Fine Structure of the Testis and Epididymis of Rats Treated with Cyproterone Acetate

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ABSTRACT Adult male rats were administered the antiandrogen, cyproterone acetate, for 4, 8 or 12 weeks, and the histology and fine structure of the testis and several parts of the epididymis were studied. After treatment for 8 or 12 weeks, the testes of treated animals displayed a great reduction in the abundance of late spermatids. Necrotic cells, many of which were identified as cap-phase spermatids, were present in the seminiferous epithelium. Sertoli cells contained many large lipid droplets and lysosome-like structures with a content of cellular debris, including parts of spermatids. Leydig cells of treated rats were smaller than those of control animals at all the intervals studied. Sperm were absent from the lumen of the middle segment, or caput epididymidis, of severely affected specimens. In the terminal segment, or cauda epididymidis, the microscopic appearance varied in different regions. In the proximal part of the cauda epididymidis, the lumen was usually clear of sperm. The epithelium was tall and the light cells were very large and distended with many dense bodies resembling lysosomes. In contrast, in the distal part of the cauda epididymidis, the lumen was filled with sperm and debris, which appeared to be derived from germ cells. It is suggested that the light cells of the epididymal epithelium may have a role in clearing the lumen in the proximal part of the cauda epididymidis, in which they are particularly large and numerous. The results suggest that in the presence of cyproterone acetate, germ cells develop up to cap-phase spermatids and then begin to undergo degeneration and death. This alteration may have an important role in the antifertility effect of the drug, but changes in the epididymis may contribute also.

The antiandrogens, cyproterone and cyproterone acetate, are of medical interest because they cause reversible infertility (Whalen and Luttge, '69; Steinbeck et al., '71; Morse et al., '73), and by suppressing androgen-dependent organs they may be useful in the treatment of various diseases, including prostatic neoplasia (Geller et al., '68; Scott and Wade, '69). Since these compounds act by competing with testosterone for androgen receptors in target cells (Fang and Liao, '69; Stern and Eisenfeld, '69; Tveter and Aakvaag, '69) they have also been useful in experimental studies on the male reproductive tract. Of the two compounds, cyproterone acetate has been the more widely used and has a greater potency, although it is said to have more progestational activity than cyproterone (Weichert and Neumann, '65; Steinbeck et al., '71). The literature on the mechanism of action of these compounds and clinical and experimental studies with them has been reviewed on several occasions by Neumann and his associates (Neumann, '66, '71; Neumann et al., '68, '69, '70).

The basis of the antifertility action of cyproterone acetate is not entirely clear, however, because several parts of the reproductive tract are affected by the drug. The weights of the testis and the accessory sex glands of animals treated with cyproterone acetate are reduced (Neumann et al., '70), suggesting that alterations in any of these organs might be involved in the decreased fertility produced by the drug. Ultrastructural studies of the prostate and seminal vesicles of animals treated with cyproterone acetate have shown that there is a large decline in the height of the epithelium and a diminution in the organelles

Accepted April 13, '76.

involved in secretion (Dahl and Kjaerheim, '74; Dahl and Tveter, '74; Loving and Flickinger, '76). Information on the structure of the testis and epididymis in the presence of cyproterone or cyproterone acetate is less complete, but studies at the light microscopic level have indicated that spermatogenesis is affected (Neumann and von Berswordt-Wallrabe, '66; Mietkowski and Lukaszyk, '69; Markewitz et al., '69; Steinbeck et al., '71; Morse et al., '73) and that the histology of the epididymal epithelium is altered as well (Prasad et al., '70, '71; Rajalakshmi et al., '71; Rajalakshmi and Prasad, '75).

The fine structure of the testis and epididymis in the presence of cyproterone acetate has not previously been investigated, and it seemed that such a study could aid in understanding the antifertility action of the drug by providing more detailed information on the stages of germ cells affected, the nature of the structural changes in germ cells and Sertoli cells, and the type of alteration in epididymal epithelium and sperm. Therefore, in the present study we examined the histology and ultrastructure of the testis and several parts of the epididymis of rats treated for up to 12 weeks with cyproterone acetate. Alterations were found in the testis, including degeneration of spermatids and changes in Sertoli cells. In the epididymis, alterations in the epithelium and luminal contents varied in the different segments. Observations on the prostate and seminal vesicle were reported in a previous publication (Loving and Flickinger, '76).

MATERIALS AND METHODS

Sexually mature male Sprague-Dawley rats weighing approximately 200 g were obtained from the Charles River Breeding Laboratories, Wilmington, Mass. The rats were caged separately in the University Vivarium. They had constant access to water and food, and the temperature was maintained at 20–22°C.

Rats were given daily subcutaneous injections of 10 mg of cyproterone acetate suspended in 0.2 ml sesame oil. This constant dose was used, rather than one that was a function of the weight of the animal, so that our results could be correlated with extensive work in the literature that has been carried out using a dose of 10 mg/rat/day (e.g., Neumann, '66; Neumann et al., '69, '70; Whalen and Luttge, '69; Steinbeck et al., '71). The cyproterone acetate was supplied through the courtesy of Dr. Eberhard Berger of Schering AG, Berlin, West Germany. Control rats were injected once daily with 0.2 ml of sesame oil.

Rats were treated for 4, 8, or 12 weeks. At each interval two to four rats treated with cyproterone acetate and two control rats were killed by cervical dislocation. The reproductive organs, including testis, caput and cauda epididymidis, ventral prostate and seminal vesicles were removed and weighed. Tissue from several normal rats was also obtained for comparison with that from treated and control animals.

Whole testes were immersed for one hour in a glutaraldehyde, formaldehyde, and picric acid fixative (Ito and Karnovsky, '68), prepared by mixing 8.5 ml of Karnovsky's fixative (Karnovsky, '65) with 1.5 ml of a saturated solution of picric acid and 10 ml of 0.1 M cacodylate buffer, pH 7.3. After one hour, pieces were cut from the testes, diced and fixed for an additional one hour. The major portion of the caput epididymidis, corresponding to the middle segment of the epididymis (Glover and Nicander, '71), was bisected and immersed in Karnovsky's fixative for one hour. The cauda epididymidis, which contains the terminal segment of the epididymis (Glover and Nicander, '71), was similarly divided and immersed in fixative. In the later experiments, care was taken to divide it into two parts, one corresponding to the proximal part of the cauda, which has the smaller diameter (probably regions 5B and 6A of Reid and Cleland, '57), and the other comprising the distal part of the cauda (region 6B of Reid and Cleland). After one hour the blocks were diced and immersed in fixative for an additional hour. After fixation for a total of two hours in aldehyde the tissue blocks were rinsed with 0.1 M cacodylate buffer and post-fixed for one hour in 1% OsO_4 in 0.1 M cacodylate buffer at pH 7.3. The tissue was dehydrated in a graded series of ethanols followed by propylene oxide and was embedded in Araldite.

Sections 1 μ thick for light microscopy were cut with glass knives, mounted on slides, and stained with 0.5% toluidine blue in 0.5% sodium borate. Thin sections

showing silver to pale gold interference colors were cut with a diamond knife, mounted on uncoated copper grids and stained with lead citrate (Reynolds, '63). The preparations were examined and photographed using a Philips EM-300 electron microscope.

The size of Leydig cells in treated and control animals was estimated in the following way. Accumulations of interstitial tissue at the junction between three or more seminiferous tubules were selected at random for study in preparations for light microscopy. Within such a region, the length and width of all the cells identifiable as Leydig cells were measured using a Zeiss ocular micrometer at a magnification of × 400. Twenty-five cells were measured from at least two treated rats and one control animal at each interval of treatment. Assuming the profiles of the cells to be elliptical, the area of the cell within the plane of section was calculated by multiplying its length 2 × width 2 × π. The mean area of the profiles of Leydig cells from treated animals was compared with that of control rats using a T-test.

RESULTS

General observations

The testes and epididymides of rats treated with cyproterone acetate were smaller than normal, and the weights of both organs were consistently less than those of control animals. After 12 weeks, the testes of treated rats weighed approximately 55–60% those of control rats, while the epididymides of treated animals weighed only 20–25% of control values.

