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Effects of Experimental Cryptorchidism on the Ultrastructure and Function of the Sertoli Cell and Peritubular Tissue of the Rat Testis

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ABSTRACT

Within 7 days of the surgical induction of cryptorchidism, the Sertoli cells demonstrated an accumulation of lipid inclusions and dilatations of smooth endoplasmic reticulum. Aggregations of large vacuoles were observed at the basal aspects of the Sertoli cells and appeared to arise from local dilatations of the intercellular spaces between opposing inter-Sertoli cell junctions. These modifications of the inter-Sertoli cell junctional complexes disappeared as the cryptorchid state persisted, though some observations suggest that the associated membranes form complexly arranged bodies. The function of the Sertoli cells was altered in the cryptorchid testis as demonstrated by severe reduction in androgen binding protein (ABP) production by the 4 week cryptorchid testis and the lack of measureable ABP within the caput epididymidis. Serum FSH and LH levels became significantly elevated within 14 days of establishing cryptorchidism, suggesting diminished feedback from the damaged testis. Continuation of cryptorchidism was associated with progressive widening and folding of the peritubular tissue of the seminiferous tubule leading to bizarre arrangements of the tunica propria. The results are consistent with the proposal that in association with degeneration of the germ cells of the cryptorchid testis, the structure and function of the Sertoli cells are acutely sensitive to the raised intra-abdominal temperature.

INTRODUCTION

The failure of the testis to descend during fetal development from an intra-abdominal position into the scrotum occurs in a wide variety of mammals and is known as cryptorchidism.

Experimentally, cryptorchidism may be induced by surgical relocation of the testes into the abdominal cavity. In this manner the sensitivity of the seminiferous epithelium to elevated temperature has been extensively investigated in the guinea pig, rabbit and rat, each of which respond to experimental cryptorchidism by exhibiting infertility (Hart, 1922; Moore, 1922, 1924a,b; Fukui, 1923; Moore and Quick, 1924).

Although the changes in the morphology of the germ cells following short term cryptorchidism have been investigated for the rabbit and rat testis (Leeson and Leeson, 1970; Plöen, 1972, 1973a,b; Saba et al., 1972), similar studies of Sertoli cell morphology within the cryptorchid testis have received little attention.

The function of the Sertoli cells of the normal testis has been recently reviewed (Fawcett, 1975) and it has been demonstrated that in response to gonadotropic stimulation the Sertoli cells produce androgen binding protein (Hansson, 1975). Recently Hagenäs and Ritzén (1976) have shown that ABP production, used as a marker of Sertoli cell function, was impaired following artificially induced cryptorchidism in the rat. The present paper reports the changes in the morphology of the seminiferous epithelium following experimentally induced cryptorchidism in the rat with specific reference to the Sertoli cells. These changes are related to the production of ABP by the Sertoli cells and to the levels of serum follicle stimulating hormone (FSH) and luteinizing hormone (LH).

MATERIALS AND METHODS

Surgical Procedures

Thirty adult male Sprague-Dawley rats were

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anesthetized with ether and the testes exposed through an incision above the inguinal canal. The epididymal fat pad was grasped with blunt forceps, care being taken to avoid damage to the testicular artery, and the testes were transferred through the inguinal canal into the abdominal cavity ∿4 cm above the normal scrotal position. The inguinal canal was closed by sutures to prevent descent of the testis into the scrotum.

Fixation and Microscopy

At intervals of 5 and 7 days, 4 and 7 weeks and 3 months following the induction of cryptorchidism, the testes of 3 rats were fixed by perfusion. Under ether anesthesia, the testes of these animals were fixed either by whole body perfusion via the ascending aorta or by cannulation of the testicular artery, fixation being performed using a mixture of 5% glutaraldehyde, 4% formaldehyde and 0.05% 2,4,6-trinitrocresol, buffered to pH 7.4 with 0.2 M sodium cacodylate (Kerr and de Kretser, 1975). Small pieces of testicular tissue were postfixed in cacodylate buffered 2% osmium tetroxide and after dehydration in a graded series of alcohols, were embedded in a 1:1 mixture of Epon and araldite. For light microscopy, sections 0.5–1 μm thick were stained with toluidine blue and photographed with a Leitz Ortholux or Orthoplan microscope using monochromatic light of 550 nm. Observations of ultrastructure were recorded from thin silver sections cut with a Reichert OMU3 ultramicrotome using glass and diamond knives, stained with lead citrate and examined with an Hitachi HU-11E or JEOL 100S electron microscope.

Radioimmunoassay

Twenty-four rats 100 days of age were rendered experimentally cryptorchid and 30 rats of the same age were used as sham operated controls. At the commencement of the study, a group of 6 intact control animals was killed by decapitation between 0830–0900 h and blood was collected from each animal. Subsequent to this on Days 7, 14, 21 and 28, 6 control and 6 experimentally cryptorchid rats were sacrificed in an identical manner and blood was collected. Serum was separated from all blood samples and stored at −20°C until assay. FSH and LH were measured by specific double antibody radioimmunoassays whose characteristics have been previously described (Lee et al., 1975). Intrassay precision ranged from 4–9% and all samples from the entire study were measured in the same assay.

Measurement of Androgen Binding Protein

Testes and caput epididymides from normal and 4 week experimentally cryptorchid rats were dissected free of connective tissue and fat and weighed. Tissues from 8 animals in each group were pooled and ABP was measured in 105,000 × g supernatants by steady state polyacrylamide gel electrophoresis as previously described (Ritzén et al., 1974).

RESULTS

Seminiferous Epithelium

Five days after the induction of cryptorchidism obvious disruption of the seminiferous epithelium was noted in comparison to the histological appearance of testes of normal rats (Figs. 1, 2). Some seminiferous tubules within the cryptorchid testis exhibited a reduction in height of the epithelium associated with the loss of maturing spermatids. Round spermatids were aggregated into large multinucleated giant cells situated close to the lumen of the seminiferous tubules and these inclusions were readily observed within the cryptorchid testes when they were examined by light microscopy and compared with normal testicular tissue. These dramatic alterations to the structure of the seminiferous epithelium following cryptorchidism were indicative of early degeneration of spermatogenic function. However, not every seminiferous tubule showed the same degree of epithelial disruption, thereby causing a patchy histological appearance.

The Sertoli cells within the 5 day cryptorchid testis also appeared strikingly altered and when examined by light microscopy many of them contained large numbers of clear vacuoles of various diameters (Fig. 3), a phenomenon not encountered within the normal seminiferous epithelium following fixation by perfusion. Ultrastructurally these vacuoles were basally aggregated within the seminiferous epithelium and appeared to be localized dilatations of the intercellular spaces found between opposing surfaces of inter-Sertoli cell junctions. The dilatations were arranged in a sequential series along the pathway of each inter-Sertoli cell junctional complex (Fig. 4). At higher magnification, the smaller vacuoles (1–4 μm diameter) were seen to be bounded by a single membrane and appeared to be expansions of the intercellular spaces between opposing Sertoli cell membranes (inset Fig. 4). Larger vacuoles (5–10 μm diameter) were often surrounded by an additional double-membrane bound layer bearing occasional electron dense particles (Fig. 5). Electron dense material was observed between the limiting single membrane of these vacuoles and the closely adjacent cisternal membrane.

