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Biochemical, histochemical and ultrastructural changes in the mouse seminal vesicle after castration

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INTRODUCTION

In man, the more obvious effects of castration have been recognized since ancient times; the distribution of body hair, the pitch of the voice, the contours of the skeleton, and the extent of the facial sinuses are familiar examples of morphological characteristics which reflect the endocrine function of the testis. John Hunter (1786) was probably the first to note the trophic relationship of the testes and the seminal vesicles and various workers since have severally described some of the morphological changes in the seminal vesicles which follow castration. This paper describes an attempt to relate the biochemical and histochemical changes in the seminal vesicle after castration to ultrastructural changes.

MATERIALS AND METHODS

Adult male Swiss white mice aged 3–9 months were used in this investigation. Eighty-six animals were used, six daily, in compiling the biochemical and histochemical series, fourteen, two daily, in the ultrastructural study. The distribution of control and castrate animals is summarized in Table 1. The techniques used were as follows:

Biochemical series. Immediately after sacrifice the seminal vesicles were excised together with their contained secretion. The wet seminal vesicle tissue from the mice killed was divided into two parts. Each part was weighed, a specimen about 3 mm^3 was taken from each vesicle for histochemical studies, the samples were reweighed and the amount of fructose in one and citric acid in the other determined. Fructose was estimated using the colorimetric method of Roe (1934). Citric acid estimation was carried out by the colorimetric method of Speck, Moulder & Evans (1946) as modified by Lindner & Mann (1960). Colorimetric determinations were carried out on a Hilger–Spekker absorptiometer. The citric acid and fructose content of the tissue was an estimate based on the weight of tissue used for the biochemical investigation.

Histochemical series. Specimens of vesicles from each animal used in the biochemical study were examined, using the following techniques:

(1) Esterases, using the substrates α-naphthyl acetate, Naphthol AS acetate, Naphthol AS.LC acetate, and Naphthol AS.D acetate. Controls for these techniques are described elsewhere (Baillie, 1964*a*).

(2) Cytochrome oxidase activity, using the G-Nadi reaction of Moog (1943).

(3) Succinate dehydrogenase activity by the method of Nachlas, Crawford & Seligman (1957), but using Nitro-BT as the coupling agent.

(4) Aryl sulphatase activity using the technique first described by Rutenberg, Cohen & Seligman (1952). The substrates employed were potassium-6-benzoyl-2-

Table 1. *Mensural data of the seminal vesicles and their contained fructose and citric acid*

Period after castration (days) ... Control	1	2	3	4	5	6	7	10
Seminal vesicle wt. (mg) 310	310	236	187	158	122	86	63	—
Mean and S.E. ±30	±41	±27	±23	±32	±12	±27	±7	—
Fructose, yield per buck in mg 1·32	0·66	0·5	0·28	0·24	0·15	0·09	0·06	—
Mean and S.E. ±0·10	±0·09	±0·07	±0·07	±0·06	±0·07	±0·01	±0·01	—
Conc. in mg/100 g of vesicle tissue 591	248	250	235	196	150	112	91	—
Citric acid, yield per buck in mg 0·49	0·56	0·39	0·34	0·12	0·11	0·08	0·04	—
Mean and S.E. ±0·05	±0·01	±0·03	±0·02	±0·01	±0·02	±0·01	±0·003	—
Conc. in mg/100 g of vesicle tissue 195	200	229	308	218	120	96	71	—
No. of animals used in biochemical series 24	6	6	6	6	6	6	6	—
No. of animals used in histochemical series 24	6	6	6	6	6	6	6	—
No. of animals used in ultrastructural series 4	—	—	—	—	2	—	—	2

Period after castration (days) ... Control	14	50	75	100	125	150	175	200
Seminal vesicle wt (mg) 310	52	48	21	31	30	27	25	25
Mean and S.E. ±30	±9	±9	±5	±7	±5	±6	±4	±5
Fructose, yield per buck in mg 1·32	0	0	0	0	0	0	0	0
Mean and S.E. ±0·10	0	0	0	0	0	0	0	0
Conc. in mg/100 g of vesicle tissue 591	0	0	0	0	0	0	0	0
Citric acid, yield per buck in mg 0·49	0	0	0	0	0	0	0	0
Mean and S.E. ±0·05	0	0	0	0	0	0	0	0
Conc. in mg/100 g of vesicle tissue 195	0	0	0	0	0	0	0	0
No. of animals used in biochemical series 24	6	2	2	2	2	2	2	2
No. of animals used in histochemical series 24	6	2	2	2	2	2	2	2
No. of animals used in ultrastructural series 4	—	2	—	2	—	—	—	2

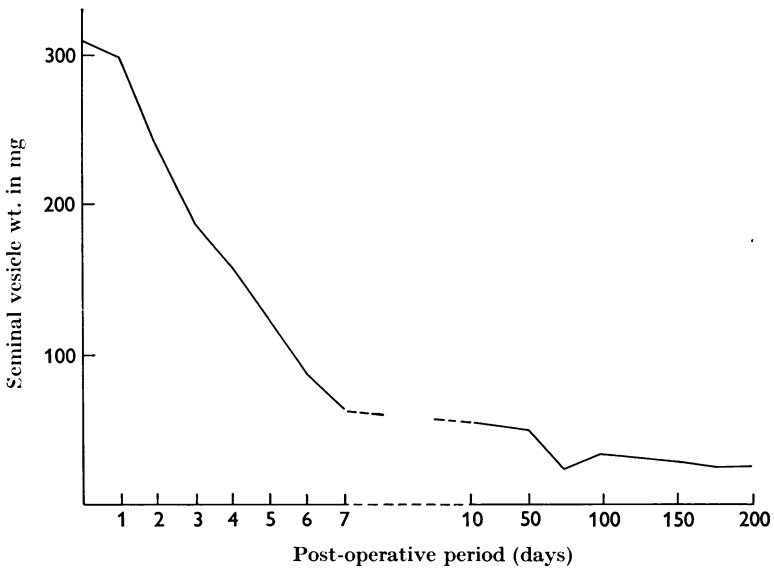


Fig. 1. Seminal vesicle weight falls rapidly during the first 7 postoperative days; thereafter a smaller reduction takes place.

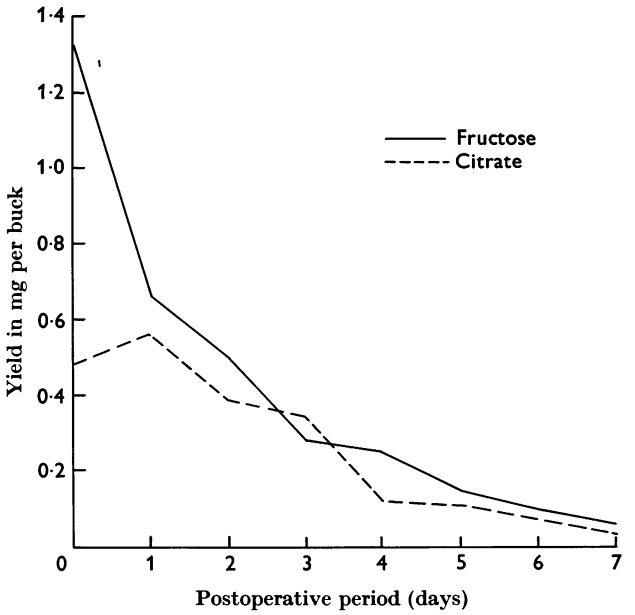


Fig. 2. The amount of fructose contained in the seminal vesicles is halved within 24 h of castration and thereafter falls steadily. The vesicle content of citrate is slower in beginning to fall.