One of three animals killed after treatment for four weeks showed microscopic changes in the testis and epididymis, while the remainder were unaltered or had only slight changes. Microscopic changes were present in all the rats prepared following 8 or 12 weeks treatment, but the animals were not uniformly affected. In the most severely affected rats, representing about half those treated, late spermatids and sperm were virtually absent in the testis and in the lumen of the caput and proximal cauda epididymidis. Other animals appeared to have been less extensively affected, because some late spermatids remained in the seminiferous epithelium and in the lumen of the caput epididymidis, although their numbers appeared to be reduced. All the treated animals displayed necrotic germ cells in the testis and the remainder of the alterations described below.

In animals treated for eight weeks or longer, the reproductive organs that had the most severe microscopic changes weighed less than those that showed less pronounced alterations. Although treated animals did not gain weight as rapidly as did control rats, the degree of microscopic change did not appear to bear a consistent relation to either the initial or the final body weight of the animals.

Testis

Normal and control animals

The normal structure of the rat testis at both the light and electron microscopic levels is well known and a detailed description is not repeated here (e.g., Brokelmann, '63; Burgos et al., '70; Dym and Fawcett, '70; Flickinger, '72b). As in other mammals, the seminiferous tubules (fig. 1) contain Sertoli cells and combinations of germ cells in different phases of development from spermatogonia through spermatocytes to spermatids and mature sperm, constituting the various stages of the cycle of the seminiferous epithelium (Leblond and Clermont, '52). Myoid cells surround the seminiferous tubules and the interstitial tissue contains blood vessels, lymphatics (Fawcett et al., '73), and Leydig cells, which have abundant smooth endoplasmic reticulum and secrete testosterone (Christensen and Gillim, '69; Christensen, '75; Neaves, '75).

Cyproterone acetate-treated rats

The main alterations in the testis of rats treated with cyproterone acetate were the presence of numerous degenerating or necrotic spermatids within the seminiferous epithelium, a decrease in the abundance of late spermatids and the accumulation of debris, parts of germ cells and numerous very large lipid droplets in Sertoli cells. In addition, Leydig cells decreased in size.

Spermatogonia, spermatocytes and early spermatids were abundant in light microscopic preparations of treated animals (fig. 2). When studied with the electron microscope, germ cells appeared to have a normal ultrastructure in stages up to cap-

phase spermatids (stages 4–7, Leblond and Clermont, '52). It is not practical to illustrate all these stages, but portions of several germ cells are visible in the field shown in figure 3. The presence of many synaptinemal complexes in the nuclei of primary spermatocytes (fig. 3) suggested that prophase of the first meiotic division proceeded in the presence of the drug. It should be noted however, that this study *did not* include a quantitative evaluation of the cellular types present, and changes in the numbers of the early stages of germ cells are not excluded.

Some cap-phase spermatids with a normal fine structure were present, but others appeared to be degenerating or necrotic. In toluidine blue-stained sections for light microscopy, some cells had an intensely staining granular cytoplasm and cytological details were obscured. At the electron microscopic level (figs. 3, 4), round cells were observed that were unusually dense and that contained disrupted cytoplasmic organelles and myelin figures. It was not possible to identify the type of cell involved in all instances, because of the disruption in morphology, but in some cases the cells were identified as cap-phase spermatids (approximately stages 6–7) by the presence and shape of the remnant of the acrosome (figs. 3, 4). The extent of disruption of normal structures in many of these cells suggested that they were dead rather than reversibly injured and thus are more appropriately termed necrotic than degenerating (Robbins, '74). Some germ cells normally degenerate during their development (Clermont and Bustos-Obregon, '68; Oakberg, '56), but in thin sections of normal and control rats these were rarely encountered. In contrast, necrotic cells were so abundant in treated rats that as many as six to eight were seen in one square of a 200-mesh grid. In addition, the cells that normally degenerate are thought to be spermatogonia or spermatocytes while many of those in treated animals were identified as spermatids.

The nature of the initial degenerative morphological changes is uncertain. Some cap-phase spermatids, however, had dilated elements of endoplasmic reticulum and the nuclear envelope had an angular profile rather than the usual round outline. It is possible that these alterations might be precursors to the more obvious necrotic changes described above.

A large decrease in the numbers of late spermatids (stages 8 and above) was manifested at the light microscopic level by a scarcity of germ cells with condensed nuclei in the seminiferous epithelium (fig. 2). In the most severely affected samples, spermatids with condensed nuclei appeared to be absent. Accordingly, when thin sections of the same blocks were viewed with the electron microscope, late spermatids of the acrosomal and maturation phases were rarely encountered. Late spermatids were not as greatly depleted in the less severely altered specimens, but in either case altered parts of late spermatids were found in the seminiferous epithelium, usually within Sertoli cells (fig. 5). These included remnants of condensed nuclear material lacking nuclear membranes, portions of the middle piece with outer coarse fibers and distorted mitochondria, and unusually dense tail sections containing an axoneme, coarse fibers and a fibrous sheath. Their density and the presence of disrupted organelles suggested that some of these remaining late spermatids had also undergone a degenerative change.

Sertoli cells of treated rats contained many membrane-bound structures resembling lysosomes in their polymorphic content of membranes, granules and amorphous substance. As described above, the source of the material within them could in some cases be determined to be of germ-cell origin because the condensed nuclear material or tail fibers of spermatids were visible. Due to the complexity of the seminiferous epithelium, it was often difficult to determine whether the necrotic spermatids lay between or within Sertoli cells, but in favorable sections portions of necrotic germ cells were seen to be encircled by the cytoplasm of a single Sertoli cell (fig. 3).

Sertoli cells of treated animals also contained many large lipid droplets (figs. 2, 6). Some lipid droplets are normally present in rat Sertoli cells at certain stages of the cycle of the seminiferous epithelium (Kerr and DeKretser, '75; Fawcett, '75). The specimens from treated animals gave the impression of an increase in the amount of Sertoli-cell lipid, however, because droplets were present in virtually all the seminiferous tubules. Some were as large as

6 μm in diameter (fig. 6) and were so closely packed in some cells as to displace other organelles and to indent the nucleus.

The Leydig cells of treated animals appeared smaller than normal, and to test this impression the dimensions of Leydig-cell profiles within sections was measured. Profiles of Leydig cells of treated animals were found to be smaller than those of normal or control rats at each interval of treatment (table 1). The decrease was not large, the sectional areas of Leydig cells of treated rats ranging between 58 and 76% of control values, but at each interval the difference between treated and control animals was highly significant (table 1). Leydig cells appeared particularly abundant in treated rats (fig. 2), perhaps as the result of shrinkage of the seminiferous tubules. When studied with the electron microscope (fig. 7), the Leydig cells of treated animals were seen to be small but to contain the smooth endoplasmic reticulum and other cytoplasmic organelles characteristic of normal rat Leydig cells.

Epididymis

Normal and control animals

The structure of the normal rat epididymis has been reviewed recently (Hamilton, '75). The columnar epithelial lining generally decreases in height along the length of the epididymis. Several cell types are present. Principal cells are the most numerous, and their features include long microvilli, apical vesicles and vacuoles, a large Golgi apparatus and both rough and smooth endoplasmic reticulum. In the middle segment in the caput epididymidis (fig. 8), the principal cells are tall, while in the terminal segment in the cauda epididymidis (figs. 10, 13) they have a shorter columnar shape. The principal cells of the middle and terminal segments are accompanied by "halo" cells, which are believed to be migrating lymphocytes (Hoffer et al., '73), and by small basal cells. The cauda epididymidis also contains significant numbers of the "light" or "clear" cells, which have a cytoplasm that is usually less electron-dense than adjacent principal cells and contains many electron-lucent vesicles and vacuoles in the apical region (fig. 12: treated animal). Vacuoles found deeper in the cytoplasm have a greater internal density as does the content of the membrane-bound bodies of the perinuclear and basal regions that resemble lysosomes (Flickinger, '72a). The nature of these light cells has been controversial, but there are indications that they are absorptive cells (Nicander, '70; Flickinger, '72a). The proximal and distal regions of the cauda epididymidis in normal rats have a similar appearance (figs. 10, 13), although the epithelium of the proximal part is slightly taller and the light cells are larger and more numerous than in the distal portion (Reid and Cleland, '57). Large numbers of sperm are normally present in the lumen of both the middle and terminal segments (figs. 8, 10, 13).