After 7 days of experimental cryptorchidism, numerous seminiferous tubules were largely depleted of germ cells with the remaining Sertoli cells exhibiting great numbers of lipid

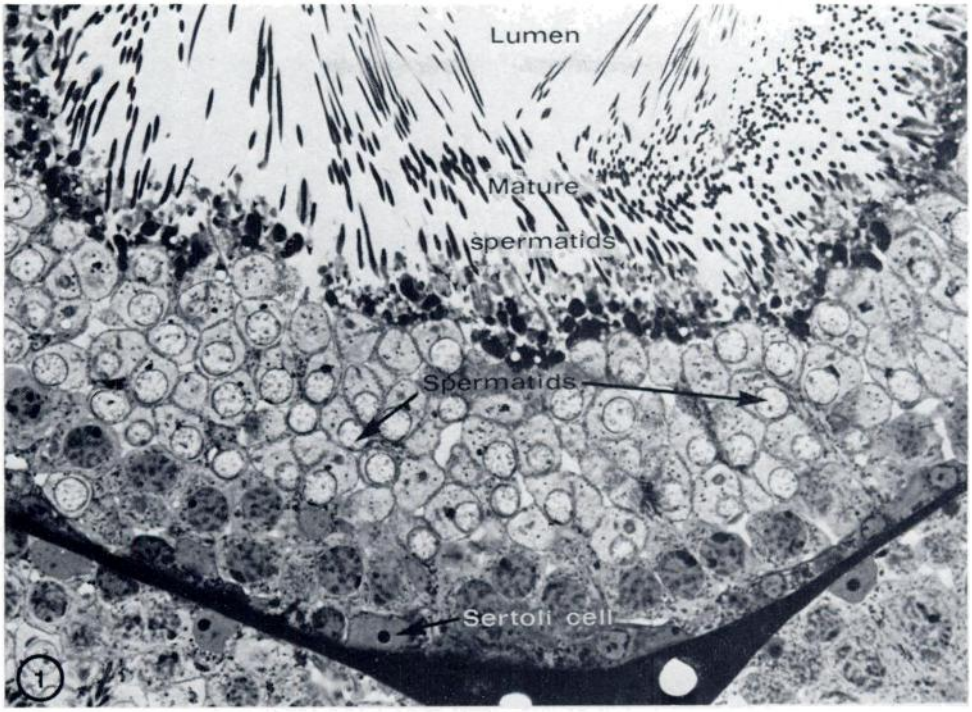


FIG. 1. Light micrograph of the normal rat seminiferous epithelium at stage 8 of the spermatogenic cycle, illustrating the release of mature spermatids into the lumen of the seminiferous tubule. A principal feature of the epithelium is the close association of all cell types throughout the depth of the tissue. X 530.

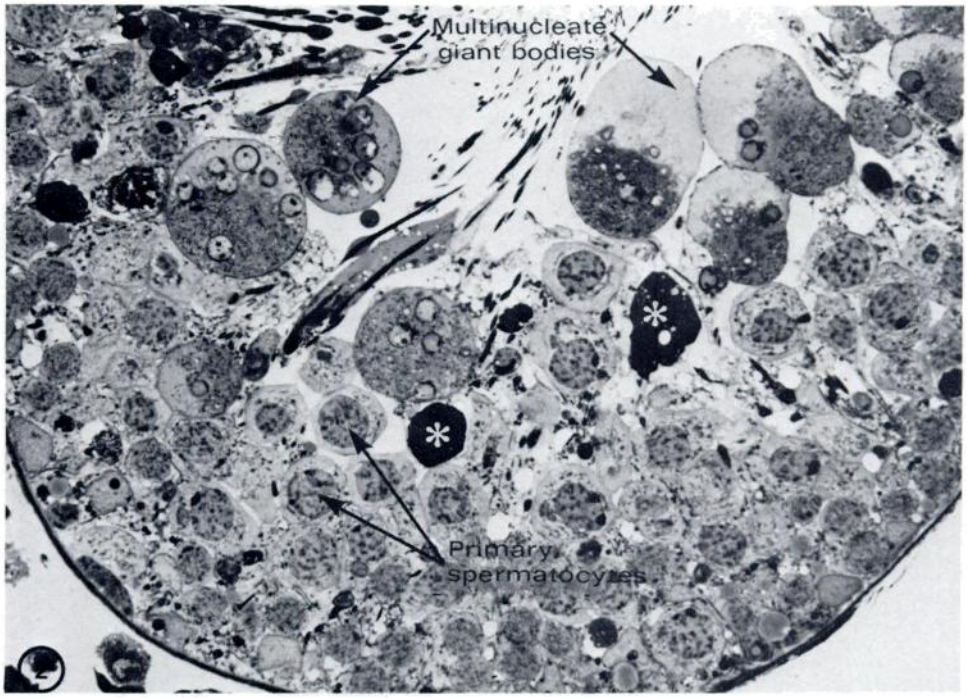


FIG. 2. Light micrograph illustrating abnormal spermiogenesis within the 5 day cryptorchid testis. Dense irregular condensations (asterisks) and aggregations of round spermatids into multinucleate giant bodies (arrows) are noted. X 530.

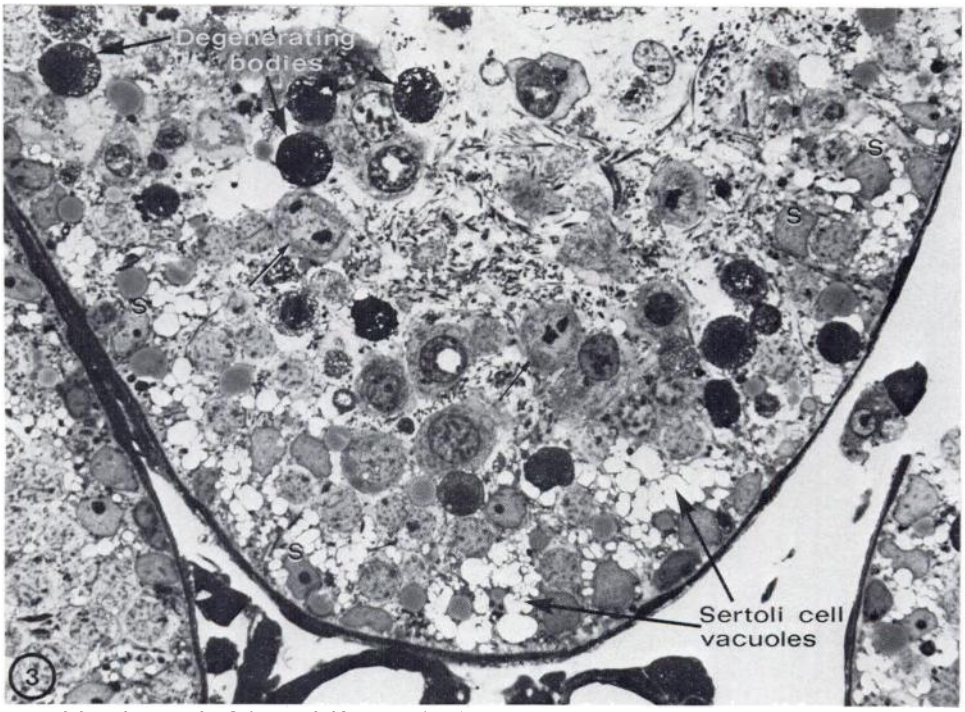


FIG. 3. Light micrograph of the seminiferous epithelium of the 5 day cryptorchid testis at stage 13 or 14 of the spermatogenic cycle as judged by the presence of large primary spermatocytes in the first meiotic division (arrows). The basal cytoplasm of each Sertoli cell exhibits many closely packed clear vacuoles. Nuclei of Sertoli cells (S) are also shown. X 530.

inclusions (Fig. 6). Ultrastructurally the Sertoli cells contained small dilated vesicles of smooth endoplasmic reticulum, very large lipid inclusions and numerous small membrane bound vacuoles, 0.5–2 μm in diameter (Fig. 7). These features of the Sertoli cells in the experimentally cryptorchid seminiferous tubules were a dramatic departure from the usual ultrastructural characteristics seen in Sertoli cells from the normal rat testis (Fig. 8) which contain small anastomotic tubules of smooth endoplasmic reticulum, numerous mitochondria and prominent inter-Sertoli cell junctions.