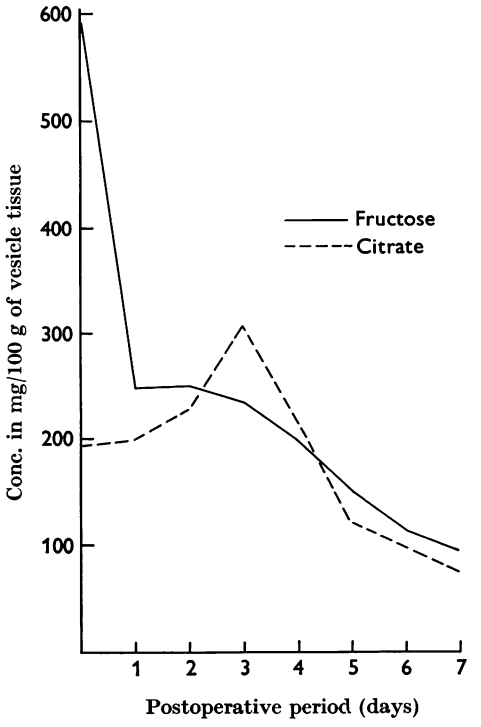
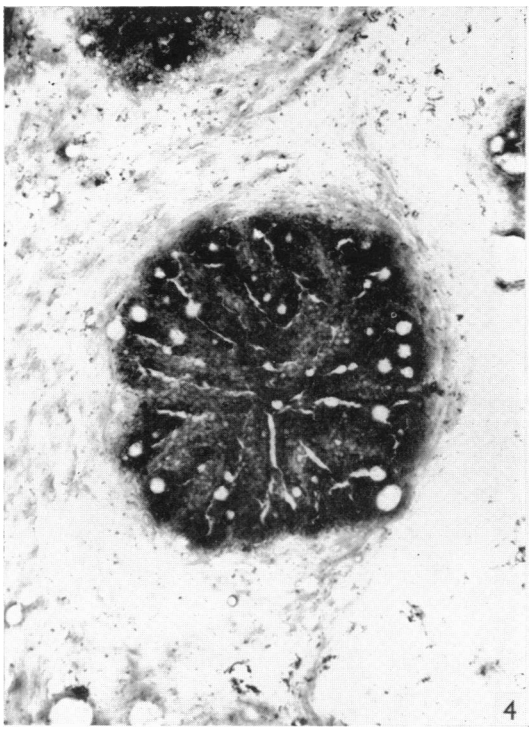


Fig. 3. The concentration of fructose in the seminal vesicles is steadily reduced in the first seven days after castration. The concentration of citrate on the other hand, rises for for the first 3 days before beginning to fall.



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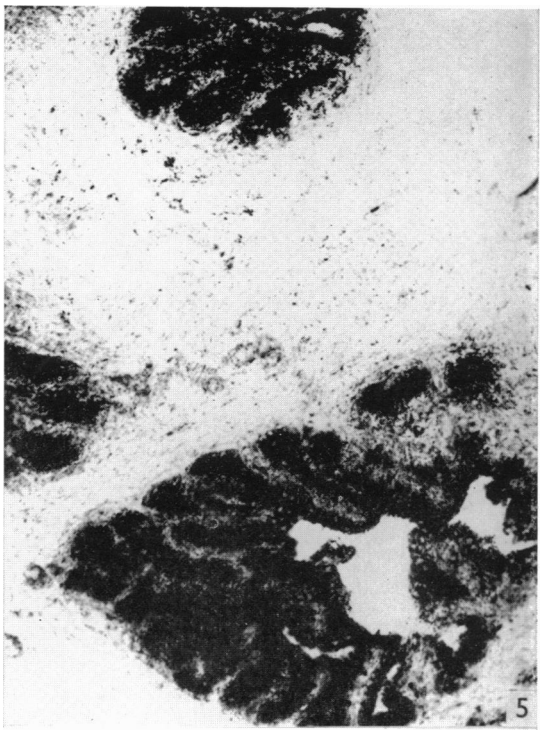

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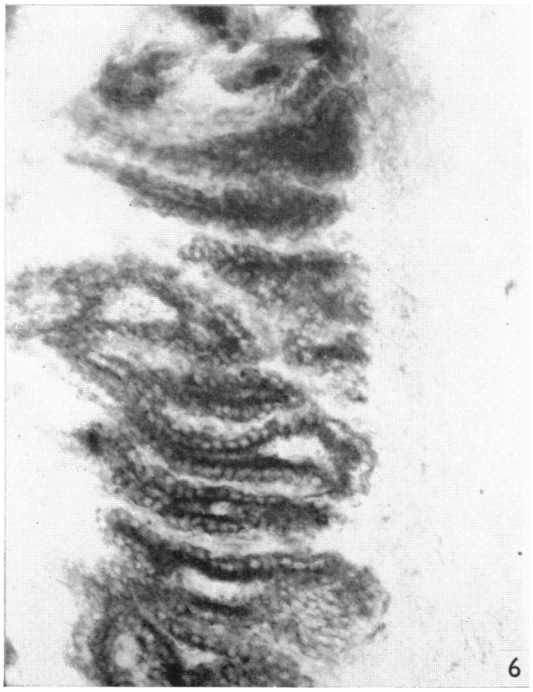

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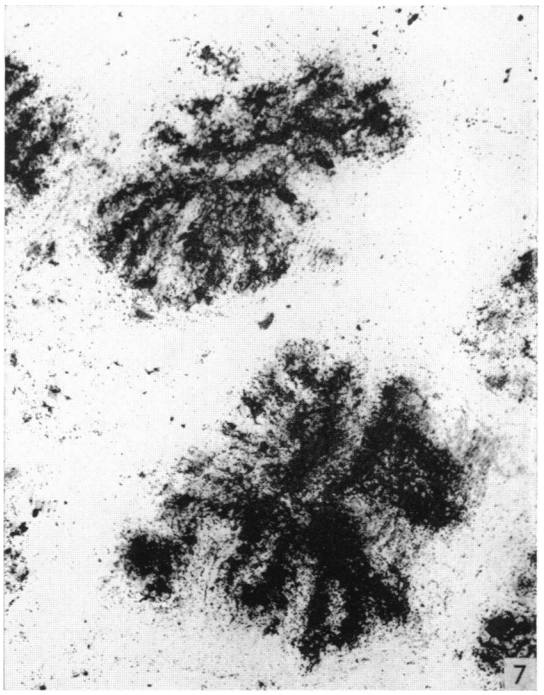

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naphthyl sulphate and potassium-6-bromo-2-naphthyl sulphate (Borden Co. Chemical Division). Post coupling in both instances was with Fast Blue salt B (G. T. Gurr Ltd.).