TABLE 1

	Weeks of treatment		
	4	8	12
Control (μ^2)	86	91	84
CA (μ^2)	50	69	53
CA, % of control	58	76	63
P	< 0.001	< 0.01	< 0.001

The mean areas (μ^2) of profiles of Leydig cells after treatment for 4 to 12 weeks with cyproterone acetate (CA). The ratio of treated to control values (× 100) is also shown for each interval. In each case the difference between the treated and the control is statistically significant at the P value indicated.

The testis and epididymis of control rats resembled those of normal animals at both the light and electron microscopic levels.

Cyproterone acetate-treated rats

The lumen of the middle segment in the *caput epididymidis* of severely affected animals treated with cyproterone acetate contained few or no sperm (fig. 9). Often only a few round cells resembling immature germinal cells remained. Despite this change in luminal content, the epithelium of this part of the epididymis remained columnar, and the principal cells retained their characteristic ultrastructural features of sterocilia, apical vesicles and vacuoles, a large Golgi apparatus and profiles of smooth endoplasmic reticulum.

The appearance of samples of the terminal segment in the cauda epididymidis varied with the location along the duct from which they were taken. In the *proximal cauda epididymidis* of treated rats, sperm were virtually absent from the lumen (fig. 11), which appeared collapsed and was occupied only by the numerous sterocilia of the epithelial cells. The apical

surface of the epithelium had an undulating contour which lent the small remaining lumen a stellate outline in cross section. The epithelial cells of treated animals had a tall columnar shape and some cells contained many granules that stained with toluidine blue. Study of the epithelium with the electron microscope revealed that the light cells of the epididymal epithelium were greatly distended with vacuoles and dense bodies (fig. 12). Some of these cells presented a very striking appearance, since even in low-magnification electron micrographs almost the entire field was occupied by these lysosome-like dense bodies.

In the *distal part of the cauda epididymidis* of cyproterone acetate-treated rats (fig. 14), the lumen had a smooth outline and was packed with masses of material consisting of large amounts of debris, some sperm and structures resembling small round cells. When viewed with the electron microscope (fig. 15), the round structures were sometimes seen to possess a nucleus, but in most cases they appeared to be simply membrane-bound spheres of cytoplasm that contained small dense mitochondria, lipid droplets, a granular material and small dense mitochondria. Thus they resembled the residual bodies normally shed in the testis by developing spermatids (Smith and Lacy, '59; Dietert, '66). Sometimes the masses of cytoplasm also contained an axoneme and sperm tail fibers. Epithelial cells or phagocytic cells were not observed in the lumen. Light cells were present in the epithelium of the distal cauda epididymidis, but as in normal rats, they were not as large or as numerous as in the proximal part of the cauda epididymidis. Principal cells in both parts of the cauda epididymidis contained their usual complement of organelles and did not appear to be significantly altered in their ultrastructure by the treatment.

DISCUSSION

Testis

The results indicate that in rats administered cyproterone acetate, germ cells develop up to early spermatid stages and then begin to degenerate and die. This finding is in accord with most of the previous light microscopic studies, which indicated that late spermatids were the most severely depleted stages in rats treated with cyproterone (Mietkowski and Lukaszyk, '69) or cyproterone acetate (Neumann and von Berswordt-Wallrabe, '66; Steinbeck, '71) and in men administered cyproterone acetate (Morse et al., '73). In one study, however, indications of altered DNA synthesis in the testes of men with prostatic cancer that were treated with cyproterone acetate suggested that the drug effects spermatogonia and primary spermatocytes (Markewitz et al., '69).

The ultrastructural observations in the present study extend previous observations by more precisely defining the stages of germ cells that are altered in the presence of cyproterone acetate. Cap-phase spermatids were the earliest stages to show degenerative changes and comprised the bulk of the necrotic cells that could be identified as to type. Furthermore, not only were late spermatids of the acrosomal and maturation phases greatly diminished in most specimens, but parts of those that remained were sometimes altered and lay within Sertoli cells, suggesting that perhaps even those late spermatids that persist or are formed in the presence of the drug are altered and may no longer be viable. It should be noted, however, that even though ultrastructural changes were found in spermatids in the present study, the observations do not rule out changes in the metabolism of earlier stages, in the rate of cell division or in the timing of spermatid development.

The results suggest that at least some necrotic germ cells are taken up and digested by Sertoli cells, since recognizable parts of germ cells were found within Sertoli cells. This is in accord with observations that Sertoli cells phagocytose degenerating germ cells under other conditions (Lacy and Lofts, '65; Vilar et al., '67; Hugon and Borgers, '66; Reddy and Svoboda, '67; Roosen-Runge and Leik, '68; Black, '71), as well as injected particulates (Clegg and MacMillan, '65), and that they retain the residual bodies shed by developing spermatids (Smith and Lacy, '59; Brokelmann, '63; Dietert, '66).

Many extremely large lipid droplets were present in Sertoli cells throughout the seminiferous tubules of treated animals. An increase in lipid in Sertoli cells occurs in other conditions in which spermatogene-

sis is disrupted, including cryptorchidism, hypophysectomy, local heating, estrogen treatment (Hanes and Rosenbloom, '11; Lynch and Scott, '51; Lacy, '62; Lacy and Lofts, '65; Collins and Lacy, '69) and the testicular feminization syndrome (Chung, '74). The lipid in Sertoli cells of rats treated with cyproterone acetate might be acquired from the cytoplasm of ingested germ cells. This is thought to be the case for the appearance of lipid in Sertoli cells at stage IX of the normal cycle of the seminiferous epithelium as the result of ingestion of many residual bodies (Niemi and Kormano, '65; Kerr and DeKretser, '75). Alternatively, the accumulation of lipid could be due to an alteration in the metabolism of the Sertoli cells themselves in the presence of cyproterone acetate, because lipid accumulation is a well-known pathological change that occurs in many cells under a variety of deleterious conditions (Robbins, '74).

Testosterone is thought to act on the seminiferous tubules and to play a role in maintaining spermatogenesis (Steinberger, '71). Since cyproterone acetate is known to compete with testosterone (Fang and Liao, '69; Stern and Eisenfeld, '69; Tveter and Aakvaag, '69), the changes in the seminiferous epithelium may be due to this antiandrogenic action of the drug. It should be kept in mind, however, that cyproterone acetate has progestational side-activity, suppressing gonadotropin release (Wiechert and Neumann, '65; Steinbeck et al., '71), and that the seminiferous tubules might also be affected in this way. As discussed further below, the observation that Leydig cells were reduced in size suggests that this possibility should be considered. In addition, the similarity between the present observations and the effects of treatment with a progestin (Flickinger, '76) or hypophysectomy (Clermont and Morgentaler, '55) lend credence to the idea that cyproterone acetate may affect the testis by suppressing gonadotropins, since in all these instances spermatids were observed to begin degeneration at a similar stage. On the other hand, the capacity of cyproterone acetate to produce changes in spermatogenesis in the testes of hypophysectomized rats that were administered testosterone and cyproterone acetate (Neumann and von Berswordt-Wallrabe, '66) argues for the supposition that the testicular effects of the drug are due at least in part to its peripheral antiandrogenic action.

In the present study, Leydig cells were found to be smaller in animals treated with cyproterone acetate than in control rats. Previous reports have differed on the effects of cyproterone acetate on Leydig cells. In some studies of cyproterone acetate-treated rats, Leydig cells were reported to remain unchanged for several weeks and then to undergo slight shrinkage (Neumann et al., '70), and a decrease in the size of Leydig cell nuclei has been observed (Heinert and Taubert, '73). In one study the Leydig cells were said to decrease in size for six weeks and then to return to normal (Steinbeck et al., '71). Other reports that rat Leydig cells remain unchanged in the presence of cyproterone acetate may reflect the use of shorter treatment periods (Junkmann and Neumann, '64; Neumann, '66), although no change was observed in the Leydig cells of men treated with cyproterone acetate for as long as 16 to 20 weeks (Morse et al., '73). An increase in Leydig cell size would be expected for a drug with an antiandrogenic action due to its competition with testosterone centrally, and this is in fact observed in animals treated with cyproterone (the free alcohol) (Mietkiewski and Lukasyk, '69; Neumann et al., '70; Steinbeck et al., '71; Heinert and Taubert, '73). The occurrence of no change or only a small change in Leydig cells in the presence of cyproterone *acetate* has been rationalized as a balancing of the antiandrogenic and gonadotropin-suppressing actions of this compound (Steinbeck et al., '71). The small but significant decrease in the size of Leydig cells observed in the present study suggests that they may have received reduced gonadotropin stimulation. It should be noted, however, that the functional state of the Leydig cells is not definitely known, because direct measurements of gonadotropin and testosterone levels were not performed in the present study, and these would provide more reliable information on the mechanism of action of cyproterone acetate than do changes in cell size.