The numbers of remaining spermatogonia seen after 4–7 weeks of cryptorchidism were few and, in conjunction with the loss of the germ cells from the seminiferous epithelium, the Sertoli cells became more closely packed. After 4, 7 and 12 weeks of artificial cryptorchidism, the ultrasturctural features of the Sertoli cells were similar in that the nuclei were pleomorphic and lobulated and contained small peripheral chromatin clumps; the slender cytoplasmic processes of the Sertoli cells showed remarkable interdigitations with adjacent Sertoli cells (Fig. 9).

The many vacuoles within the Sertoli cell cytoplasm which had been a striking feature of the seminiferous tubules at 5 and 7 days of cryptorchidism were not observed after 4 weeks. Occasionally the interface between adjacent Sertoli cells exhibited unusual configurations of regularly aligned parallel arrays of smooth membranes which appeared to gain attachment to the plasma membranes of neighboring Sertoli cells (Fig. 12). Collections of these parallel arrays of membranes were also observed in the basal aspect of the Sertoli cell cytoplasm, where they took attachment from the junctional complex between adjacent Sertoli cells (Fig. 13). At higher magnification these membranous bodies consisted of alternate layers of double-membrane smooth cisternae between which were seen bundles of electron-dense filaments. Occasionally the terminal segments of cisternae showed attached ribosomal type particles (Fig. 14).

Tunica Propria

Accompanying the marked structural changes to the seminiferous epithelium, the boundary

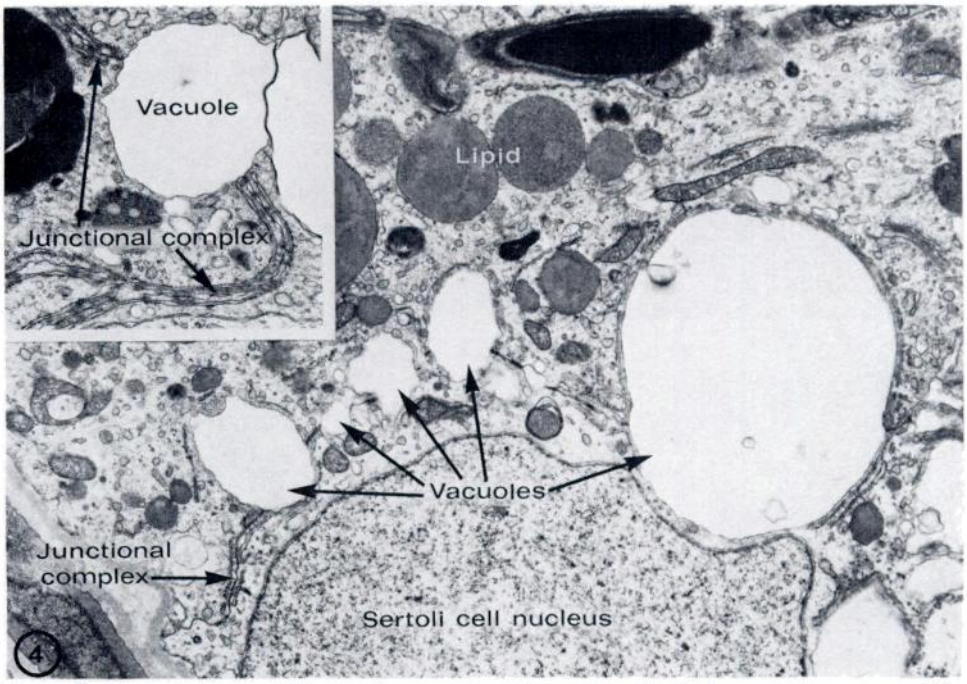


FIG. 4. Electron micrograph of the basal aspect of 2 adjacent Sertoli cells from a 5 day cryptorchid testis. A junctional complex is present at the interface between apposing cytoplasmic surfaces of each Sertoli cell; arranged in sequence in relation to the inter-Sertoli cell junction are 5 dilatations corresponding to the vacuoles seen with the light microscope. Inset: Higher magnification of an inter-Sertoli cell junctional complex illustrating expansion of the inter-cellular space between 2 Sertoli cells forming a single membrane bound vacuole. Note that the vacuole is connected on each side by intact profiles of junctional complexes. X 8200.

tissue of the seminiferous tubules exhibited remarkable changes in morphology. In the normal testis the tunica propria consists of the thin basement membrane adjacent to the seminiferous epithelium external to which the extracellular space contains a few collagen fibers in close proximation to the attenuated processes of the myoid cells, each cell being invested by a thin homogeneous band of basement membrane-type material. External to the myoid cells is a further extracellular space bearing collagen fibers, exterior to which is the parietal endothelium of the lymphatic sinusoids (Fig. 8).

As early as 7 days after cryptorchidism, the tunica propria is widened due to an increase in the width of the extracellular space which contains increased quantities of collagen; thickening of the basement membrane-type material lining the myoid cell surface is also observed. Marked folding of the basement membrane of the seminiferous tubule can also be seen (Fig. 10). With increased duration of the cryptorchid state, widening of the tunica propria is further increased and at times exceeds 20 μm. This is due to the bizarre folding of basement membrane together with remarkable convolutions of the myoid cells and their basement membrane-type lining. These glycoprotein layers become less closely apposed to the myoid cell surface and collagen accumulates within the widened extracellular spaces (Fig. 11).

Androgen Binding Protein

Rats which had been cryptorchid for 4 weeks had a mean testis weight of 0.64 g which was 39% of the weight of a testis from a normal adult rat (Table 1). Caput epididymal weight was also markedly decreased in the 4 week cryptorchid rats due to the absence of sperm in the lumen of the epididymis and possibly also due to a decrease in stimulation by androgens.

The concentration of ABP/mg protein in the testis of 4 week experimentally cryptorchid rats was similar to that measured in normal animals (Table 1). However, calculation of the total

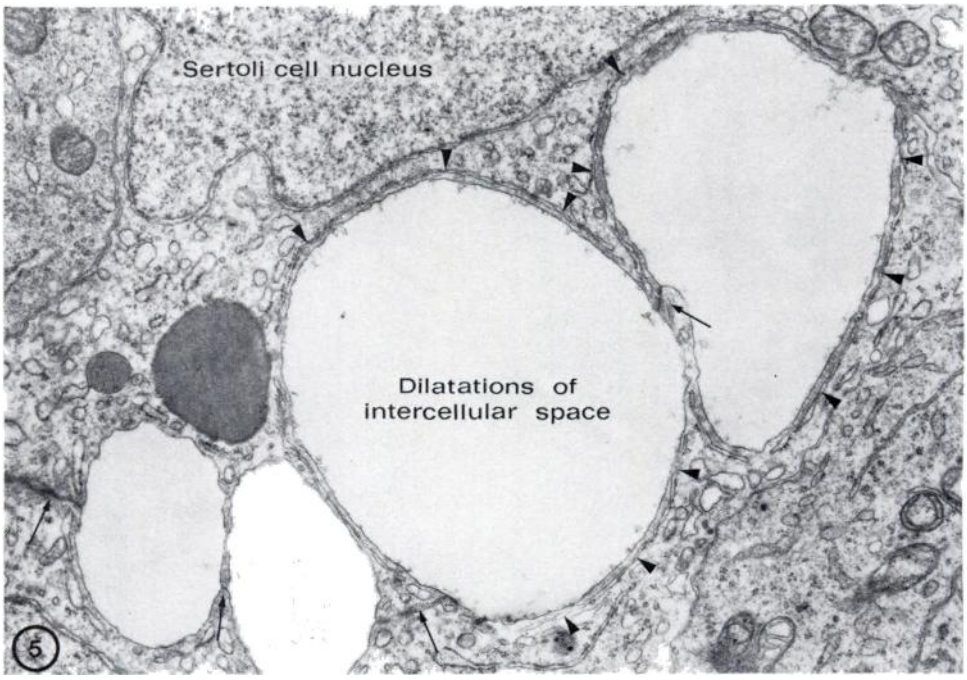


FIG. 5. Electron micrograph of the 5 day cryptorchid testis, illustrating a series of larger dilatations of the intercellular space associated with an inter-Sertoli cell junctional complex. The vacuoles are joined in sequence by very short electron dense membranes (arrows). The subsurface cisternae have been displaced laterally around the membrane bounded borders of each vacuole (arrowheads). Small particles, probably representing ribosomes, are attached to parts of the cisternal membrane. X 16,000.

