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Response of the seminiferous epithelium of the rat testis to withdrawal of androgen: evidence for direct effect upon intercellular spaces associated with Sertoli cell junctional complexes

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Abstract. The morphological response of the Sertoli cells to partial or complete withdrawal of testosterone was studied in adult rats following hypophysectomy or administration of ethane dimethanesulphonate (EDS), a toxicant known to destroy selectively the Leydig cells of the testis. To assess the role of germ cells in effecting changes to Sertoli cells following withdrawal of testosterone, germ cell-deficient rats with Sertoli-cell-only testes (SCO) were treated with EDS to remove the source of testosterone. At 6 days after hypophysectomy or 4, 6 and 8 days after EDS treatment, stage VII and VIII seminiferous tubules showed degenerating germ cells and numerous basally-located vacuoles approximately 1–15 μm in diameter. Ultrastructural analysis indicated that most of the vacuoles were multiple focal dilations of the intercellular space associated with Sertoli cell junctional complexes. In SCO rats, treatment with EDS resulted in a significant ($P < 0.05$) increase in the formation of many vacuoles particularly in the base but also in the trunk of the Sertoli cells and again electron microscopic analysis showed multiple, localized expansions of the intercellular space associated with Sertoli cell junctional complexes. The appearance of intercellular spaces in SCO testes following androgen withdrawal cannot be attributed to shrinkage of degenerating germ cells since the seminiferous tubules did not contain germ cells. It is concluded that withdrawal of androgen induces early morphological alterations of the Sertoli cell junctional complexes in which the sites of membrane fusions representing tight junctions remain intact whereas the intercellular spaces exhibit major focal dilations. The results are discussed in relation to the fluid secretion by the seminiferous tubules which is regulated by the Sertoli cells.

Key words: Testis – Sertoli cells – Testosterone – Hypophysectomy – Ethane dimethanesulphonate – Rat (Sprague-Dawley)

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Introduction

The sensitivity of the seminiferous epithelium to a wide variety of factors which disrupt the process of spermatogenesis has provided a useful approach to the study of the biology of the Sertoli cells and their role in regulating germ cell development (Bartlett et al. 1988; de Kretser and Kerr 1988; Sharpe et al. 1990; Steinberger 1971). Histological examination of fixed preparations of the seminiferous tubules is the chief method used for evaluating quantitative and qualitative alterations of germ cells and Sertoli cells (Russell 1983; Schulze 1984) but interpretation of the morphological patterns of response is dependent upon two factors. First, testes should be perfusion-fixed since this technique minimizes or avoids mechanical disruption of the tissue and prevents swelling or shrinkage artefacts which very often occur within the seminiferous epithelia of immersion-fixed testicular fragments. Secondly, it is necessary to distinguish between alterations of the Sertoli cells and damage to or degeneration of the germ cells, because an initial lesion in one cell type may cause secondary morphological alterations of the other. In studies concerned with the hormonal control of spermatogenesis in the rat testis, stages VII–VIII of the spermatogenic cycle (Le blond and Clermont 1952) are the first to show histological alterations following the partial or complete withdrawal of testosterone, achieved by surgical hypophysectomy (Russell and Clermont 1977; Ghosh et al. 1991) or selective elimination of the Leydig cells with the Leydig cell toxicant ethane dimethanesulphonate (EDS) (Bartlett et al. 1986; Kerr et al. 1985, 1993). Either method results in early degeneration of specific germ cells (pachytene primary spermatocytes and step 7 and 19 spermatids) together with the appearance of intra-epithelial vacuoles. The latter are attributable in part to the necrosis of degenerating germ cells in a vacuolated space outside the Sertoli cells (Russell and Clermont 1977) and to the appearance of intracellular vacuoles within the basal Sertoli cell cytoplasm (Ghosh et al. 1991).

Accumulation of vacuoles in association with experimental impairment of spermatogenesis is a commonly observed phenomenon and has been described as a non-specific response of the seminiferous epithelium to numerous unrelated insults to the human and mammalian testis (Fawcett 1975).

Vacuoles increase in size and numbers in response to testicular toxicants (Creasy et al. 1987); hCG treatment (Kerr and Sharpe 1989a, b), vasectomy (Flickinger 1981), experimental cryptorchidism (Kerr et al. 1979), and vitamin A deficiency (Rich and de Kretser 1977). Based upon the fact that intra-epithelial vacuoles appear soon after the experimental withdrawal of testosterone the aims of this study were 1) to identify the subcellular origin of these vacuoles, and 2) to determine whether the appearance of vacuoles following withdrawal of testosterone is a primary effect upon the structure of the Sertoli cells, or a secondary consequence of necrosis and shrinkage of degenerating germ cells.