Epididymis

In the caput epididymidis, sperm were absent from the lumen of the severely

affected animals. Since the epithelium did not appear to be significantly altered in its complement of organelles and there were no indications of infiltration of phagocytic cells, the lack of sperm seems most likely due to the decreased production of sperm by the testes. Sperm present in the caput when treatment began may have been transported normally to the cauda epididymidis.

The situation was different in the cauda epididymidis, where sperm are normally stored. In the proximal part of the cauda, the numbers of sperm in the lumen were greatly reduced or absent, and the lumen occupied only a very small space. Sperm may have been moved to more distal parts of the male duct system. However, the presence in the epithelium of this segment of many large light cells, seemingly distended with remarkably large numbers of dense bodies which resemble lysosomes (Flickinger, '72a), suggests that the uptake of material by the epithelium may have contributed to the clearing of the lumen. Some support is lent to this conjecture by comparing the effects of the treatment on the proximal and distal parts of the cauda epididymidis.

In the distal part of the cauda epididymidis, the lumen was not clear of material as in the proximal part, but instead contained large amounts of cellular debris and some sperm. These differences in luminal content may be related to differences in the cellular composition and activity of the epithelium in these two parts of the epididymis. In their detailed study of the histology of the rat epididymis, Reid and Cleland ('57) noted that the light ("clear") cells were particularly large and numerous in the proximal part of the cauda epididymidis (their regions 5 and 6A) and they appeared more active than in the distal part of the cauda, as indicated by a greater degree of vacuolization. Similarly, in the present study, although light cells were found in the distal part of the cauda epididymidis of treated animals, they did not appear to be as numerous or as large as in the proximal portion. Thus, if light cells function in absorption, their greater size and abundance in the proximal part of the cauda epididymidis might account for the clearing of the lumen in this region, while the retention of luminal material in the distal part of the cauda epididymidis could reflect lesser absorptive activity by these cells. Since parts of sperm were not recognized in the epithelial cells, the material taken up may have been confined to soluble substances and fluid, or sperm might have broken down in the lumen prior to absorption by the epithelium. In any event, no evidence was obtained for migration of phagocytic cells into the epithelium or the lumen of either part of the cauda epididymidis.

The absence of sperm in the caput epididymidis and the presence of material in the lumen of the cauda epididymidis were also observed previously in a light microscopic study of rats treated with cyproterone acetate (Rajalakshmi and Prasad, '75). Other changes such as the reported depletion in "secretory granules," vacuolation of cells and nuclear pycnosis are less readily related to the ultrastructural observations made in the present study, but perhaps the different methods of specimen preparation and study account for some of the differences. "Cells" found in the lumen of the cauda epididymidis were previously interpreted on the basis of light microscope observations as exfoliated epithelial cells (Rajalakshmi and Prasad, '75). Our observations, however, suggest that these structures have a different origin. They did not bear a resemblance to the epithelial cells in their fine structure, nor did they appear to be phagocytic cells that might have migrated into the lumen. Their nature is not definitely established, but they usually were masses of cytoplasm that lacked a nucleus, and their morphology was reminiscent of that of the residual bodies of Regaud (Smith and Lacy, '59; Dietert, '66), which are normally shed by the developing sperm in the testis. This, along with the occasional presence of parts of sperm tails suggests that these luminal structures consist of germ cell cytoplasm either derived from sperm in the epididymis or from the remnants of earlier stages of germ cells shed by the seminiferous epithelium following the depletion of late spermatids.

Antifertility effects of cyproterone acetate

Administration of cyproterone acetate at the dose used in the present study results in infertility in rats within seven weeks

(Whalen and Luttge, '69). The results of the present study suggest that an important basis for this antifertility action is an alteration in spermatogenesis, resulting in the degeneration of cap-phase and later spermatids. Changes in the epididymis may also contribute, however, since the lumen of the cauda epididymidis appeared to be blocked with a mass of cellular debris and degenerating sperm. In support of the contention that the epididymis is involved is the observation that very small doses of cyproterone acetate released from implanted Silastic capsules (232 μg/day) cause infertility, non-motile sperm and alterations in epididymal histology and sialic acid content, in the absence of any change in the testis, sex accessory glands or libido (Prasad et al., '70, '71; Rajalakshmi et al., '71). In addition, diminution in the volume and possible changes in the character of the secretions of the accessory glands might also play a contributory role in the antifertility effect of cyproterone acetate (Loving and Flickinger, '76).

ACKNOWLEDGMENTS

The authors are indebted for technical assistance to Ms. Sharon Odum. This research was supported by a contract (NO1-HD-1-2506) with the National Institute for Child Health and Human Development, and a grant from the Population Council (M74.82).

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PLATE 1

EXPLANATION OF FIGURES

1 Light micrograph of normal rat testis. The seminiferous tubules contain different stages of germ cells, including primary spermatocytes (P) and many late spermatids with condensed nuclei (S). $\times 145$.

2 Light micrograph of the testis of a rat treated with cyproterone acetate for 12 weeks. Spermatogonia, spermatocytes (P), and early spermatids (S) are present, but the condensed nuclei of late spermatids are virtually absent. Many lipid droplets (L) lie in the basal region of the seminiferous epithelium. Although their nature cannot be appreciated at this low magnification, other densely staining structures in the seminiferous epithelium (arrows) can be seen, in electron micrographs (figs. 3, 4), to be degenerating or necrotic spermatids. $\times 145$.