(5) Alkaline and acid phosphatase activity using a modified Gomori technique (Pearse, 1960).

(6) Lipofuscin using the Schmorl (Pearse, 1960) reaction.

Ultrastructural series. Small pieces of seminal vesicle tissue were fixed for 1 h at 4 °C in either 1% potassium permanganate (Luft, 1956) or 1% osmium tetroxide (Zetterqvist, 1956) buffered at pH 7·4. The tissues were dehydrated in ethanol and embedded in araldite by the short method of Luft (1961). Thin sections were cut using the Huxley ultramicrotome, mounted on copper grids without films and stained in 1% methanolic lead acetate. Sections were examined in the Philips EM 75b and EM 200 electron microscopes.

The experimental animals (Table 1) suffered bilateral orchidectomy through an aseptic mid-line abdominal incision under full surgical anaesthesia. The epididymis was left *in situ* and the wound closed in layers.

RESULTS

After castration the seminal vesicle weight falls from the control level of 310 mg to 63 mg on the seventh post-operative day. Thereafter a slow fall over the next 68 days (Table 1, Fig. 1) occurs and the weight of the vesicles of castrated animals settles at 25–30 mg.

Biochemical observations. The average amount of fructose found in the seminal vesicle of the control bucks was 1·32 mg. This was approximately halved within 24 h of castration (Table 1, Fig. 2) and then fell more slowly to 0·06 mg 7 days after operation. No fructose was detectable on the fourteenth or any subsequent post-operative day. In the same way the concentration of fructose fell from a control level of 591 mg/100 g of vesicle to 248 mg on the first postoperative day.

A concentration of 91 mg/100 g was recorded on the seventh postoperative day and this sugar was not found subsequently (Table 1, Fig. 3).

The yield of citric acid per buck rose from the control level of 0·49 to 0·56 mg on the first postoperative day and then fell slightly over the ensuing 2 days (Table 1, Fig. 2). Thereafter the citric acid yield fell steadily to 0·04 mg on the seventh postoperative day and it was not detectable on the fourteenth day. The concentration of citric acid per 100 g of seminal vesicle rose from the control level of 195 to 308 mg 3 days after operation (Table 1, Fig. 3). It thereafter was regularly reduced to 71 mg on the seventh day and zero by the fourteenth day.

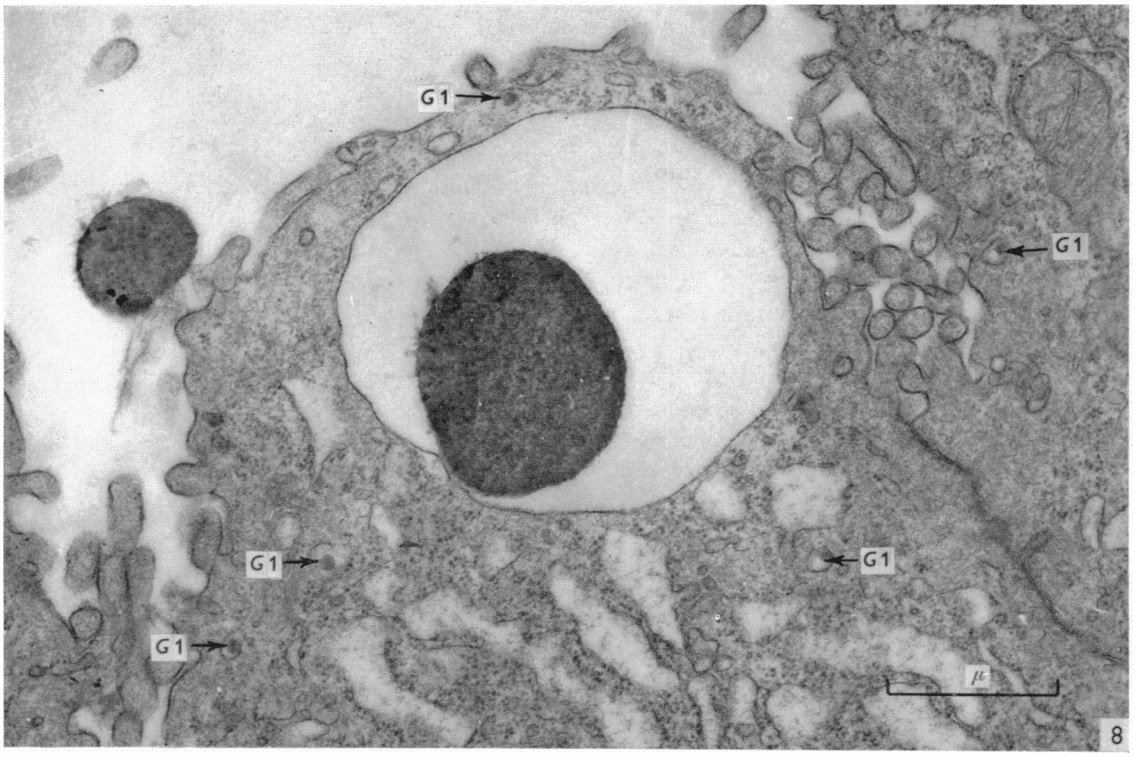
Histochemical observations. Sections stained with haematoxylin and eosin indicate

Fig. 4. Seminal vesicle 200 days after castration. Intense esterase activity is visible in the epithelium. α-naphthyl acetate. × 90.

Fig. 5. Succinic dehydrogenase activity in the epithelium of the seminal vesicle 200 days after castration. × 90.

Fig. 6. Aryl sulphatase active in the seminal vesicle epithelium 100 days after castration. × 180