Materials and methods

Alterations of the seminiferous epithelium

Adult male Sprague-Dawley rats (300–350 g body weight) were maintained in the laboratory colony under standard conditions. Five animals were hypophysectomized under general ether anaesthesia via the parapharyngeal route by making a small hole in the sphenoid bone (Waynforth 1980). Following recovery from anaesthesia animals were housed at 30° C, fed normal rat food and given 5% sucrose in 0.9% saline as drinking water. Hypophysectomized rats were killed 6 days later as described below. Fifteen animals were given a single intraperitoneal injection of ethane dimethanesulphonate (EDS, 7.5 mg/100 g body weight) in dimethyl sulphoxide-water (1:3 V/V), known to destroy selectively the Leydig cells within 3 days and to reduce the intratesticular levels of testosterone below the limits of detection by radioimmunoassay (Barlett et al. 1986). Groups of 5 rats were subsequently killed on days 4, 6, and 8 after EDS injection. Adult male rats with Sertoli cell-only (SCO) testes were obtained by X-irradiation of foetuses *in utero*. Pregnant female rats of known gestational age (determined by time-mating) were given whole body X-irradiation (1.5 Gy, RS20/55M irradiator, Gravatom Industries, Gosport, Hants, UK) on day 20 of gestation to destroy selectively the proliferation of gonocytes within the foetal testis (Maddocks et al. 1992). New-born male pups were reared to 100 days of age. The Sertoli cells in these testes exhibit junctional complexes which form an intact blood-testis barrier (Means et al. 1976). Five of these animals were killed with no further treatment and another group of 5 received an EDS injection as described above and sacrified 6 days later.

Tissue fixation

Testes were fixed by vascular perfusion using a method described previously (Kerr et al. 1985). Under deep ether anaesthesia, the thoracic aorta was cannulated, the right atrium was cut and testes perfused for for 30 s with 0.9% physiological saline, followed by 30 min perfusion with 3% glutaraldehyde, 2% formaldehyde and 0.01% picric acid buffered in 0.1 M sodium cacodylate, pH 7.4. From each fixed testis five cubes 2 mm on edge were prepared from 5 different slices of the testis. Tissue cubes were rinsed in the same buffer, post-fixed for 2 h in osmium tetroxide, stained en bloc with uranyl acetate, dehydrated in graded concentrations of ethanol, cleaned in propylene oxide and embedded in a 1:1 mixture of Araldite: TAAB 812 (TAAB Laboratories, Reading, UK). Polymerized blocks were sectioned at <1 μm, stained with toluidine blue and examined by light microscopy. Ultrathin sections were cut with diamond knives, stained with lead citrate and examined with a JEOL 100S electron microscope. The completeness of hypophysectomy was confirmed by inspection of the sella turcica for pituitary fragments (none found), a comparison of adrenal weights with age-matched intact animals and the appearance of degenerating germ cells within the seminiferous epithelium of Stage VII–VIII tubules. In all EDS-treated animals, Leydig cells were absent.

Quantitative histological analysis

Semithin stained sections of cross-sectioned seminiferous tubules from 5 tissue blocks selected from random locations within each testis were examined by light microscopy using a 40× oil-immersion objective lens. In sections from hypophysectomized and EDS-treated animals, all stage VII and VIII tubules were identified and using an eyepiece graticule with a square lattice pattern of 441 points, the occurrence of intra-epithelial vacuoles was measured by a point-counting technique in which lattice intersection points superimposed over vacuoles were counted. Volumetric density (Vv, %) of vacuoles was expressed as a percentage of the total number of points superimposed over each selected seminiferous tubule. Tubules not showing vacuoles were scored as a zero percentage and included in the raw counts. For SCO testes lacking all germ cells, 10 tubules were randomly measured per section. Average volumetric densities per tubule were calculated for each testis. To convert vacuole volumetric densities into actual volumes per cross-sectioned tubule, the mean area (in μm^2) of each tubule analysed was measured using an image analyser (Houston Instruments, Texas) linked to the microscope. The cross-sectional areas of seminiferous tubules in 6 day hypophysectomized rats and in 6 to 8 days EDS-treated rats were similar and ranged between 6.6 ± 0.7 to $7.0 \pm 1.0 \times 10^4$ μm^2 (mean±SD). In SCO and SCO/EDS treated rats, the values were 3.2 ± 0.3 and $2.8 \pm 0.2 \times 10^4$ μm^2 (mean±SD) respectively. In each testis the volume of vacuoles per cross-sectioned tubule (mean±SD) was then calculated by multiplying Vv by mean tubule area. The means (±SD) for the animals belonging to each group were determined. The mean (±SD) value for each treatment group was normally distributed. Analysis of variance was used to determine a significant overall difference between groups. Comparison of data between groups was made with Student's *t*-test.