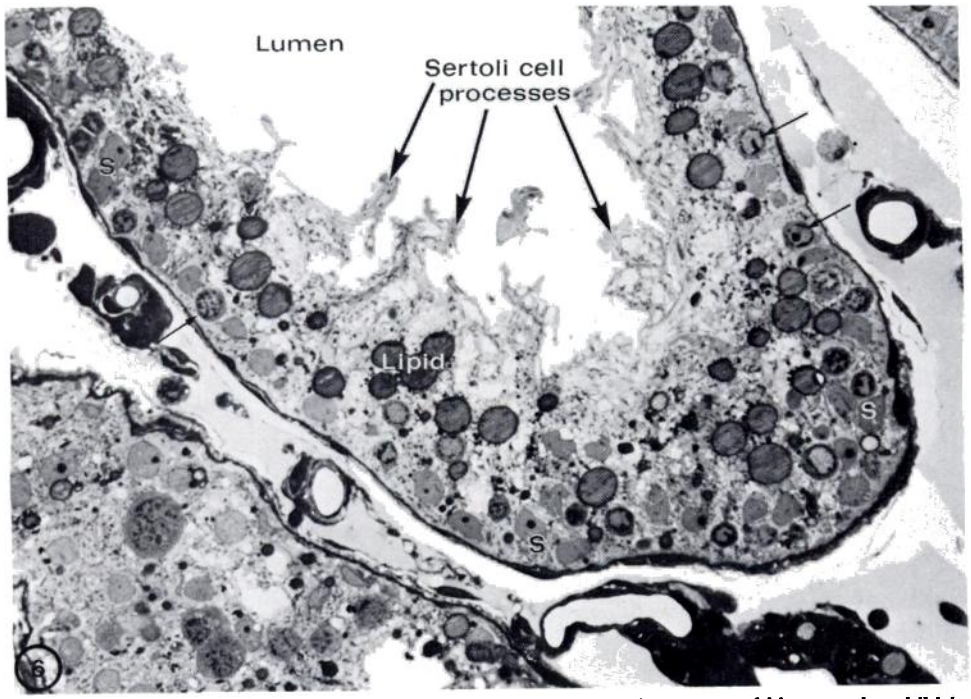


FIG. 6. Light micrograph of the seminiferous epithelium of the 7 day cryptorchid rat testis exhibiting the loss of most of the germ cells. The epithelium contains spermatogonia (arrows), Sertoli cells (S) with cytoplasmic extensions protruding into the lumen and a large number of dense lipid inclusions. X 420.

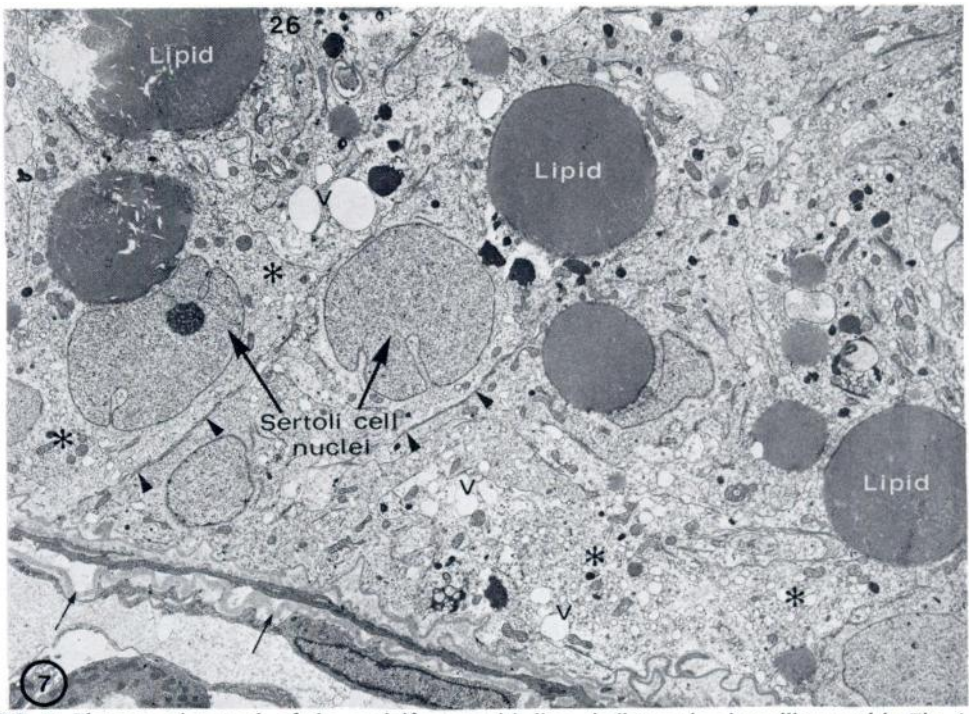


FIG. 7. Electron micrograph of the seminiferous epithelium similar to the tissue illustrated in Fig. 6. **The Sertoli cells contain large spherical lipid inclusions and small dilated vesicles of smooth endoplasmic reticulum (asterisks). Images resembling intact junctional complexes between Sertoli cells are observed (arrowheads). Numerous small vacuoles scattered throughout the basal aspect of the Sertoli cells are shown (V). Note the unusual folding of the glycoprotein layers (arrows) within the tunica propria. X 3100.**

amount of ABP/testis demonstrated that ABP content in the cryptorchid testis was only 29% of that in the normal testis. Increased amounts of an androgen binding component migrating with an Rf similar to that of albumin were noted following electrophoresis of testicular cytosols from the cryptorchid rats. The secretion and transport of ABP to the epididymis is reflected by its concentration in the caput. No ABP activity was detectable in the caput epididymis of the 4 week experimentally cryptorchid animals, suggesting a diminished production rate of ABP in the testis.

Gonadotropin Levels

Serum FSH levels were significantly elevated ($P<0.01$) in cryptorchid animals after 14 days of cryptorchidism (Table 2). Serum LH levels were transiently depressed in cryptorchid rats compared with control levels after 7 days of cryptorchidism ($P<0.05$), but as testicular failure proceeded, cryptorchid serum LH became significantly elevated ($P<0.01$) at and beyond Day 14 (Table 2).