Fig. 7. Acid phosphatase 200 days after operation in the epithelium of shrunken gland diverticula. × 90.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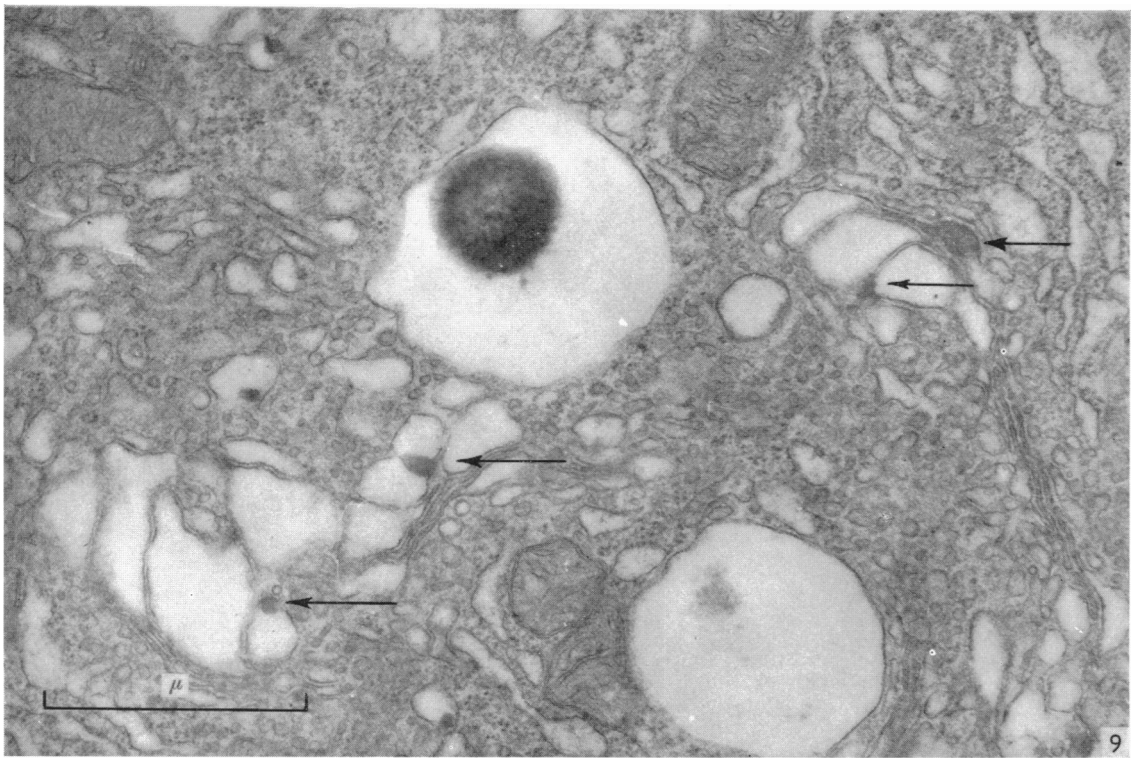
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that in the first few days after castration the secretion contained in the seminal vesicles disappears, and the gland diverticula come to have a shrunken contracted appearance. The epithelial cells themselves progressively lose height, becoming almost cuboidal after 14 days. Over the remainder of the period covered by this material the connective tissue of the vesicle appears to increase in amount, but this is probably due to the progressive atrophy of the glandular epithelium.

Esterase activity is demonstrable in the epithelium of the control seminal vesicles with all substrates used. With α-naphthyl acetate a dense reaction developed quickly in the entire epithelium of the vesicle, obscuring all cytological detail. Castration had no effect on this enzyme (Fig. 4). With Napthol AS acetate fine dye particles are seen throughout the cytoplasm of the epithelial cells and this activity persists after castration. An essentially similar picture was obtained with the esterases demonstrable with Naphthol AS.LC and Naphthol AS.D acetates.

Cytochrome oxidase activity was easily demonstrated in the vesicle epithelium and connective tissue and no changes were seen after castration. Succinic dehydrogenase activity is located mainly as diformazan in the cytoplasm of the epithelial cells of the seminal vesicles of the control animals, but occasional connective tissue cells are reactive. Castration does not appear to diminish the activity of this enzyme and intense succinic dehydrogenase activity can be seen in the epithelium even two hundred days after castration (Fig. 5).

Aryl sulphatase activity is a prominent feature of all the vesicles (pre- and post-operative) studied and occurs in the epithelium, being mainly found in the basal regions of the cell (Fig. 6).

Acid phosphatase activity is also readily seen in the epithelium of the control seminal vesicles and remains intensely reactive even 200 days (Fig. 7) after operation. Alkaline phosphatase occurs principally in the stroma and basement membranes of epithelium and blood vessels and is unaffected quantitatively or qualitatively by castration.

Ultrastructural observations. In control animals, the fine structure of the seminal vesicle shows little variation and two fairly uniform cell types can be recognized, columnar cells and basal cells. After osmium fixation the columnar gland cells contain an elaborate granular endoplasmic reticulum and a prominent supranuclear Golgi apparatus, in which small fragments of secretory material are often trapped (Fig. 9). Above the nucleus there are large membrane-bound vacuoles, each containing a dense secretion granule which is often attached at one point to the limiting membrane (Fig. 8).

The granule does not fill the vacuole, and is surrounded by a pale halo which may contain flocculent material. In the zone of cytoplasm directly underlying the apical cell membrane small vesicles occur, each containing a dense granule, again probably secretory material (Fig. 8). Small dense pleomorphic bodies, perhaps lysosomes, are

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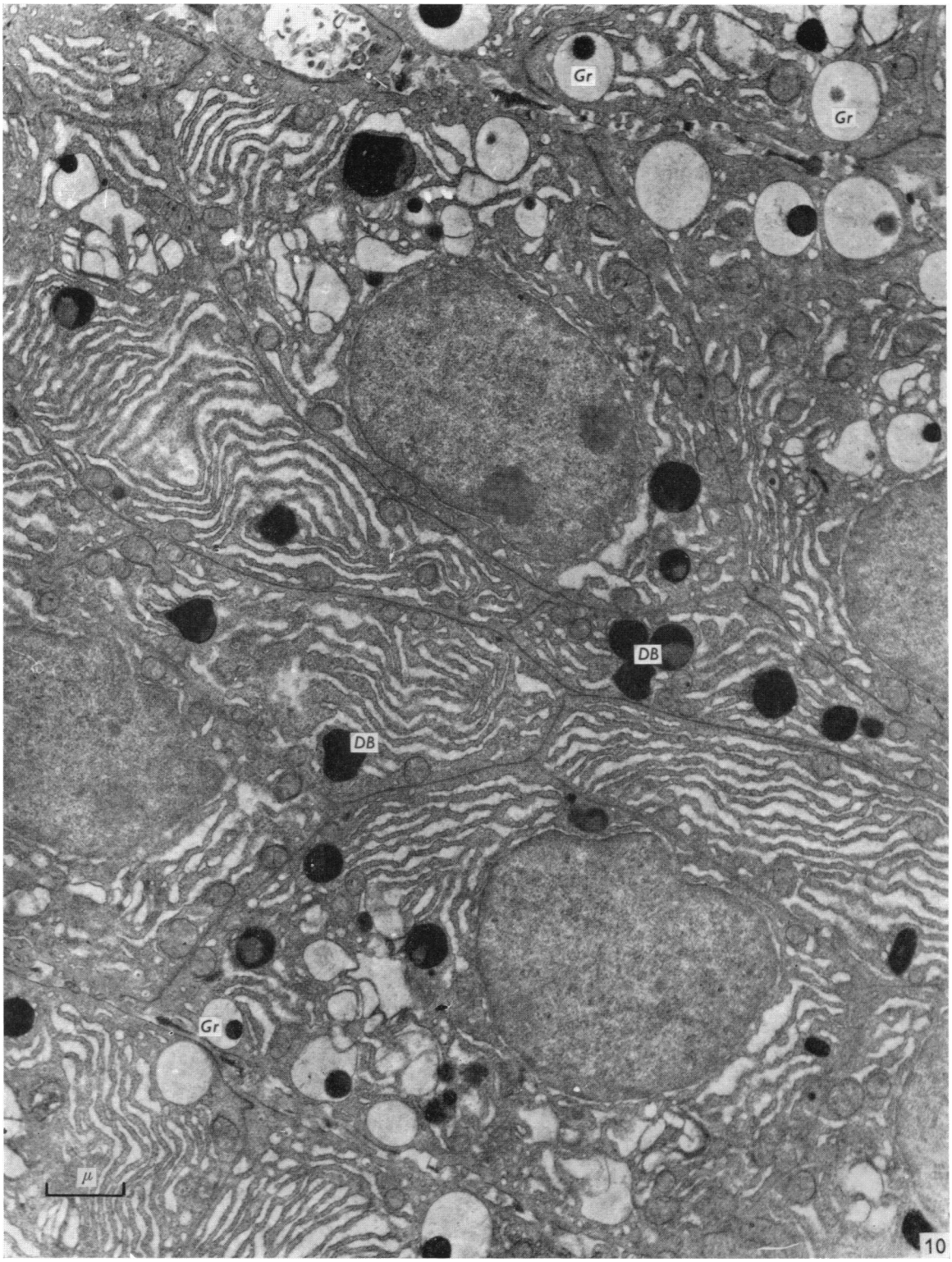
Fig. 8. Apical border of epithelial cell from control seminal vesicle. Osmium fixed. A single large granule is seen with its typical vacuole and surrounding 'Halo'. In addition, small granules are seen (*G* 1) in the apical zone. A discharged secretion granule lies free in the lumen. A number of microvilli are present on the cell surface.