Results

Testis of control rats

Seminiferous tubules showed normal morphology and extracellular spaces within the seminiferous epithelium were rarely observed. The creation of empty-looking spaces due to shrinkage of cells with hyperosmotic solutions was checked carefully in stage VII and VIII tubules. None were found.

Testis of 6-day-hypophysectomized rats

Basally-located vacuoles were noted in the seminiferous tubules at stages VII–VIII of the spermatogenic cycle, but not at other stages (Fig. 1a). Vacuoles were circular or irregularly-elliptical in shape and variable in size ranging from approximately 1 to 15 μm in diameter. Most vacuoles occurred as single entities clustered in multiple rows at or slightly below the level of the Sertoli cell nuclei, but occasionally a crescent-shaped space was associated with dense granular bodies representing degen-

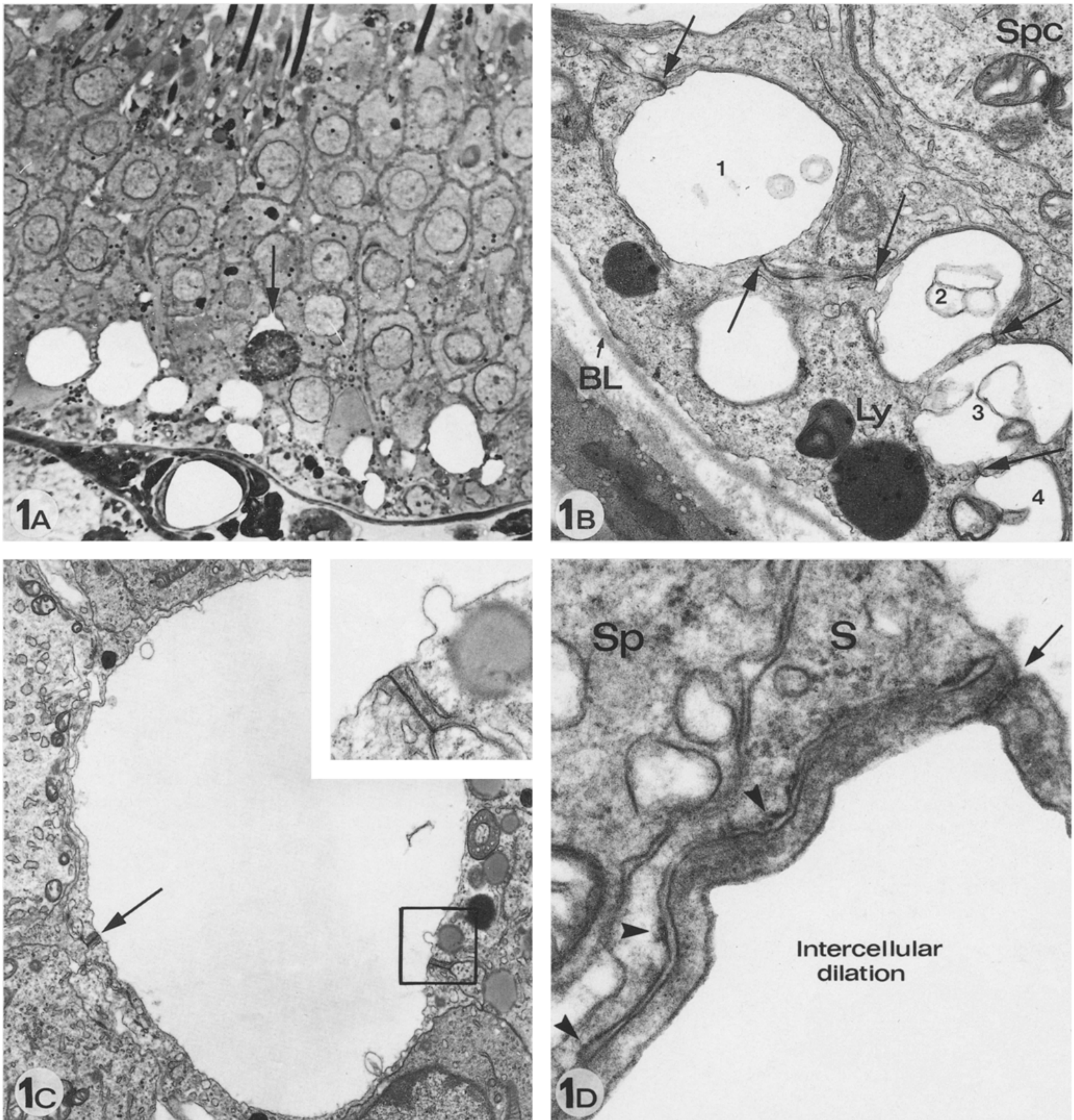
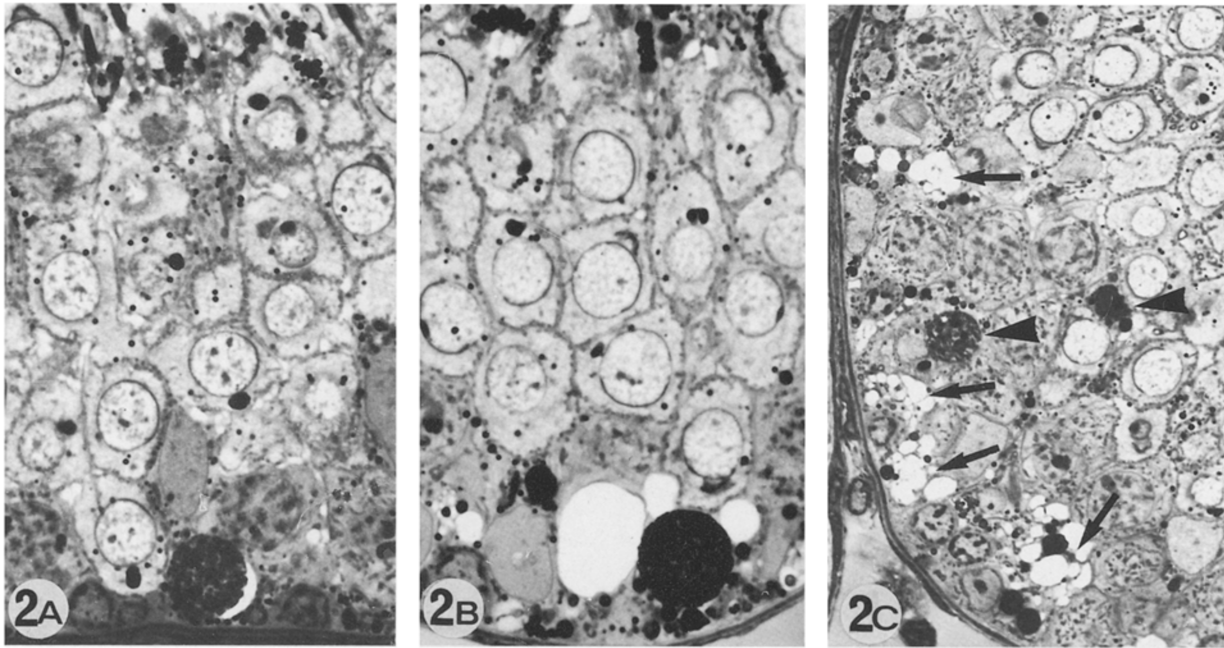


Fig. 1A–D. Seminiferous epithelium 6 days post-hypophysectomy. **A** Stage VII tubule showing large basally-located vacuoles. Note degenerating germ cell flanked by a crescent-shaped space (*arrow*). ×880. **B** Vacuoles are focal intercellular dilations of Sertoli cell junctional complexes which terminate (*arrows*) between spaces labelled 1 to 4. Part of a spermatocyte (*Spc*) is shown together with Sertoli cell lysosomes (*Ly*) and the basal lamina (*BL*). ×14700. **C** A large intercellular space between segments of junctional complexes (*arrow* and *square*). ×7500. *Inset*: High magnification of square showing termination of a junctional complex. ×25000. **D** Intercellular space with a flanking, parallel ectoplasmic specialization of endoplasmic reticulum with lateral ribosomes (*arrowheads*). Small segment of opposing membranes of a junctional complex remains adherent (*arrow*). *S* Sertoli cell cytoplasm; *Spc* spermatocyte cytoplasm. ×55000

erating germ cells. Ultrastructural analysis of the most basally situated vacuoles revealed that they were expanded intercellular spaces within the area occupied by the cytoplasm of the Sertoli cells and were separated by segments of the inter-Sertoli cell tight junctions (Fig. 1b). The repeated squences of alternate intercellular spaces and tight junctions showed that the spaces were focal dilations along the length of the junctional complexes and were clearly intercellular i.e., between adjacent Sertoli cells. Larger spaces were also focal expan-



