected intact condition has been omitted.

TABLE 5

ONE WAY ANALYSIS OF VARIANCE:
COUNTS OF SECRETION GRANULES

Source	DF	SS	Mean square	Variance estimate
Between groups	4	1021.7072	255.4268	8.528
Within groups	125	3744.0158	29.9521	
Total	129	4765.7231		

The variance arising between samples is highly significant due to the reduction in the number of granules produced by the epithelial cells in animals twelve months of age and older. There is no probability that a reduction this great could occur by chance. It is also noteworthy that large doses of testosterone in the experimental animals did not overcome this change.

The multiple ANOVA testing (for 2 variables: age and androgen injections) determined that the decrease in counts of granules was unaffected by androgen administration as shown in Table 6.

TABLE 6

2 WAY ANOVA

COUNTS OF SECRETION GRANULES*

Source of variation	sum of square	DF	mean square	F	significance of F
main effects	19.287	2	9.644	.383	.683
age	17.000	1	17.000	.674	.414
injection	2.002	1	2.002	.079	.779
2-way interaction					
age :injection	45.337	1	45.337	1.798	.183
explained	64.624	3	21.541	.855	.468
residual	2420.016	96	25.208		
TOTAL	2484.640	99	25.097		

*Those conditions described in TABLE 4 which did not include androgen injections are not analyzed. Since the significance of F reported in TABLE 5 is due to the reduction in the number of granules produced in 12-month-old and older animals as compared to 6-7-month-old animals, and since the 6-7-month-old animals were not injected and were omitted, TABLE 6 shows no significant F value.

DISCUSSION

Results of the present study indicate that a variety of morphologic alterations occur in the epithelial cells of the seminal vesicle with age and in response to castration and experimental administration of exogenous androgen. In the epithelial cells of the intact rats there is an age-associated accumulation of pigment, or lipofuscin, disorganization of the Golgi components and a decrease in the relative volume of the secretory products of the cells. Non-epithelial cells such as macrophages and lymphocytes enter the epithelium and undergo a progressive proliferation in the aging animals. The age-associated changes in the epithelial cells are irreversible and do not appear to be due to the reduction of the circulating androgen since the senescent changes are not reversed by androgen augmentation. There is relatively little mitotic activity in the epithelial cells in the normal aging animals, however cellular proliferation increases following the experimental administration of exogenous androgen both in the castrated animals and in their intact controls. A rhythmicity in the cell cycle of the epithelial cell was not evident. The hormonal induced mitotic cycle occurred approximately 72-76 hours following the injection of androgen with little or no mitotic activity observed prior to that time. The epithelial cells in the rats castrated for 4-6 months un-

dergo accretional growth following hormonal administration but the age-associated deleterious changes remain essentially unaffected by this treatment. The generally suggested criterion (bioassay) for determining cellular response to androgen which involves measurement of the restoration of cell heights was found to be less sensitive than the estimation of the secretory activity of the cells by the counting of secretion granules.

The most prominent age-associated cytologic alteration in the epithelial cells is the accumulation of the age pigment. The age pigment has been referred to as lipofuscin, lipo-pigment, wear and tear pigment (Timiras, 1972), secondary lysosomes and residual bodies (deDuve and Wattiaux, 1966; Brandes, 1966a). In this discussion the term lipofuscin will be used in referring to age pigments. There have been numerous studies dealing with the morphologic, chemical composition as related to the possible functional significance of lipofuscin. At the light microscopic level, lipofuscin granules are rounded to oblong in shape and measure 1-5 μm, increasing in size with age. They are located in their perinuclear region or are scattered throughout the cytoplasm (see Strehler, 1964, for review). In unstained sections, lipofuscin appears brownish in color. Lipofuscin stains red with the periodic-acid Schiff (PAS) reaction, purple with Gomori's aldehyde fuchsin, and pink with Oil-Red-O and black with

Sudan Black-B which suggests that lipofuscin consists of lipids and mucocomplexes (Pearse, 1972).