Fig. 9. Golgi apparatus. Control, osmium fixed. At several points (arrows) dense material, probably secretion, lies between the membranes.



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often seen in the cytoplasm. The mitochondria show no structural specialization, although their matrix is of moderate density. They have no particular orientation or distribution in the cell. The base of the cell is smooth and closely applied to the basement membrane (Fig. 11) and microvilli are present on the apical surface in variable numbers (Fig. 8).

The basal cells are often seen lying on the basement membrane sandwiched between the chief cells, with a characteristic pale and poorly organized cytoplasm in which dense granules are sometimes seen.

After permanganate fixation the appearance of the tissue is similar although there is, as usual, selective preservation of membranes and some loss of detail in the cytoplasm. There is often deep invagination of the nuclear membrane of the basal cell, not so prominent after osmium fixation.

Five days after castration there is a reduction in cell size but substantial changes in fine structure are not yet present. In most cells the granular endoplasmic reticulum is still well organized and the Golgi apparatus is as extensive as in the controls (Fig. 10).

There is, however, a reduction in the secretory material entangled in the Golgi apparatus and the diameter of the large secretion granules is reduced although the vacuoles in which they lie are no smaller. The small secretory granules lying just below the cell surface are still seen. The pleomorphic dense bodies present in the controls are now more prominent than before.

Ten days after castration there is a further reduction in the size and number of secretion granules. More of the large vacuoles which previously contained granules appear empty apart from traces of flocculent material, and in others the granules which remain are small, while few of the smaller apical type of secretion granule remain. There is little change in the Golgi apparatus, but the cisternae of the granular endoplasmic reticulum are now reduced in length and their organization is lost to some extent. In many cells there appears to be an increase in the number and length of the microvilli on the cell surface, while at the base of the epithelium some infolding of the cell membrane is now seen, affecting both chief and basal cells.

Fifty days after castration structural changes are moderately advanced. The granular endoplasmic reticulum is now poorly organized, and the Golgi apparatus is reduced in size. Dense bodies are prominent, some measuring as much as 10 μ in diameter. Although large empty vacuoles are often seen, evidence of secretion is generally absent, but in some cells both small and large secretion granules can still be found. The infolding of the base of the epithelium is now more obvious. The appearance after 100 days is essentially similar.

Two hundred days after castration there is a further extension of the changes described above. The cells are now much reduced in volume, the small apical granules have disappeared, and the large secretion granules are also absent from most cells, although empty vacuoles, similar to those in which granules were found in the controls, are still present about the nucleus in most cells (Fig. 13). The cisternae of the granular

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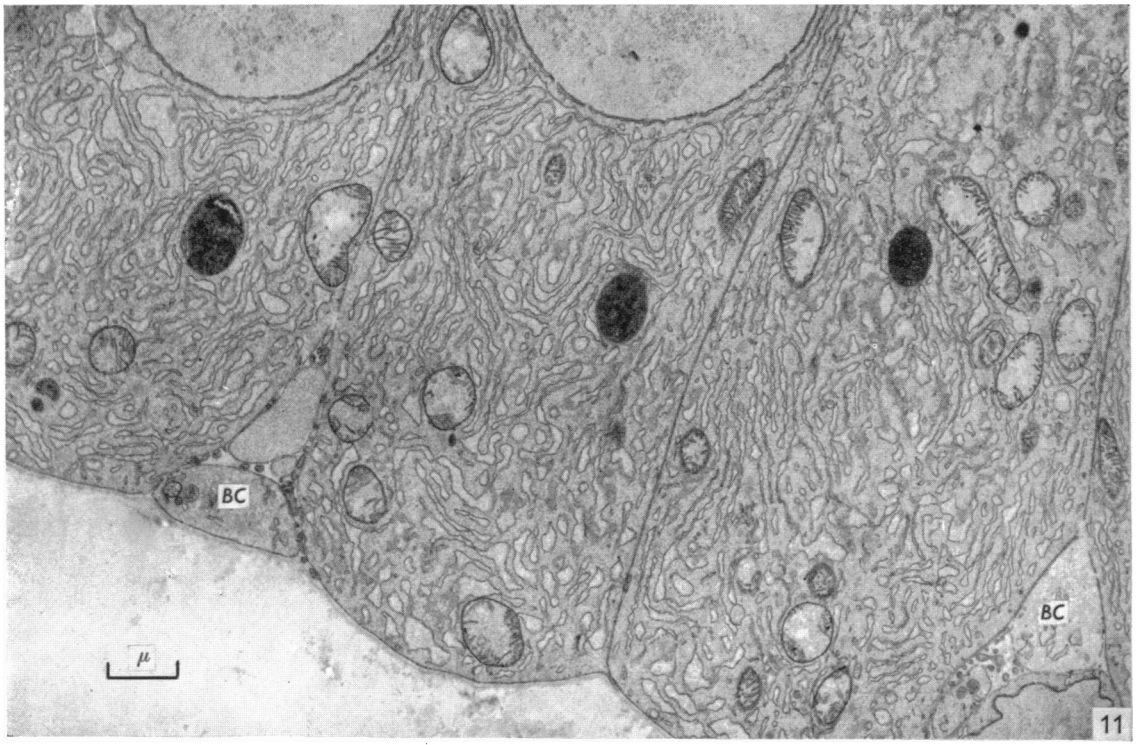
Fig. 10. Five days after castration, osmium fixed. The complex organization of the cytoplasm still remains, but the secretion granules (*Gr*) are rather smaller than in the control. The vacuoles containing the granules are not reduced in size. Dense bodies (*DB*) are more prominent than in the control.