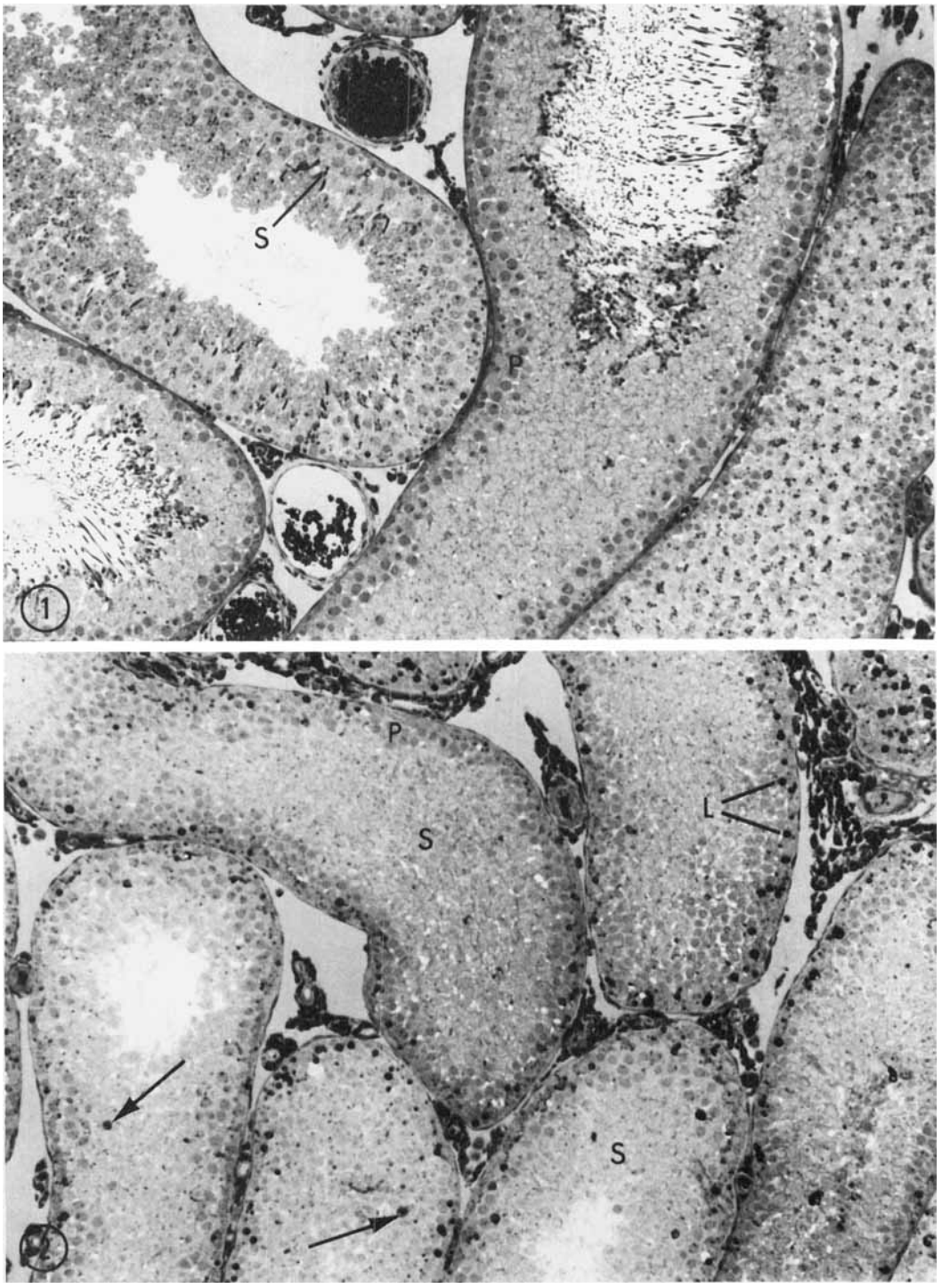

S
P
1
P
S
L
S
2

PLATE 2

EXPLANATION OF FIGURE

3 Low-power electron micrograph of the testis of an animal treated for 12 weeks with cyproterone acetate. A degenerating cell (D) within the seminiferous epithelium is surrounded by the cytoplasm of a Sertoli cell. Most of the degenerating cell is very dense, but the shape of the remaining acrosomal vacuole (A) suggests that it is a late cap-phase spermatid, stage 6 or 7 (Leblond and Clermont, '55). A few small lipid droplets (L) are present in the Sertoli cell. The two cells at the top of the figure are primary spermatocytes, probably in prophase of the first meiotic division, because synaptinemal complexes (C) are visible in their nuclei. N, nucleus of Sertoli cell; G, a spermatogonium. $\times$9,800.

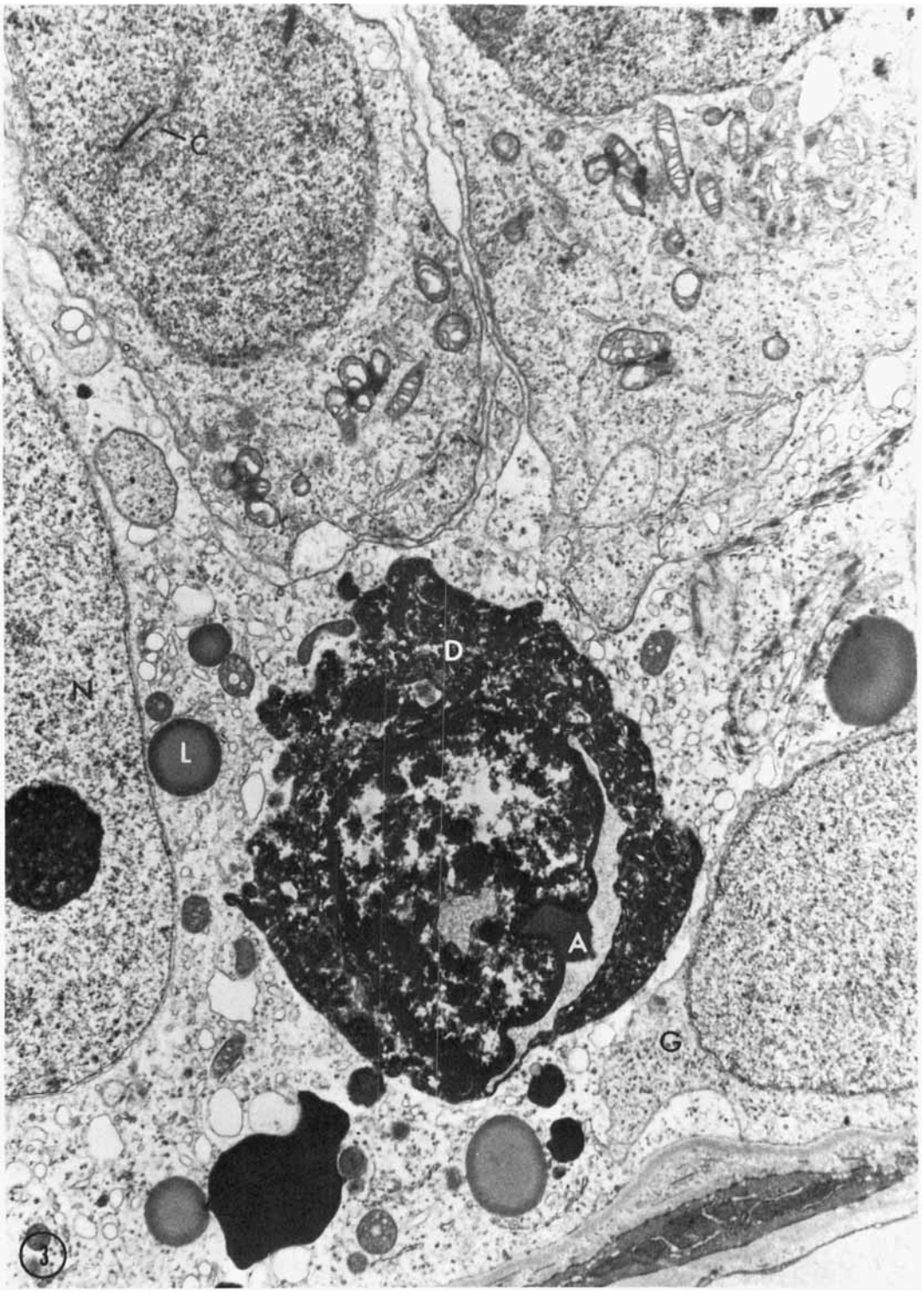

C
D
N
L
A
G

PLATE 3

EXPLANATION OF FIGURES

4 A degenerating, or necrotic, cell in the seminiferous epithelium of a rat treated with cyproterone acetate for 12 weeks. The cell is abnormally dense and its organelles are disrupted. However, the round nucleus (N) and the cap-shaped acrosomal vacuole (A) can be identified and suggest that the cell is a late cap-phase spermatid. The degenerating cell is surrounded by Sertoli cell cytoplasm (C) and is bounded by a membrane (arrow). × 11,900.

5 Electron micrograph of a portion of a Sertoli cell of an animal treated for 12 weeks. Parts of spermatids lie within the Sertoli cell. Groups of coarse fibers indicate the presence of parts of the tail region (T). An elongated dense structure (N) resembles a remnant of the condensed nucleus of a late spermatid. × 16,800.