DISCUSSION

This study has shown that induction of artificial cryptorchidism involves degeneration of the germ cells of the seminiferous epithelium and also causes significant changes in the structure and function of the Sertoli cells. Many of the ultrastructural changes which occur in the Sertoli cells as a result of cryptorchidism were observed in the 5 and 7 day cryptorchid testis, resulting in accumulation of lipid droplets and many vacuoles, changes in the morphology of the smooth endoplasmic reticulum and modifications to the structure of the inter-Sertoli cell tight junctions. These early morphological changes in the Sertoli cells are associated with physiological alterations leading to disordered Sertoli cell function as shown by a decrease in ABP production by the testis and the absence of detectable ABP in the caput epididymis of the 4 week cryptorchid testis compared with intact testes. The diminished capacity of the Sertoli cell to elaborate ABP confirms the recently reported results of Hagenas and Ritzen (1976) and indicates severe

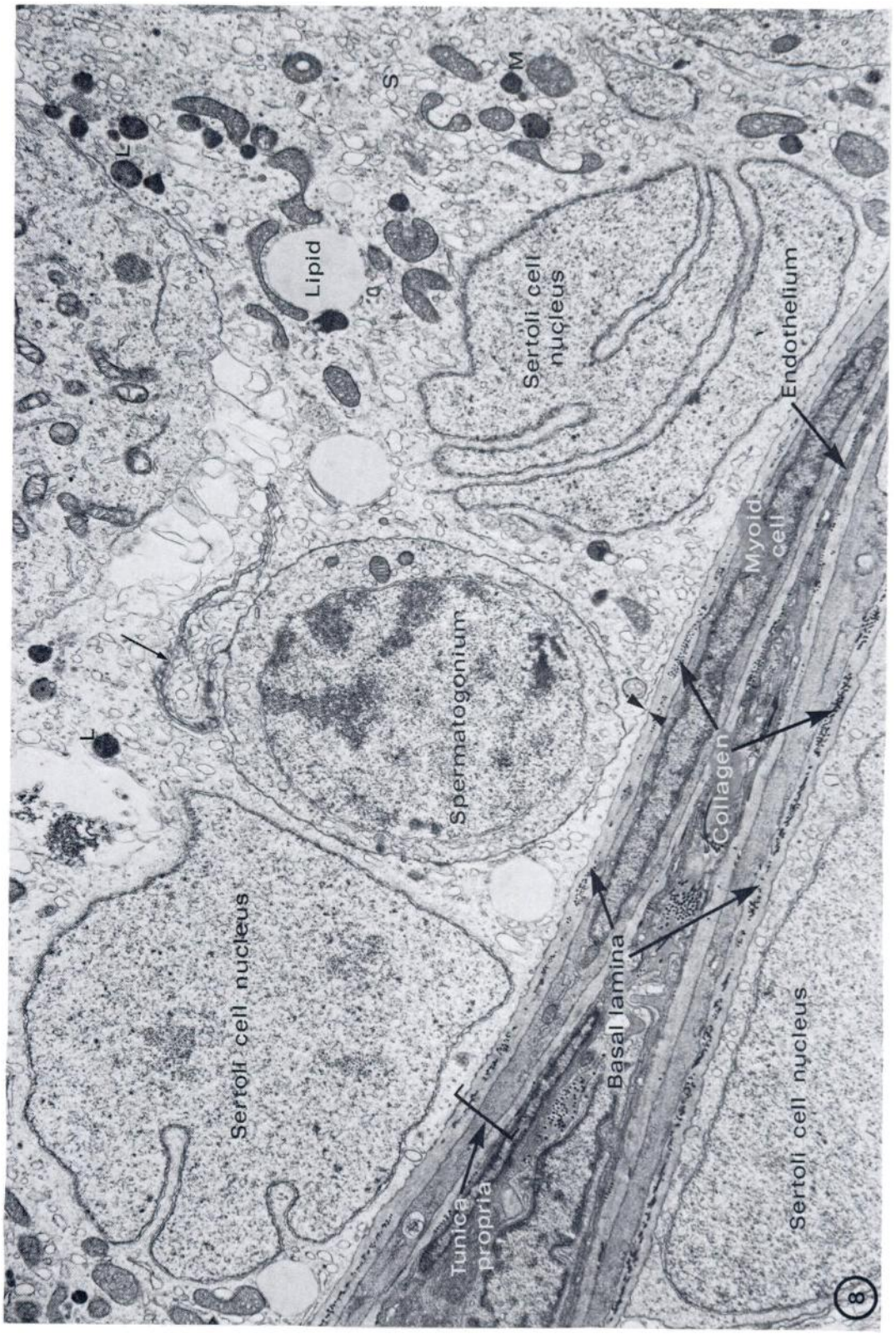
damage to Sertoli cell function in the cryptorchid state.

Although significant damage to the seminiferous epithelium is related to an elevation of serum FSH levels, our studies also show that accompanying the rapid breakdown of spermatogenesis, the Sertoli cells though present are also altered by cryptorchidism. The elevation of serum FSH levels in conjunction with cryptorchidism may be related to a depressed feedback signal from the seminiferous epithelium, perhaps elaborated by the Sertoli cells, a suggestion supported by the observation that despite profound losses of germinal cells, the serum FSH levels remained less than values in castrates (range 1800–3600 ng/ml) as previously reported by others (Amatayakul et al., 1971; Swerdloff et al., 1971; Altwein and Gittes, 1972, 1973; Walsh and Swerdloff, 1973). Recent studies by Rich and de Kretser (1977) have suggested that the feedback regulation of FSH secretion may be a function of the Sertoli cell since differing degrees of induced damage to the seminiferous epithelium of adult rats resulted in greatly diminished production of ABP and elevation of serum FSH levels. The possibility that the damaged Sertoli cell is responsible for the diminished feedback signal which regulates the secretion of FSH is further supported by the demonstration that media from the culture of rat Sertoli cells selectively inhibit the release of FSH from cultured pituitary cells (Steinberger and Steinberger, 1976).

The finding of significantly elevated levels of serum LH in association with experimental cryptorchidism may be due to the nonspecificity of the FSH feedback signal or perhaps due to an alteration of interstitial cell function. Kerr et al. (1979) have shown that in response to in vivo hCG stimulation, the secretion of testosterone by the cryptorchid testis is decreased in comparison to the normal testis. Recently Damber et al. (1978) have demonstrated that the concentration of testosterone in spermatic venous blood in the 14 week cryptorchid testis was less than half of the levels in blood draining from the intact testis. They also showed that the elaboration of testosterone from the spermatic vein in cryptorchidism was ∿13% of the measured testosterone outflow from the normal testis. Other work has demonstrated that the concentration of estradiol receptors, localized in the Leydig cells, was markedly increased in the cryptorchid rat testis (Abney et al., 1977) suggesting the possibility of an alteration in interstitial cell function which may in turn influence gonadotropin secretion.

The early phase of experimental cryptorchidism resulted in the occurrence of many vacuoles within the Sertoli cell cytoplasm, an observation often reported in association with abnormalities of spermatogenesis (Russell and Gardner, 1973; Ramamurthy and Fawcett, 1975). The origin of vacuolization of the Sertoli cell cytoplasm has previously received little attention, but in the present study it seemed that these vacuoles arose from 2 sources: dilation of the smooth endoplasmic reticulum and localized expansions of the narrow intercellular space between opposing inter-Sertoli cell junctions. The latter proposal is supported by the observation that the vacuoles were bounded by a single membrane and were concentrated in large numbers in the basal cytoplasmic area of the Sertoli cells, a position in which inter-Sertoli cell junctions are normally formed. Commonly 20 or 30 vacuoles were counted as individual dilatations of the inter-Sertoli cell space along a single junctional complex between adjacent Sertoli cells in the 5 or 7 day cryptorchid testis. As cryptorchidism persisted, the seminiferous tubules became progressively devoid of germ cells and the collapse and shrinkage of the tubules probably contributed to the close packing of the remaining Sertoli cells. The compacting of the cytoplasm of neighboring Sertoli cells may have been partly responsible for the subsequent disappearance of the vacuoles; possibly they became rearranged into the parallel arrays of cisternae seen within the Sertoli cell cytoplasm after 4 weeks of cryptorchidism. The origin of the Sertoli cell cytoplasmic vacuoles and their

FIG. 8. Electron micrograph of Sertoli cells of a normal rat testis. Sertoli cells rest upon the basal lamina of the seminiferous tubule, their nuclei being large, deeply indented and positioned in the basal cytoplasm of the cell. The apposed surfaces of adjacent Sertoli cells are characterized by junctional complexes (arrow), although a small area of interface between the Sertoli cells appears to be bound by a single membrane (arrowheads). Small lipid inclusions, mitochondria (M), smooth endoplasmic reticulum (S) and lysosomes (L) are present in Sertoli cell cytoplasm. A spermatogonium is also shown. The peritubular tissue also is illustrated. ×7500.