At the fine structural level, lipofuscin granules are bounded by a limiting membrane containing polymorphic internal structures such as fine granular particles, vacuoles, and aggregates of membranous material. There is, however, considerable variation in the morphology of lipofuscin in different cell types. In the epithelial cells of the seminal vesicle of the rat the lipofuscin granules can be classified into two categories. Some granules consist of lamellated membranous material associated with prominent lipid-like droplets, whereas others show a coarsely granular material in association with membranous and vacuolar components. The factors underlying the differences in the morphology of the lipofuscin granules are unclear. However, differences in the cellular sources producing the lipofuscin may be involved (Toth, 1968).

An overwhelming body of evidence suggests that lipofuscin arises by the process of autophagocytosis involving lysosomes (Essner and Novikoff, 1960; Koenig, 1963; Strehler, 1964; Samorajski et al., 1965; Brandes, 1966a; deDuve and Wattiaux, 1966; Sohal and Allison, 1971). Focal areas of the cytoplasm are initially isolated within limiting membranes which arise from preexisting cisternae of endoplasmic reticulum albeit other membranous sources have been suggested (see

Arstola and Trump, 1969, for review), resulting in the formation of autophagic vacuoles. Autophagic vacuoles coalesce with primary or secondary lysosomes. Hydrolytic enzymes induce the degradation and digestion of enclosed organelles (de Duve and Wattiaux, 1966). The undigested residues are indistinguishable from lipofuscins (Brandes, 1966a) hence, the lipofuscin can be identified as secondary lysosomes of residual body variety according to the classification of de Duve and Wattiaux (1966). The lipofuscin granules in the epithelium of the seminal vesicle also appear to arise from the autophagocytosis of the cytoplasm with the limiting membranes arising from granular endoplasmic reticulum. Although the lysosomal nature of the lipofuscin was not confirmed cytochemically, their morphological appearance is essentially identical to similar structures in other tissues.

It should be mentioned that variations of the sequence mentioned above have also been reported (Brandes, 1966a) de Duve and Wattiaux, 1966; Frank and Christensen, 1968; Quataker, 1971). Lipofuscin accumulation does not seem to be limited only to the tissues of aging animals. Fine structural studies of serially propagated cells grown *in vitro* also reveal an increase in autophagic vacuoles and secondary lysosomes in the late passage cells (Robbins *et al.*, 1970; Brock and Hay, 1971; Brandes *et al.*, 1972; Lipetz and Cristofalo, 1972). It therefore seems that lipofuscin formation can be

considered as a specific aging change. The results of the present study confirm this view.

The nature of the factors responsible for the increase in cellular autophagy in the hormone target cells of old organisms is unclear. The process by which lipofuscin arises is termed cellular autophagy. The nature of the factors responsible for the increase in cellular autophagy in old organisms is unknown. However, reports relating circulating hormone levels and lipofuscin formation are available. Autophagy and formation of secondary lysosomes, i.e. lipofuscin are reported in the ventral prostate after castration (see Brandes, 1974, for review; Maggi et al., 1970; Helminen, 1975) in endometrial epithelium and stroma in the spayed rabbit (Smith and Henzel, 1969) in pituitary mammotrophic cells after weaning (Smith and Farquhar, 1966) during involution of the mammary gland (Helminen and Ericsson, 1968) and in seminal vesicle, epididymis, and prostate epithelium after castration and/or hypophysectomy (see Brandes, 1974, for review). Thus it is recognized that discontinuation of a trophic hormone's action can augment cellular autophagy and lipofuscin formation. These changes are reversed by hormone administration in young adult animals. In the present study no appreciable change in lipofuscin content of the cells of the aging animals was induced by exogenous androgen, not even when excessive amounts of hormone were given to normal intact animals. It therefore seems that lipofuscin in the epithelial

cells of the seminal vesicle is a product of the underlying mechanisms of aging rather than directly due to the circulating levels of androgen.

The effects of lipofuscin accumulation on cellular function still remains a matter of conjecture. Lipofuscin accumulation is induced following pathologic and physiologic stimuli (Sulkin and Srivany, 1960; Howes et al., 1971; Zeman, 1971). A direct relationship between lipofuscin accumulation and cellular loss of function is as yet not established. Whatever the effects of lipofuscin accumulation on the physiological potential of cells may be, it is generally acknowledged that lipofuscin in aging cells represents local cytoplasmic autolysis which reduces the native cytoplasm and represents a process of cytoplasmic wasting (Strehler, 1964; Brandes, 1966a; Sohal and Sharma, 1972). In the epithelium of the seminal vesicle the accumulation of lipofuscin granules results in the diminution of the granular endoplasmic reticulum which could contribute to the observed decrease in the synthetic abilities of the cells.