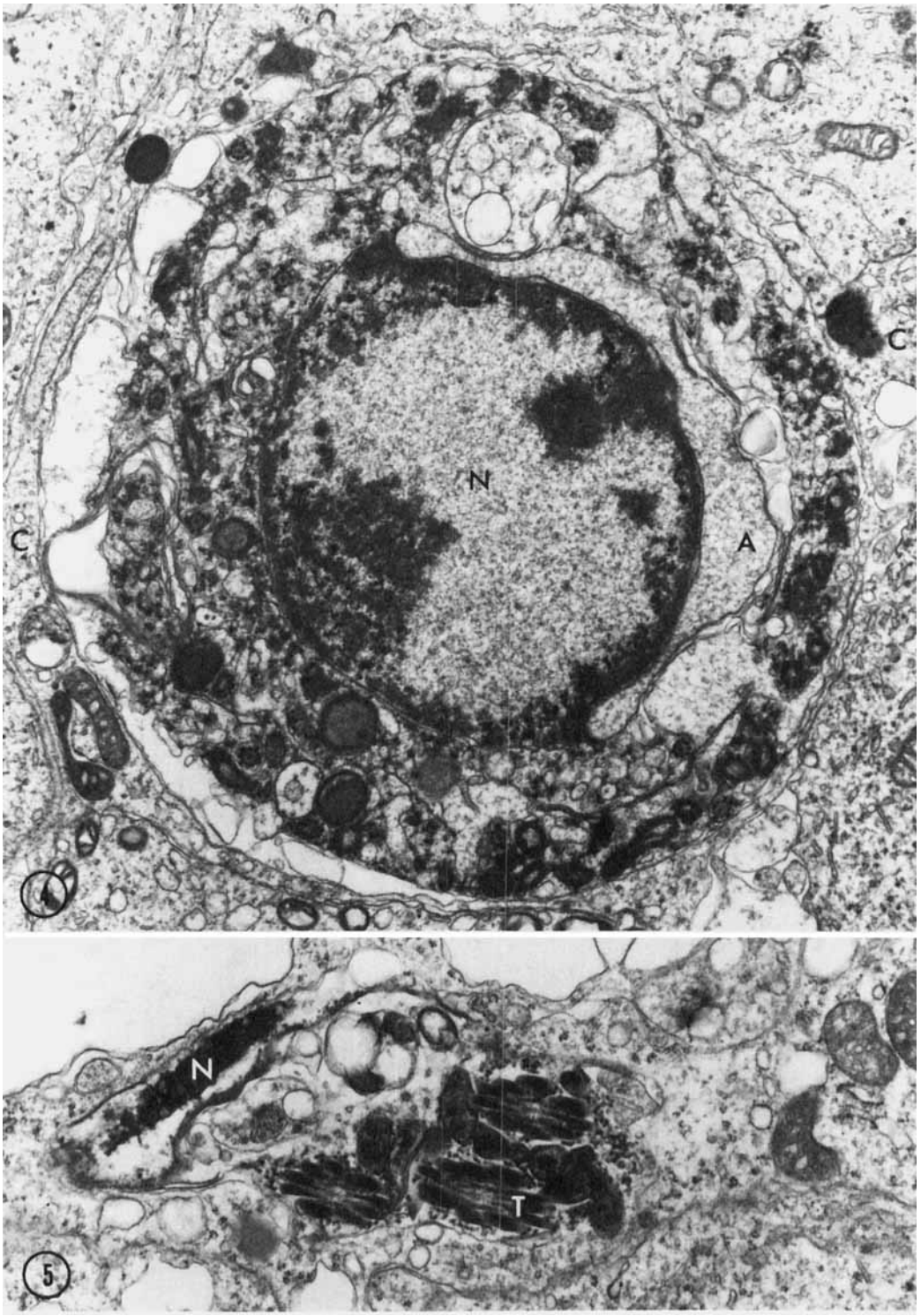
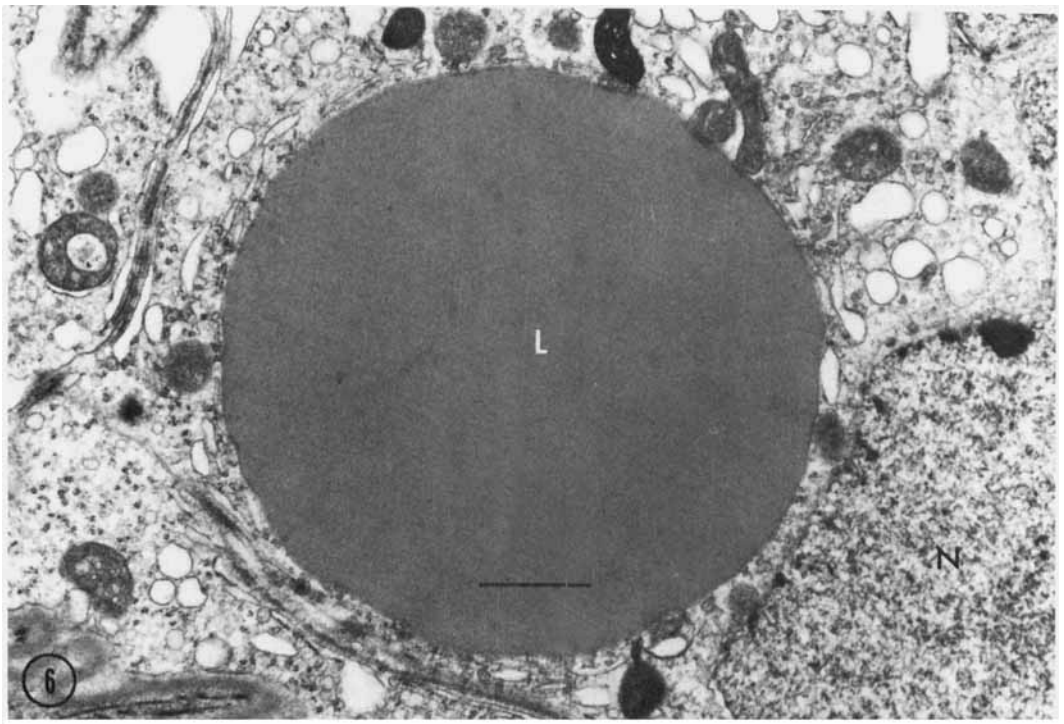

N
C
C
A
4
N
T
5

PLATE 4

EXPLANATION OF FIGURES

6 A large lipid droplet (L) in the cytoplasm of a Sertoli cell from a rat treated with cyproterone acetate for 12 weeks. Lipid droplets are very numerous in the Sertoli cells of treated animals. Some attain such a large size as to give the impression of displacing other organelles and indenting the nucleus (N). The length of the bar is 1 μm. $\times$ 13,700.

7 Low-power electron micrograph of Leydig cells from a rat treated with cyproterone acetate for 12 weeks. Measurements of Leydig cells in light microscopic preparations showed that in treated rats they were smaller than in normal animals. In the electron microscope, although the Leydig cells appeared small, they contained the normal organelles. E, smooth endoplasmic reticulum; M, mitochondria; N, nucleus. As in this micrograph, Leydig cells of normal, treated, and control rats usually appeared dense when fixed in the aldehyde mixture. $\times$ 13,500.


L
N
6

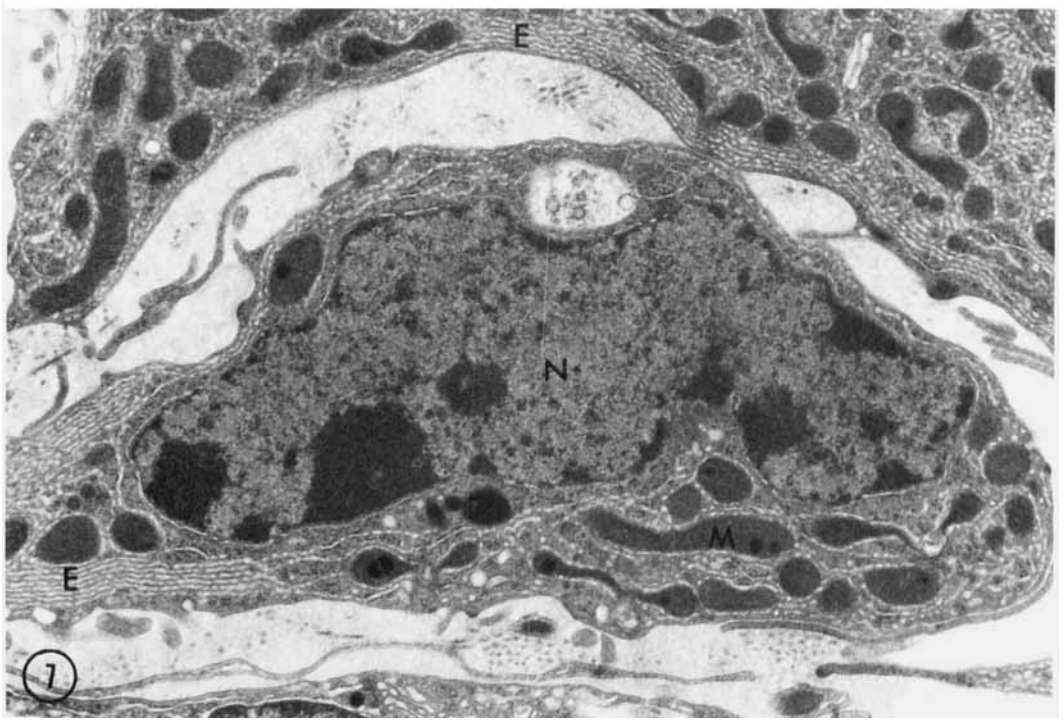

E
N
M
E
7

PLATE 5

EXPLANATION OF FIGURES

Figs. 8–11 Light micrographs of the caput and proximal cauda epididymidis of normal and treated rats.