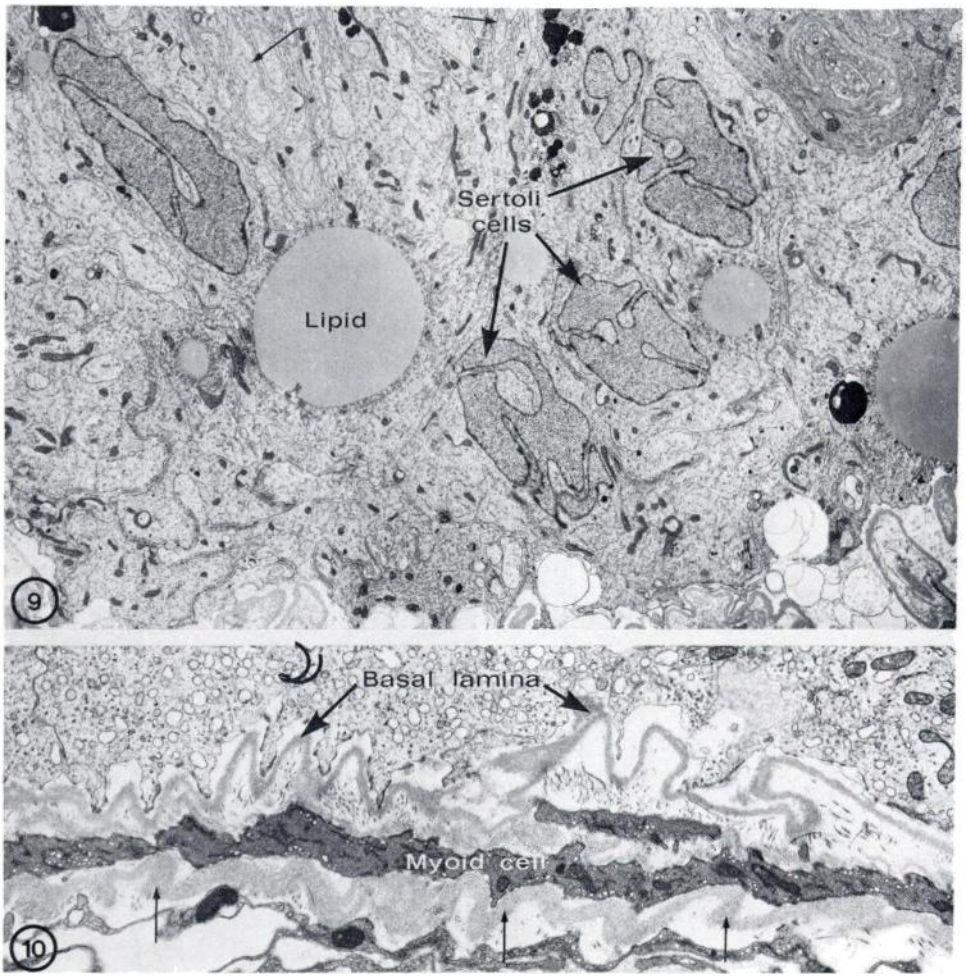


FIG. 9. Electron micrograph of Sertoli cells of the 4 week cryptorchid rat testis, illustrating the bizarre configurations of Sertoli cell nuclei, large lipid inclusions in the Sertoli cell cytoplasm and the complex interdigitations between the cytoplasmic extensions of the Sertoli cells (arrows). X 2700.

FIG. 10. Ultrastructure of the tunica propria of a seminiferous tubule after 7 days of cryptorchidism. Prominent folding and thickening of the basal lamina and glycoprotein coats of the myoid cells (arrows) are shown. X 6000.

proposed relationship to the cisternal complexes is illustrated diagrammatically in Fig. 15.

This sequence of changes may be the response of the Sertoli cell to rapid germ cell degeneration within the seminiferous epithelium which results in a rapid shrinkage of the seminiferous epithelium. The complex membranous arrays (Figs. 12, 13) are frequently seen close to the lumen of the tubule and may represent a "concertina" mechanism to allow the Sertoli cell to store redundant membrane prior to reutilization or degradation.

It is of interest that the large collections of vacuoles of this type were only of a transitory nature since they had disappeared by 4 weeks after the induction of cryptorchidism. The temporary morphological disruption of the inter-Sertoli cell junctions in the cryptorchid testis may imply that the permeability of the blood testis barrier is altered by the raised intra-abdominal temperature. This proposal is in keeping with the observations of Hagenas et al. (1977) who reported that in the 7–12 day cryptorchid rat testis, lanthanum tracer penetrated deeply through the inter-Sertoli cell junctions for distances exceeding 20 μm from the basal lamina of the seminiferous tubule. It is clearly necessary to obtain evidence concern-

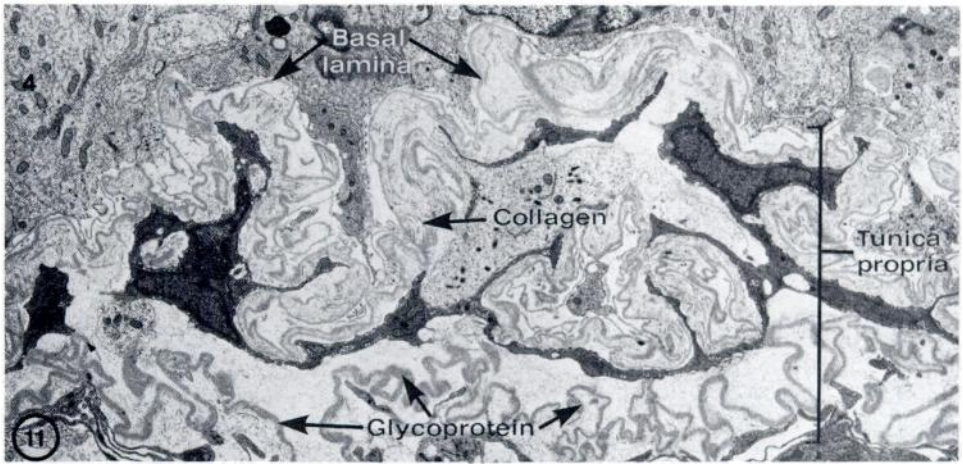


FIG. 11. Ultrastructure of the tunica propria after 3 months of cryptorchidism, showing the complex arrangement of peritubular cells. Note the expanded width of the tunica propria in comparison to the normal and 7 day cryptorchid testis. X 3300.