Disorganization of the Golgi components has been observed in a variety of cells of old organisms (Sulkin and Kuntz, 1952; Rowlatt and Franks, 1973). The most recognized function of the Golgi is the packing and concentration of secretory proteins (Dalton, 1961; Jamieson and Palade, 1967). Also, there is an addition of a carbohydrate moiety to the

secreted products in the cisternae of the Golgi complex (Neutra et al., 1966; Hagopian et al., 1968; Rambourg et al.; 1969). A disorganization in the Golgi membranes may represent a corresponding disturbance in the secretory activities of the cells. Present studies on the number of secretory granules indicate that there is indeed a reduction with age in the secretory activity of the cells. This change occurs simultaneously with the decrease in the volume of granular endoplasmic reticulum and may indicate a decreased protein synthesizing ability of the aging cells. Decline in the rate of protein synthesis has been reported in a variety of animals with age (Adelman, 1971; Rockstein and Baker, 1974; Gordon and Finch, 1974; Cicero et al., 1972). The age-associated changes in the cellular organelles may be a manifestation of the decline in the ability of the aging cell to synthesize proteins. It is tempting to speculate that this could represent the irreversible change not overcome by androgen administration in aging seminal vesicle epithelial cells.

The fate of the lipofuscin is that some is extruded while some is retained inside the cell. Some of the extruded material lies in intercellular spaces also invaded by non-epithelial cells. An interesting aging alteration in this regard is the increased incidence of mononuclear cells, macrophages and lymphocytes, in the lumen of the gland and in the interstices of the epithelial cells. In the seminal vesicle,

macrophages ingest the extruded lipofuscin as well as the remnants of the exfoliating cells. The mechanism by which the macrophages are attracted to the seminal vesicle is unknown although much information is available regarding their capacity to injest and eliminate effete and dying cellular elements (Rabanovitch, 1968; Van Furth, 1970a, b). Necrotic cells may be responsible for attraction of macrophages since there is a correspondence between the degeneration of cells and the increased macrophage activity. It also seems that the macrophages serve an additional purpose of eliminating excessive extruded debris in the form of lipofuscin. The presence of the lymphocytes in the seminal vesicle is rather intriguing. It is well known that lymphocytes play an immunologic role and respond to antigens (Sprent, 1973a, b; Roelants, 1972; Uhr, 1970; Urso and Makinodan, 1963). It is also well understood that macrophages and lymphocytes interact in immune responses (Huber, 1970; Sharp, 1960; Unanue, 1972). Although the significance of that observation remains unknown it is tempting to speculate that an age-associated change may occur in the seminal vesicle which could represent an altered antigenic pattern. Whether this phenomenon is indicative of an autoimmune process or not should be further investigated.

Because the epithelial cells of the seminal vesicles retain the ability to undergo mitosis it is imperative to

examine their proliferation capacities as a function of age. That the ability of cells to divide undergoes a decline with age has been well documented (LeBlond, 1964; Buetow, 1971). Buetow (1971) has shown that cellular proliferation and rate decline in a variety of tissues in humans and rodents. His conclusions have been confirmed by Cameron (1972a, b). Another experimental approach bearing on the age-associated proliferation capability of the cells has been provided by serial transplantation studies. Mouse mammary glands were serially transplanted into the gland free fat beds of young mice (Daniel et al., 1968). The growth rates of the glands were found to decline with age. The growth rate was also found to decline with age in salivary glands in response to isoproterenol stimulation (Adelman, et al., 1972) as well as in the mouse immune system. According to Cristofalo (1973) the decline in proliferation capacity represents a gradual functional failure with age. It should, however, be mentioned that the animals do not apparently die because of the decline in cellular multiplication since the serially transplanted tissues last longer than the life span of the recipient (Krohn, 1966). Thus the reduced proliferation capacity of the cell cannot be directly associated with the age-associated mortality rate but instead may be an indication of other deleterious changes in the cells which result in the inability of the organism to adapt to stessful stimuli.