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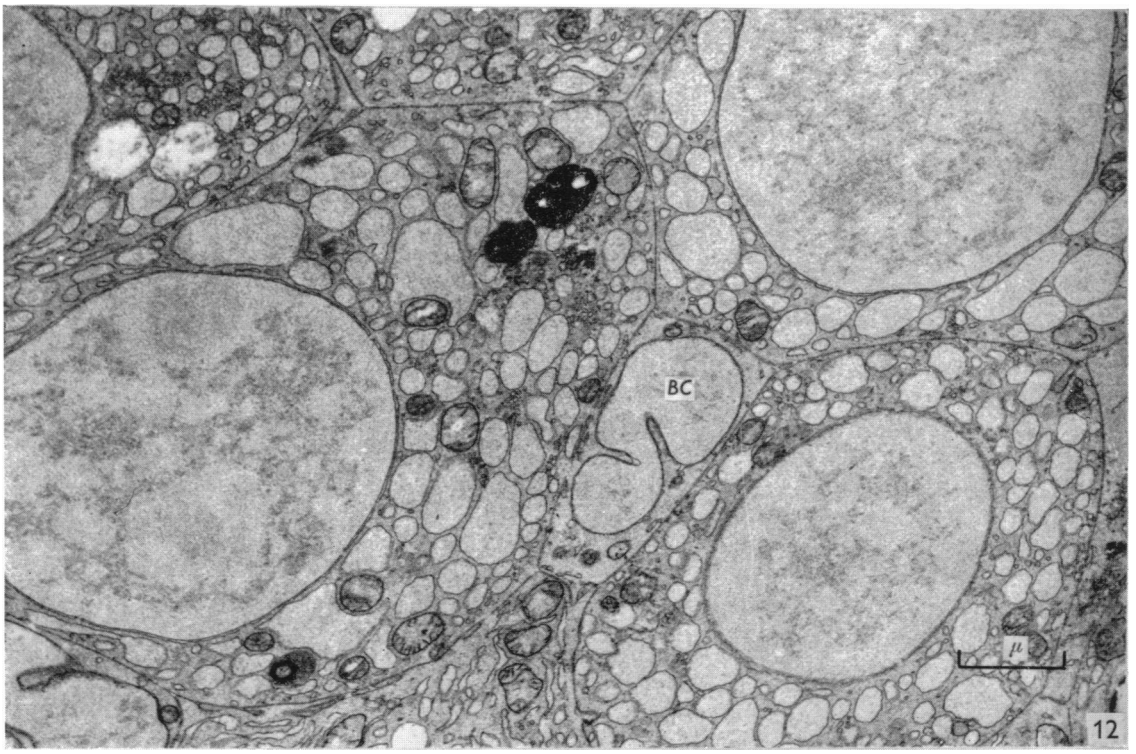

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endoplasmic reticulum are poorly organized, short and often dilated, the reticular pattern seen in the controls being absent (Fig. 12).

The Golgi apparatus is less elaborate, and is no longer associated with secretory material (Fig. 13). Dense bodies, often foamy and vacuolated, are now prominent, and often occupy much of the cytoplasm (Fig. 15). The infolding of the base of the epithelium is now quite elaborate, and affects both cell types equally, but the basement membrane is still closely applied to the crenated cell membrane (Fig. 14). Microvilli are no longer prominent on the cell surface. Marked changes in the mitochondria are not seen.

A striking feature of this group compared with the controls is the marked lack of uniformity. Adjacent to cells which show some or all of the degenerative changes described above, other cells are occasionally found (Fig. 16), which contain typical secretion granules and a well organized Golgi apparatus and granular reticulum.

DISCUSSION

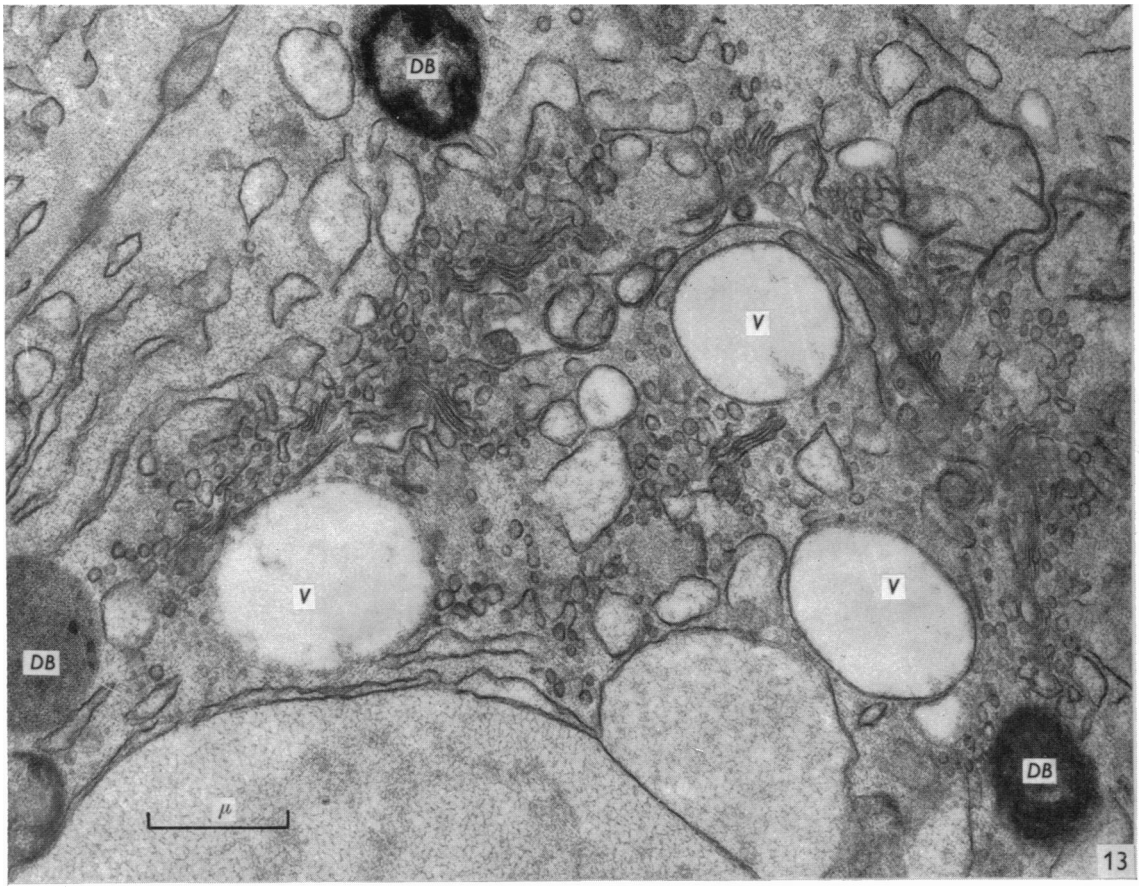
John Hunter first drew attention to the atrophy of the seminal vesicles which follows castration and the weight loss noted in the present investigation requires no comment.

Castration produces a fall in the amount of citric acid and fructose in the urine (Burrows, 1949) and having regard to the dependence of these constituents of the seminal vesicle on the level of circulating androgens in the bull (Lindner & Mann, 1960) a reduction after castration was expected. Of especial interest, however, is the different response of these two parameters of male sex hormone function to castration. While the amount of fructose contained in the seminal vesicle falls immediately and regularly after castration, the amount of citric acid remains fairly steady till the fourth day when it falls suddenly. It seems reasonable to suggest that the production of these two constituents stops when the testes are removed, and their differing behaviour subsequently may indicate some difference in the mechanism of their storage and release. Circumstantial evidence pointing to such a situation has already been noted in another context (Baillie, 1964*b*).