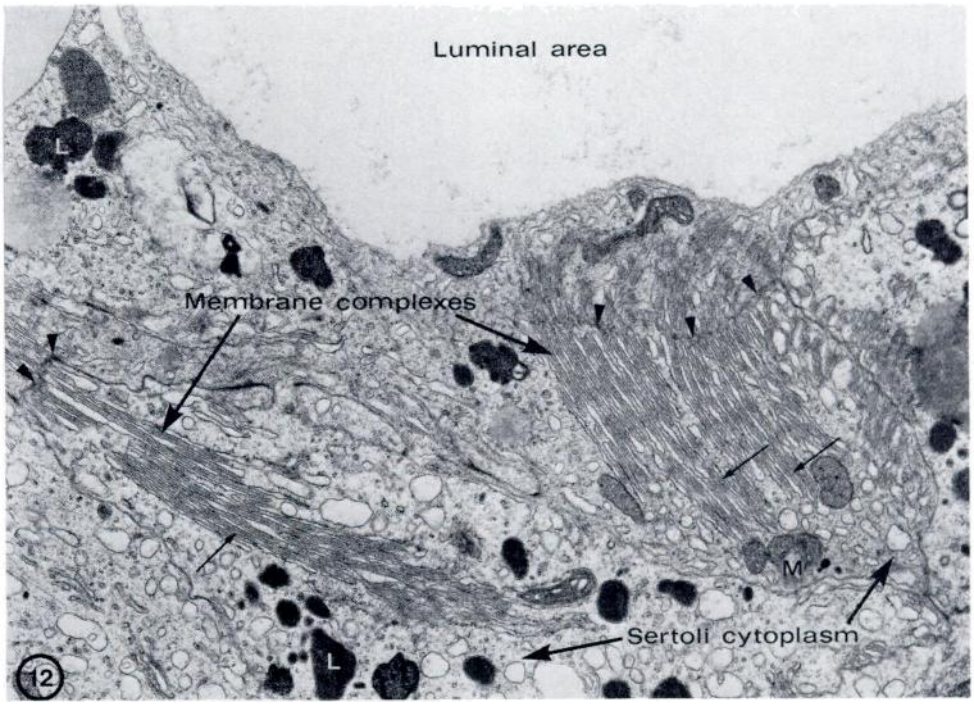


FIG. 12. Electron micrograph of the adluminal area of a seminiferous tubule of a 4 week cryptorchid testis. Arising from a central electron dense pathway (arrowheads) within Sertoli cell cytoplasm are 2 separate collections of parallel arrays of smooth membranes which project into the surrounding cytoplasm. Dense material is localized between the individual elements of the membranous complex (arrows). Mitochondria (M) and lysosomal-type bodies (L) are seen within the Sertoli cell cytoplasm. X 12,000.

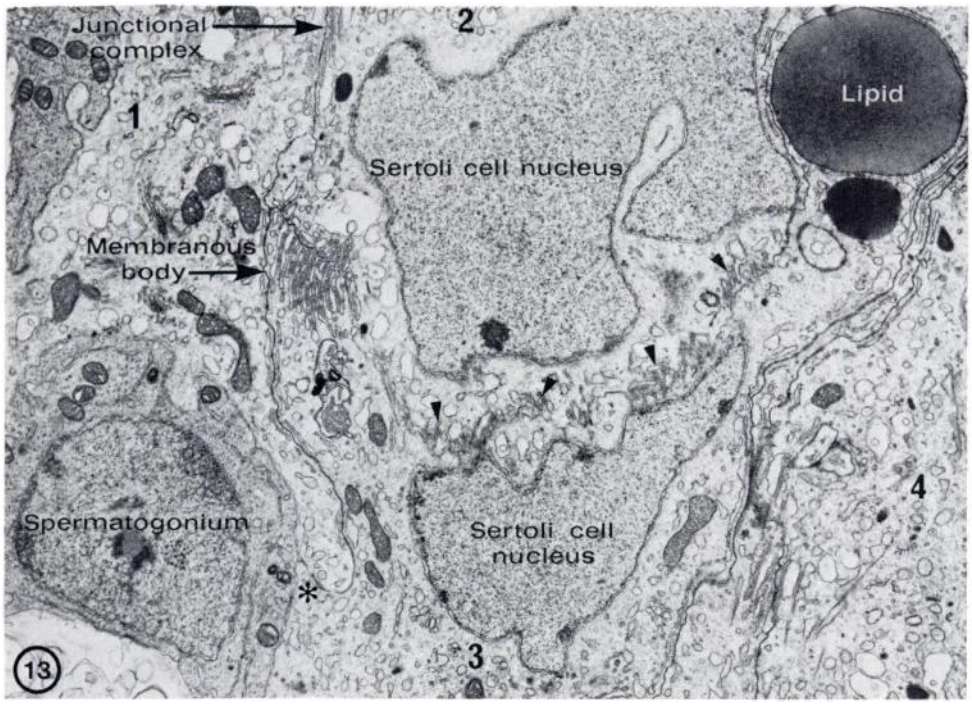


FIG. 13. Electron micrograph of the basal aspect of 4 Sertoli cells (marked 1, 2, 3, 4) of a 4 week cryptorchid testis. An inter-Sertoli cell junction arises from the surface of a spermatogonium (asterisk) and exhibits an aggregation of flattened saccules resembling cisternae of smooth endoplasmic reticulum. Beyond the membranous body, the inter-Sertoli cell junction appears to reform. A collection of small vesicles and membranes (arrowheads) is also seen traversing the cytoplasmic space between 2 Sertoli cell nuclei. X 6500.

ing the integrity of the inter-Sertoli cell junctions 5–10 days after inducing cryptorchidism when vacuolization is in progress.

Progressive folding and widening of the peritubular tissue of the cryptorchid testis was observed in this study confirming and extending earlier reports (Leeson and Leeson, 1970; Saba et al., 1972). Similar responses in the morphology of the peritubular tissue have been reported in numerous forms of testicular damage (Lacy and Rotblat, 1960; Idänpaanheikkila, 1966; Leeson, 1966; Collins and Lacy, 1969; Kaya and Harrison, 1975). The distortion of the peritubular tissue in cryptorchidism may be a result of accomodation to the progressive decrease in diameter of the seminiferous tubules, necessitating the contraction of the peritubular tissue within a smaller area than is normal.

Other work suggests that the organization of the peritubular tissue is influenced by hormonal stimulation since peritubular fibrosis often occurs within the pathological human testis of oligospermic or azoospermic patients (Bustos-Obregón and Holstein, 1973; Bustos-Obregón, 1974; de Kretser et al., 1975) and abnormalities of pituitary secretion are commonly associated with these conditions. In those cases where it has been shown that disorganization and widening of the peritubular tissue occurs in conjunction with the withdrawal of gonadotropic stimulation, such as is found after hypophysectomy or artificial regulation of photoperiod (Ross and Grant, 1968; Vilar, 1968; Berndston and Desjardins, 1974), it is not clear whether the thickening of the peritubular tissue is a result of hormonal change or is due to the physical collapse of the seminiferous tubules.

The functional significance of the excessive fibrosis of the peritubular tissue in experimental cryptorchidism is not understood and although the structure and physiological properties of the peritubular tissue probably respond to the hormonal milieu within the damaged testis, no generalizations concerning the factors which govern the role of the peritubular tissue in the cryptorchid state can be offered as yet.