That the cells have a finite proliferative capability has

been established by studies on the *in vitro* replications of normal human diploid fibroblasts (Hayflick and Morehead, 1961). These studies have shown that cells grown *in vitro* undergo a rapid proliferation followed by a decline in multiplication ability ultimately resulting in the loss of the culture.

In the epithelial cell layer of the seminal vesicle of aging animals there is an apparent increase in the number of cells showing incorporation of [^{3}H]thymidine which is indicative of DNA synthesis in the epithelial cell but not of cellular division. Synthesis of DNA by differentiated cells is known to occur elsewhere (LeBlond, 1964). Apparently such cells remain arrested in the G_2 phase of the cell cycle (Bloch, 1958). Whether the epithelial cells of the seminal vesicle undergo a similar DNA synthetic process or not is unknown. the lack of any enhanced mitotic activity with age lends support to the view that the [^{3}H]thymidine incorporation may represent additional biosynthesis of DNA without subsequent division of the chromosomes. Another possiblity is that the cell population exhibiting the [^{3}H]thymidine incorporation does not entirely belong to the epithelium of the seminal vesicle but a proportion of it may consist of the macrophage and lymphocytes which are also observed in this area. It is well known that both are types which undergo mitosis with appropriate stimuli (Unanue, 1970). The general shape of some of the nuclei showing [^{3}H]thymidine labelling is

essentially identical to that of the lymphocytes. Furthermore, some of the labelled cells appear amidst the unattached population of lymphocytes and macrophages and do not appear to form a layer like the epithelial cells. It therefore seems that the increase in the labelling of the cells in the seminal vesicle may be indicative of the proliferation of cell populations other than the epithelial cells. Autoradiographic studies at the electron microscopic level are needed in order to clarify this point.

The study of growth and renewal of cell populations during post-natal life has led to the classification of cells into three different groups: static, expanding, and renewing cell populations (LeBlond, 1964).

Static cell populations are composed of homogenous groups of cells in which no mitotic activity can be detected and in which total DNA content remains the same. Only nerve cells belong to this group. These cells persist until the organism dies, undergoing senescent changes linked to the senescence of the individual.

Expanding cell populations are homogenous groups of cells showing scattered mitoses in numbers that account for the increase in the total DNA content. This definition implies that the life of the cell is as long as that of the individual and that mitosis occurs only in order to facilitate the growth of the tissue. Such cell populations are

characterized by the incorporation of [^{3}H]thymidine but the rate of such incorporation declines with age at a rate which is higher than that for the other tissues of the body considered together. These cells are further characterized after [^{3}H]thymidine labelling by a few scattered labelled cells undergoing mitosis. These dividing cells are fully differentiated and produced only as needed. An impressive feature of expanding cell populations is that the slow mitotic index can readily be increased with appropriate stimuli. For example, partial extirpation of the liver or unilateral nephrectomy give rise to rapid regeneration and hypertrophy. In the thyroid gland the injection of thyroid stimulating hormone produces a wave of mitotic activity (De Robertis et al., 1965).

Renewing cell populations are homogenous groups of cells in which mitosis is abundant and exceeds that required for the total increase in DNA content. The rapid renewal of cells in the intestine and in the epidermis can be cited as examples. Complex controls, such as chemical, mechanical, and hormonal action, regulate the duration of life in labile cells.

The epithelium of the seminal vesicle of the aging rat will proliferate to a limited extent after androgen administration. The characteristics of this observation best match with those of the expanding cell populations.

In conclusion, the present study presents evidence from descriptive data and statistical analyses that an irreversible change has occurred in the epithelium of the seminal vesicle of the aging rat which is not overcome by androgen administration. The autoradiographic studies of this work reveal the lack of a cyclic mitotic pattern in the epithelial cells and suggest that there is an incorporation of [^{3}H]thymidine into dividing non-epithelial cells residing in the epithelial layer. Although the labelling index of the epithelial cells is low, a few cells can be induced to divide by androgen administration which places the epithelium in the category of expanding cell populations. The age-related increase in non-epithelial cells, i.e. macrophages and lymphocytes, suggests that the antigenic nature of the epithelium undergoes changes as the rats age. Autoradiographic studies at the electron microscopic level and immunologic techniques for the identification of antigens are needed for identification of cell types in mitosis and clarification of the suggested immune response.

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