The fine structure of the seminal vesicle and the changes in fine structure seen after castration in the present series agree in the main with other reports (Deane, 1963) although Deane & Porter (1960) followed changes only up to 4 weeks post-castration, while others (Allison, 1964; Szirmai & Van der Linde, 1962) do not state the duration of their experiment. Diminution in cell size, reduction in the size and number of secretion granules, and increase in prominence of dense bodies can all be noticed at 5 days, while disorganization of the granular endoplasmic reticulum and crenation of the cell base are first seen at 10 days. All of these changes are progressive, being well established by fifty days and pronounced by 200 days after castration. Although the mitochondrial depletion and distension of Golgi vesicles reported by Allison (1964)

Fig. 11. Base of epithelium. Control, permanganate fixed. The cytoplasmic membranes are well preserved, and show elaborate organization. Small portions of basal cell (*BC*) cytoplasm are seen between the chief cells.

Fig. 12. General view of epithelium. Two hundred days after castration, permanganate fixed. There is disruption of the endoplasmic reticulum. A basal cell (*BC*) is seen with its typical indented nucleus.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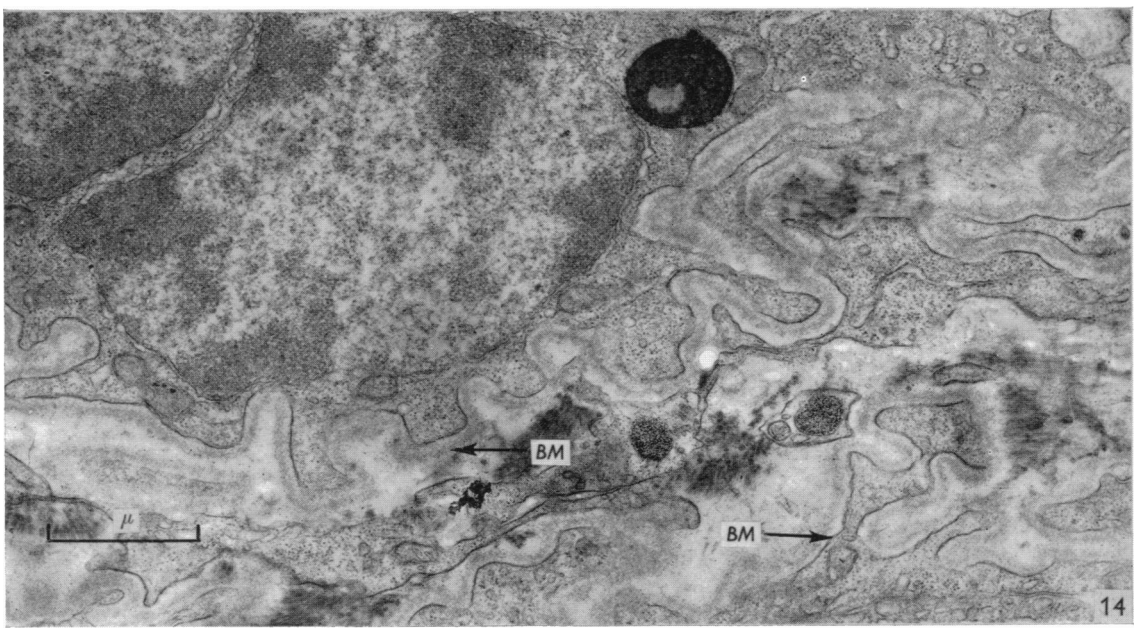
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were not seen in the present study, there is no doubt that the fine structural appearances indicate almost complete functional collapse of the seminal vesicle epithelium.

The behaviour of the histochemical characteristics surveyed in the present study has not previously been reported following castration. In contrast with the fine structural observations, no gross changes can be seen in any of these reactions, even 200 days after castration. The present material does not admit of quantitative estimation, however, and the continuing presence of a given colour reaction does not exclude the possibility of a reduction in the amount of the responsible enzyme present in the cell cytoplasm. Moreover, molecular integrity is not synonymous with physiological activity. The complete absence of changes in the histochemical constitution of the seminal vesicle cells after castration suggests that once the epithelial cells differentiate they retain much of their complement of enzymes even if not functionally active. It is clear as a corollary of this that the presence of such histochemically demonstrable enzymes is not a reliable indication of function.

Gohary, Cavazos & Manning (1962) have studied histochemically the distribution of PAS reactivity, cytoplasmic basophilia, sudanophilia and phospholipids in the epithelium of the castrated hamster seminal vesicle. The increase in sudanophilia and phospholipids noted by these authors may correspond with the increasing prominence of pleomorphic dense bodies seen in the electron micrographs. Allison's (1964) suggestion that they are pigment is not supported by the negative lipofuscin reaction, but pigments other than lipofuscin may be involved. Deane & Porter (1960) studied the relationship of cytoplasmic basophilia and ribosome population in the mouse seminal vesicle after castration, and found that the marked decrease in basophilia was not accompanied by any reduction in ribosome population density in the intercisternal cytoplasm. Despite this, it seems likely that the progressive loss of organization of the granular reticulum does bear some relationship to the loss of basophilia.

Infolding of the cell membrane at the base of an epithelial cell generally suggests enhanced metabolic activity, as, for example, in the kidney tubule (Rhodin, 1958) the choroid plexus (Millen & Rogers, 1956) and the acid-secreting cells of the stomach (Vial & Orrego, 1960; Ito & Winchester, 1963; Toner, 1963), but in these cases the basement membrane does not follow the infolding of the cell membrane. The crenation of the base of the seminal vesicle epithelium following castration is clearly of a different nature, and may simply reflect the reduction in the volume of the epithelium which has taken place.

Deane & Porter (1960) describe vacuoles in the cytoplasm following castration, and suggest that they are formed by the absorption of material from the lumen of the gland. In the present study there was no evidence to suggest such absorption, and it seems more likely that these are simply empty secretion vacuoles. The appearances at 5 and 10 days after castration make it clear that loss or resorption of the typical