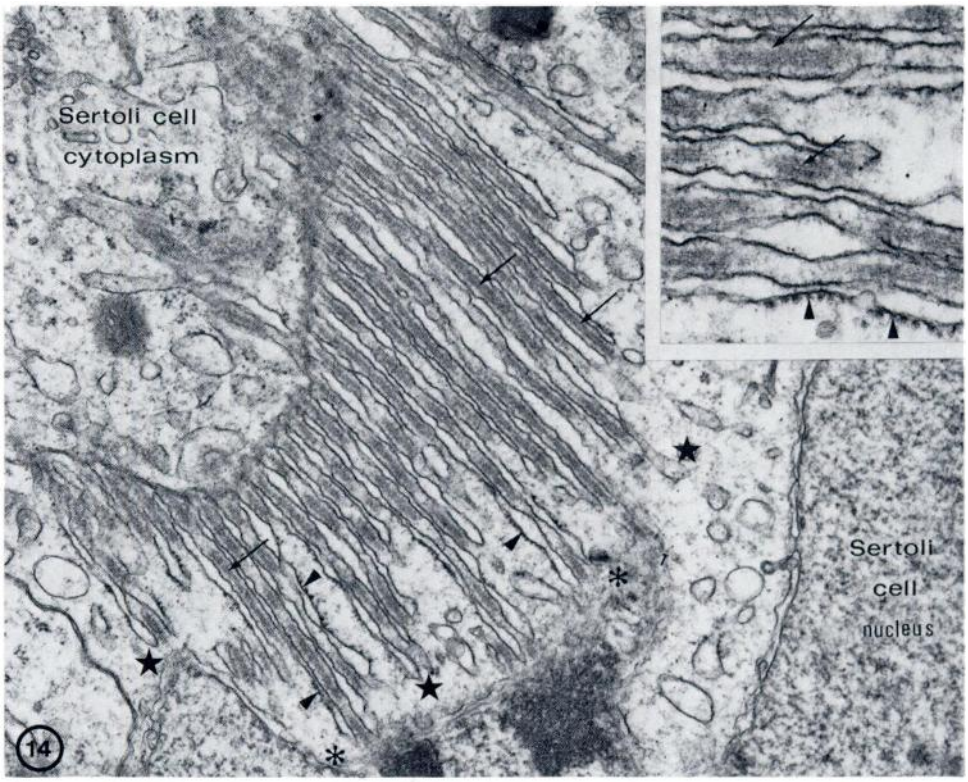


FIG. 14. Electron micrograph of a membranous complex within a Sertoli cell of a 4 week cryptorchid testis. Saccules are of 2 types, some consisting of smooth membranes and others bearing small electron dense particles (arrowheads) resembling ribosomes, which are commonly attached upon only one surface of the saccule. The extremities of the saccules are occasionally closed (stars) or extend to the nuclear membrane of the Sertoli cell nucleus (asterisk) where they appear indistinct. Electron dense matter between each saccule (arrows) is suggestive of bundles of filaments. X25,000. Inset: Bundles of filaments (arrows) are identified between individual saccules, one of which bears ribosomal-type particles upon one surface (arrowheads). X80,000.

TABLE 1. Effect of cryptorchidism on levels of ABP and testicular and caput epididymal weight.[a]

	Testis			Epididymis		
	Testis weight (g)	pmol/mg protein	pmol/ testis	Caput epididymal weight (mg)	pmol/mg protein	pmol/ caput
Normal adult	1.65 ± 0.06	0.40	16.71	178 ± 10	6.24	31.12
4 week cryptorchid	0.64 ± 0.02*	0.36	4.88*	97 ± 8*	ND	...

[a]Organ weights are expressed as mean ± SEM (n = 8) and ABP data as mean value of duplicate estimations of 105,000 × g supernatants perepared from tissues pooled from 8 animals. *P<0.001; ND, not detectable. Statistical difference between mean control and cryptorchid values was assessed using a 1-tailed t test.

TABLE 2. Response of serum FSH and LH to experimental cryptorchidism.[a]

	Serum FSH (NIAMDD-FSH RP1) (ng/ml)		Serum LH (NIAMDD-LH I1) (ng/ml)	
	Normal	Cryptorchid	Normal	Cryptorchid
Basal	255 ± 17	...	1.46 ± 0.3	...
Day 7	247 ± 13	342 ± 49	2.0 ± 0.2	1.33 ± 0.14*
Day 14	215 ± 11	528 ± 81**	1.32 ± 0.14	3.08 ± 0.5**
Day 21	230 ± 20	549 ± 52**	1.0 ± 0.21	3.3 ± 0.3**
Day 28	248 ± 15	464 ± 67**	1.1 ± 0.15	3.4 ± 0.6**

[a]Serum FSH and LH levels in intact and experimentally cryptorchid rats. The values for serum LH have previously been documented in Kerr et al. (1979). Values represent mean ± SEM. Statistical difference between mean control and cryptorchid values was assessed using the unpaired t test. *P<0.01; **P<0.01.

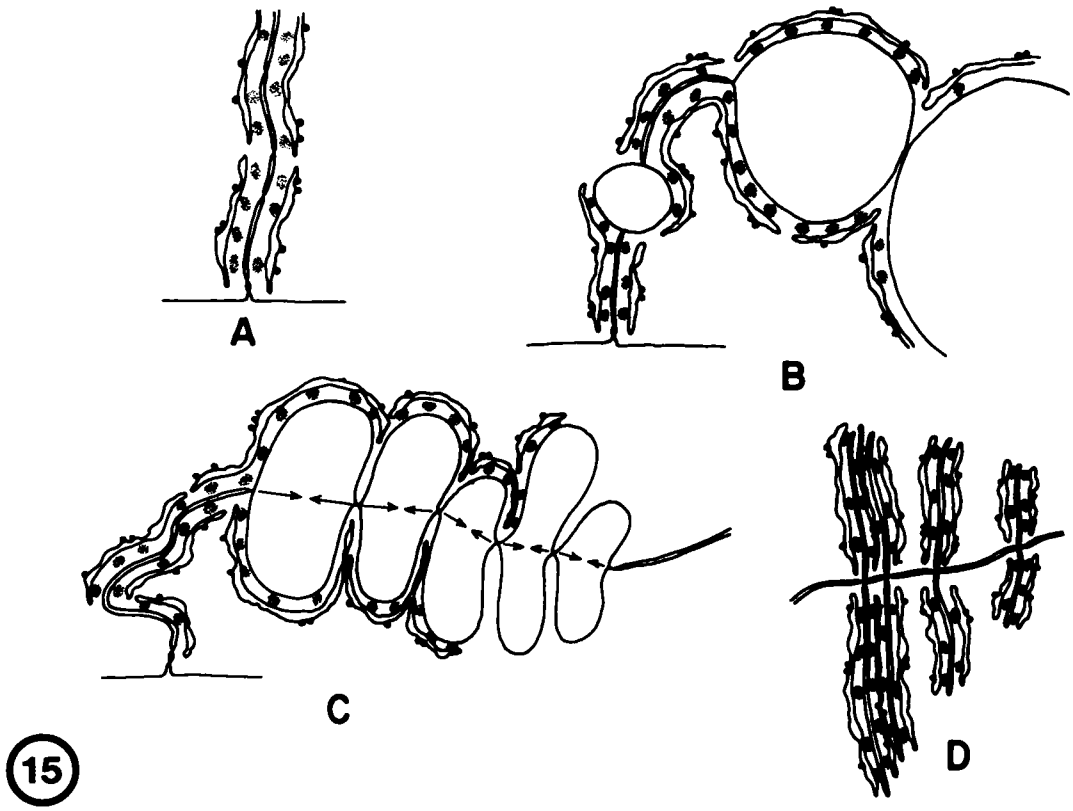


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FIG. 15. Diagrammatic representation of a proposed relationship between the appearance of vacuoles and complex membranous bodies associated with inter-Sertoli cell tight junctions. The hypothesis is based upon observations of the Sertoli cells as cryptorchidism persisted. A) An inter-Sertoli cell junctional complex within a normal testis. The intercellular space between adjacent Sertoli cells is frequently fused at intervals forming tight junctions. Cisternae of endoplasmic reticulum are separated from the cell surface by hexagonal groups of filaments. B) After 7 days of cryptorchidism, the intercellular junctions expand between the sites of plasma membrane fusion, giving rise to multiple sites of dilation along the pathway of each junctional complex. The subsurface cisternae expand with the dilated intercellular space, thus usually encompassing each vacuole. C) With the prompt depletion of germ cells in the sustained cryptorchid state, the Sertoli cells collapse and become compacted. The vacuoles probably undergo compression forces which produce distortion and they behave like a "concertina" mechanism, being squeezed along the pathway of the junctional complex. D) After 4 weeks of cryptorchidism, the vacuoles are compressed into thin membrane bound saccules, aggregated in parallel where they form alternate layers with their accompanying smooth cisternae and bundles of filaments.

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