8 The caput epididymidis of a normal rat. The columnar epithelium (E) encricles a lumen (L) that contains many sperm. ×275.

9 Caput epididymidis of a rat treated with cyproterone acetate for 12 weeks. The lumen (L) is virtually devoid of sperm. The epithelium is columnar, although it appears slightly shorter than in the case of the normal rat shown in figure 8. ×275.

10 Proximal part of the cauda epididymidis of a normal rat. The epithelium contains principal cells (P) and numerous light cells (arrows). Many sperm are present in the lumen (L). ×265.

11 Proximal part of the cauda epididymidis of a rat treated for 12 weeks with cyproterone acetate. Sperm are absent from the lumen (L), which is small and has a stellate outline. The epithelium is tall and its surface has an undulating contour. The pale-staining nuclei of the light cells (arrow) lie closer to the lumen than those of the principal cells, probably as the result of accumulation in the basal cytoplasm of dense bodies, visible in the electron microscope (fig. 12). ×265.

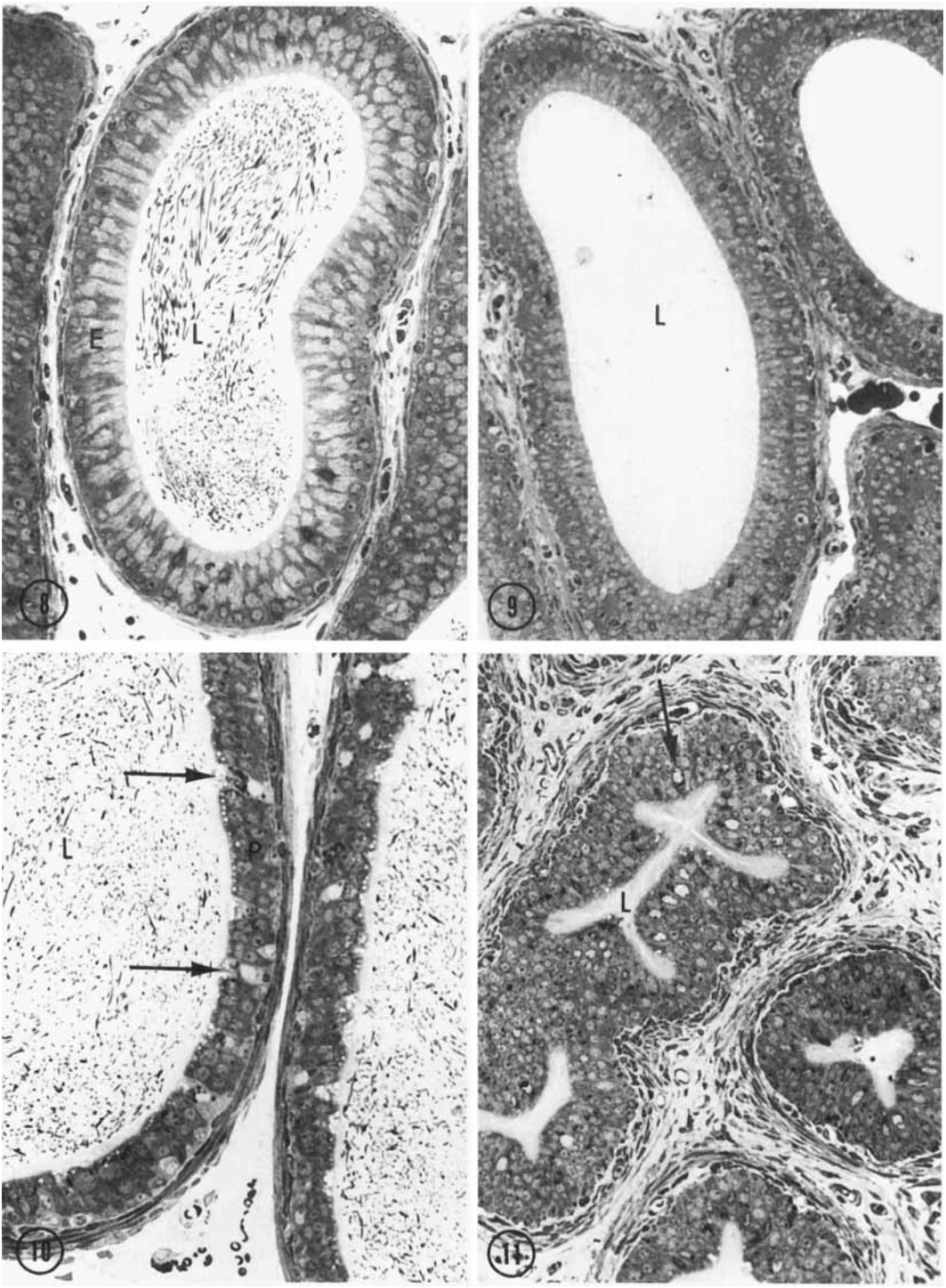

E
L
8
L
9
L
P
10
L
11

PLATE 6

EXPLANATION OF FIGURE

12 Low power electron micrograph of a portion of a light cell from the proximal cauda epididymidis of a rat treated with cyproterone acetate for eight weeks. The apical cytoplasm contains many vesicles and large vacuoles (V). Deeper in the cytoplasm, the content of the vacuoles becomes more dense and they are interspersed with dense bodies (D) that resemble lysosomes. The light cells in this part of the epididymis of treated rats were exceptionally distended with dense bodies, both in the supranuclear and basal cytoplasm. Some low magnification fields several times the size of this one were almost completely filled with dense bodies. N, nucleus; L, lumen. $\times$ 9,700.