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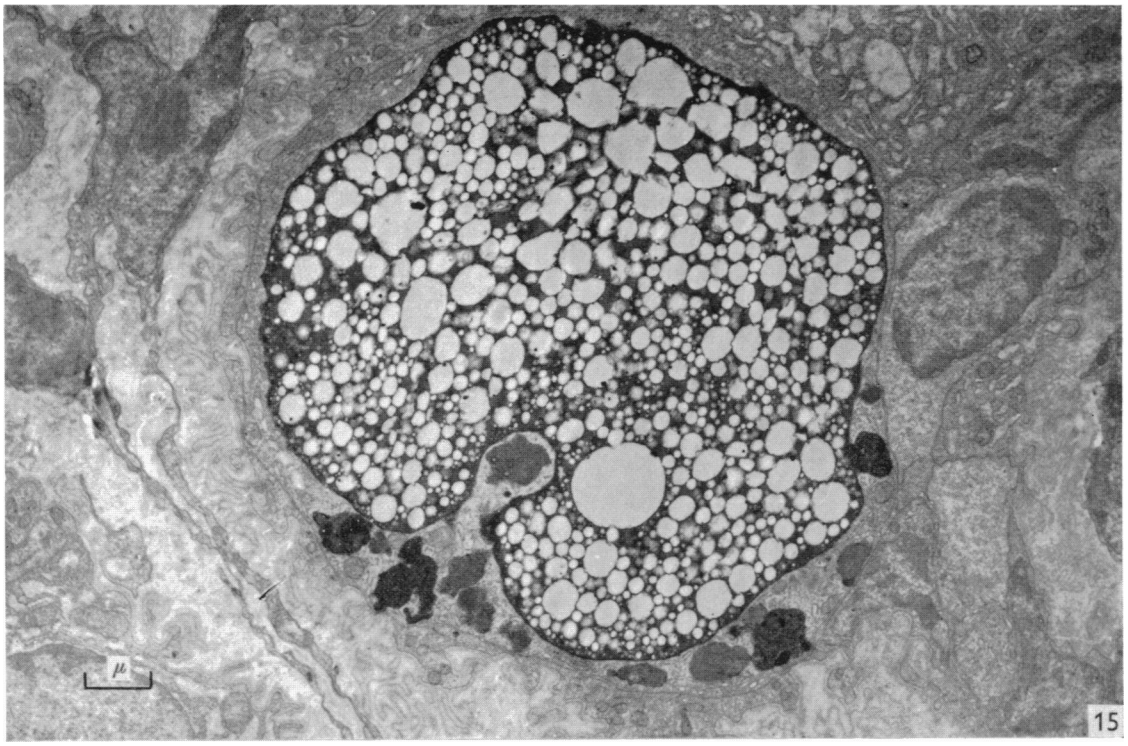
Fig. 13. Golgi apparatus. Two hundred days after castration; permanganate fixed. The apparatus is rather condensed compared with controls. Several small dense bodies (*DB*) are seen. Three empty vacuoles (*V*) are seen, similar in appearance to the vacuoles which contain large secretion granules in the control epithelium. Compare with Fig. 9.

Fig. 14. Basement membranes (*BM*) and cell base. Two hundred days after castration, osmium fixed. The crenation of the cell base is obvious, but the basement membrane follows the contours of the cell membrane.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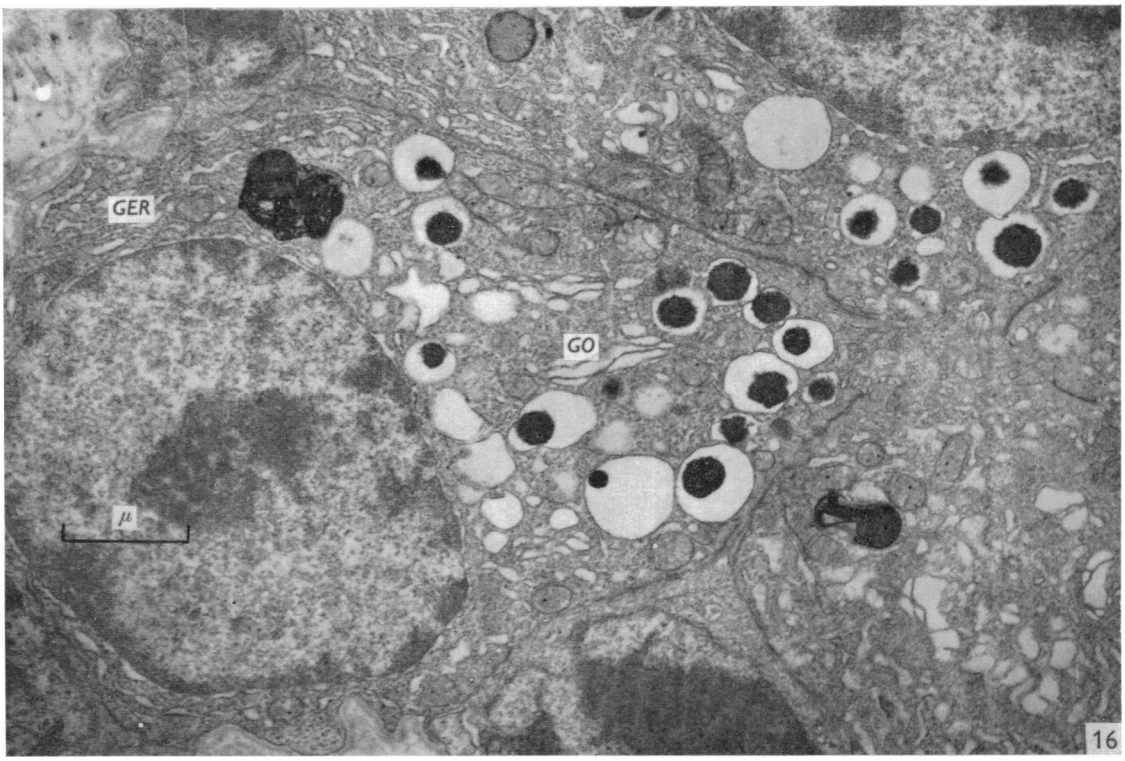
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dense secretion granules can occur without any reduction in the diameter of the enclosing vacuole.

Two hundred days after castration, occasional cells have some fine structural features which suggest normal activity, while adjacent cells show all the signs of loss of function. This lack of uniformity, in striking contrast with the controls, suggests that some cells may have an enhanced sensitivity to the androgen available from extra-testicular sources.

SUMMARY

1. The weight of the seminal vesicles, their contained fructose and citric acid and their histochemistry and ultrastructure have been studied in adult male Swiss white mice for a period of 200 days after castration.

2. Seminal vesicle weight falls immediately and regularly after castration to one-sixth of the control level within 7 days.

3. The amount of fructose present in the vesicles is halved within 24 h of castration and this sugar is not detectable after 7 days. The vesicle content of citrate remains fairly stable for 4 days after operation and then falls steadily, to disappear by the fourteenth day.

4. Esterase activity, visualized with α-naphthyl acetate, Naphthol AS acetate, Naphthol AS.LC acetate and Naphthol AS.D acetate is unchanged after castration. Nor were any changes seen in cytochrome oxidase, succinic dehydrogenase, aryl sulphatase and acid or alkaline phosphatase in the seminal vesicle epithelium after castration.

5. After castration the electron microscope reveals that in the seminal vesicle epithelium cell volume is progressively reduced and the endoplasmic reticulum and Golgi apparatus become less well organized and prominent. Secretory granules disappear leaving empty vacuoles, and the cell base becomes crenated. There is an increase in the number and prominence of pleomorphic dense bodies.

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Fig. 15. Dense bodies. Two hundred days after castration, osmium fixed. In one cell there can be seen a single large vacuolated body and several smaller irregular dense inclusions.

Fig. 16. Two hundred days after castration, osmium fixed. Two of these cells contain a number of typical large secretion granules lying in vacuoles, and in one cell the Golgi apparatus (*GO*) and endoplasmic reticulum (*GER*) are well organized. Such cells are rare after two hundred days. Adjacent cells showed all signs of degeneration. Figs. 15 and 16 are from the same section.

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