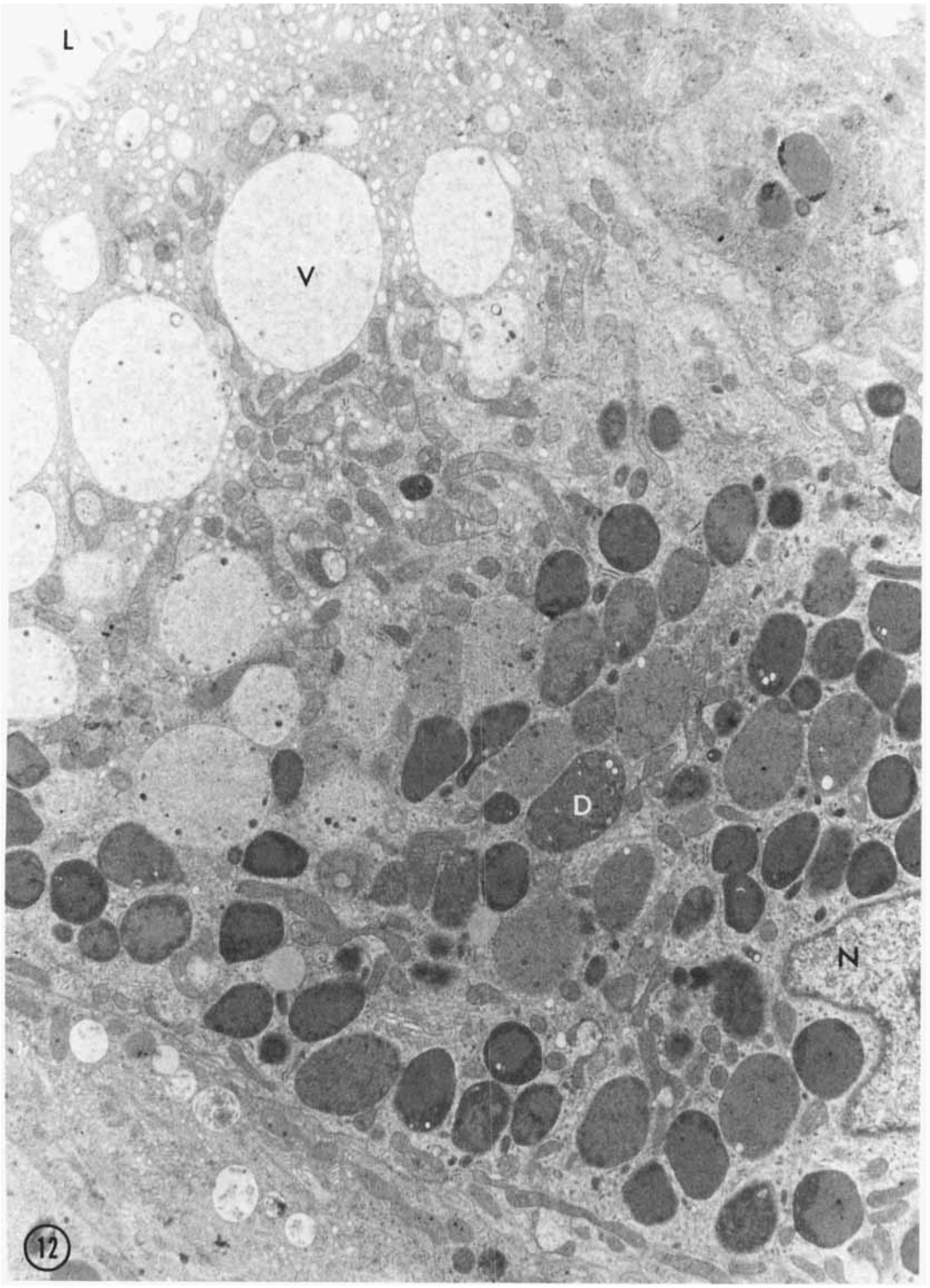

L
V
D
N
12

PLATE 7

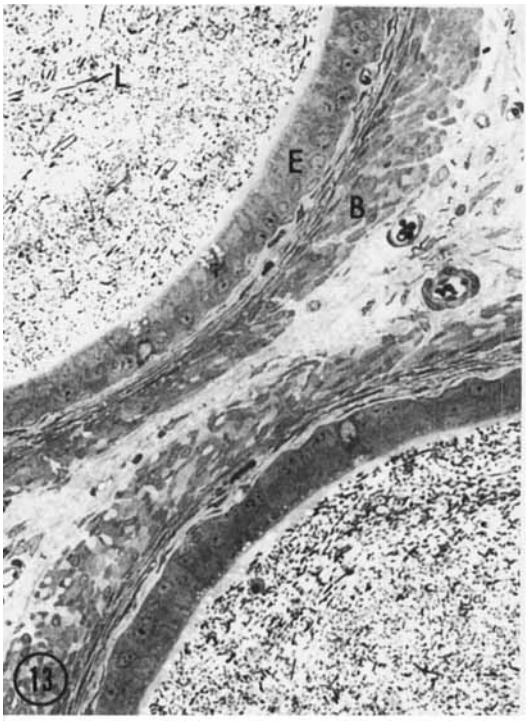
EXPLANATION OF FIGURES

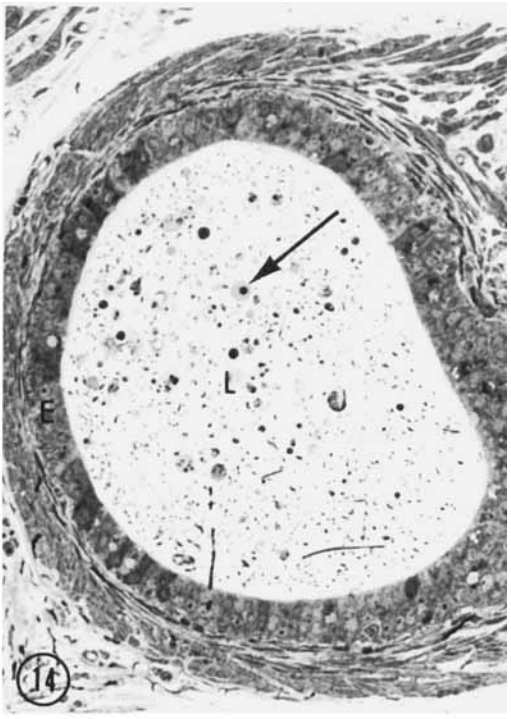
Figs. 13–15 Illustrations of the distal part of the cauda epididymidis in normal and treated rats.

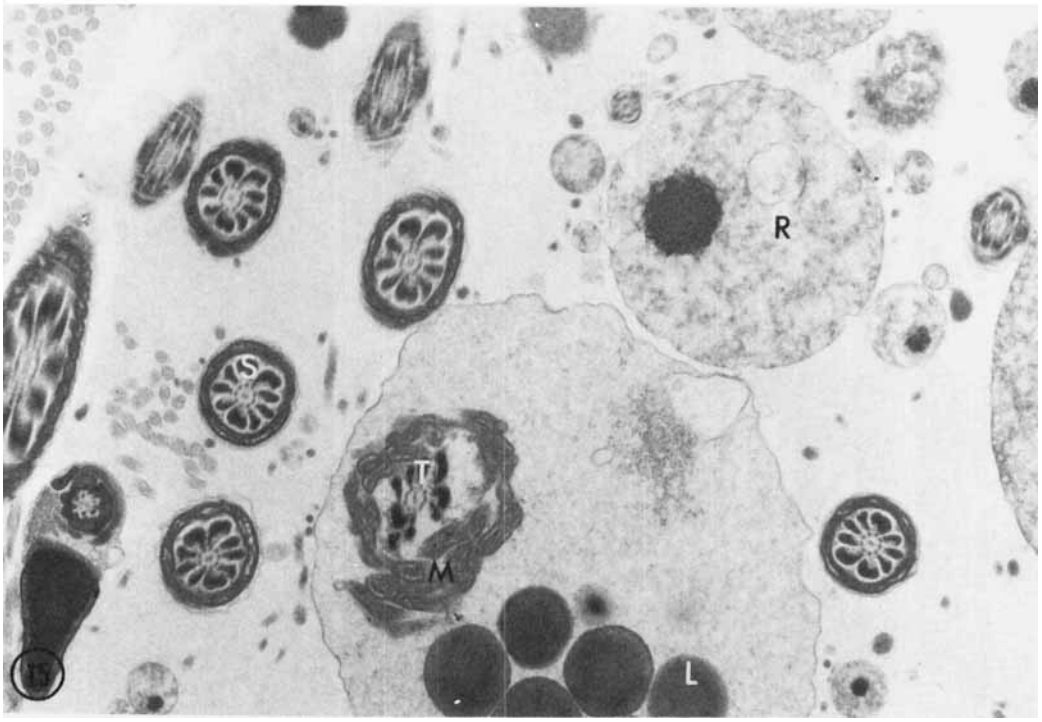
13 Light micrograph of the distal cauda epididymidis of a normal rat. The low columnar epithelium (E) lines a large lumen (L) that contains many sperm. A prominent layer of smooth muscle (B) surrounds the epithelium in this region. ×265.

14 Light micrograph of the distal part of the cauda epididymidis of a rat treated with cyproterone acetate for 12 weeks. The lumen (L) contains round structures (arrow), debris and sperm. It has a circular outline, being surrounded by a smoothly contoured low columnar epithelium (E). ×265.

15 An electron micrograph of some of the material in the lumen of the distal cauda epididymidis of a rat treated with cyproterone acetate. The numerous round bodies (R) appear to be masses of cytoplasm. Some contain lipid (L), mitochondria (M) and granular material. This example also contains parts of a sperm tail (T). Many sperm are also present. They appear relatively well-preserved in this specimen which was prepared after only four weeks' treatment. Others, especially at later intervals, had broken membranes and showed other evidence of disintegration. ×13,600.


L
E
B
13


L
E
14


R
S
T
M
L
15