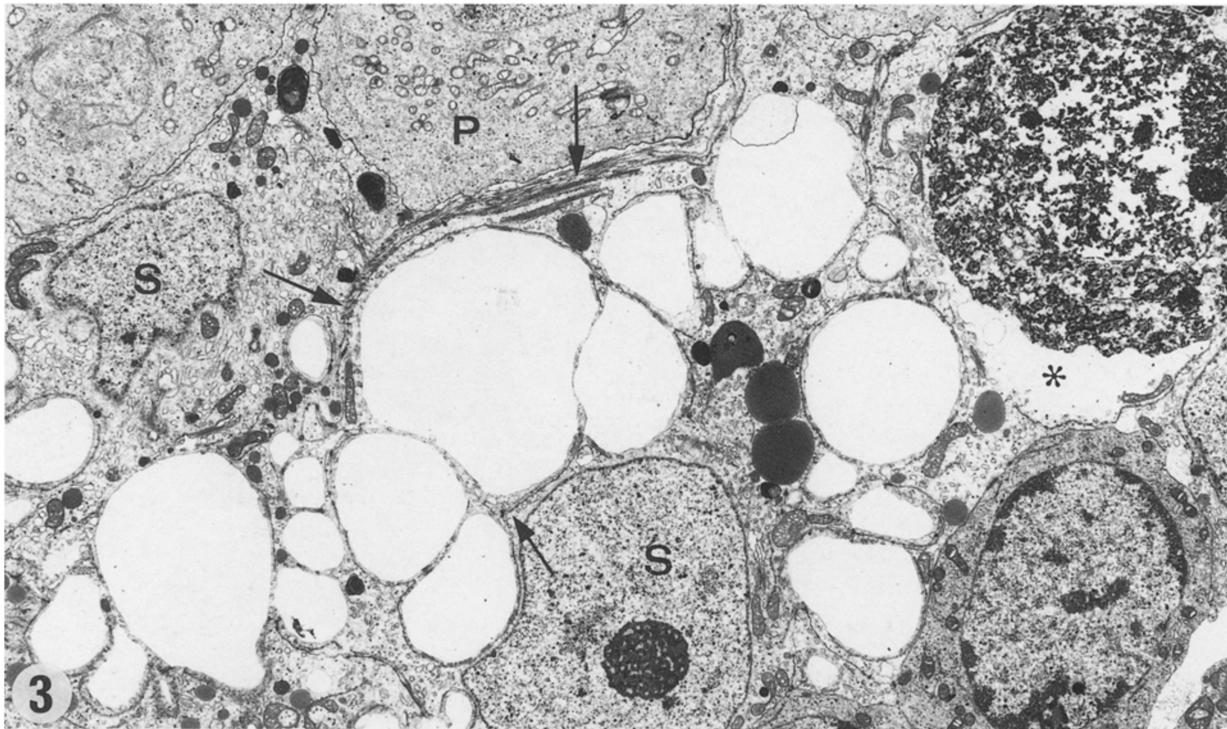


Fig. 2A–C. Stage VII tubules after EDS treatment. **A** 4 days post-EDS showing a degenerating germ cell bordered by a crescent-shaped space. ×1100. **B** 6 days post-EDS showing a degenerating germ cell and adjacent circular spaces. ×1200. **C** 8 days post-EDS showing focal aggregations of basally-located spaces (*arrows*) and degenerating germ cells (*arrowheads*). ×800

Fig. 3. Ultrastructure of a stage VII tubule 8 days post-EDS showing many tissue spaces between Sertoli cell nuclei (*S*) and primary spermatocytes (*P*). Note the space associated with a pyknotic germ cell (*asterisk*). Sertoli cell junctional complexes (*arrows*) are indicated. ×4300

sions of the intercellular space between membranes of Sertoli cell junctional complexes (Fig. 1c). Depending upon the plane of section, the ectoplasmic specializations which course parallel to opposing Sertoli cell membranes were seen to flank the circumference of the spaces (Fig. 1d).

Testis of EDS-treated rats

Removal of the Leydig cells and thus the source of testosterone was followed by degeneration of germ cells initially in stage VII seminiferous tubules. In addition, 3 types of vacuoles or empty-looking spaces were noted.

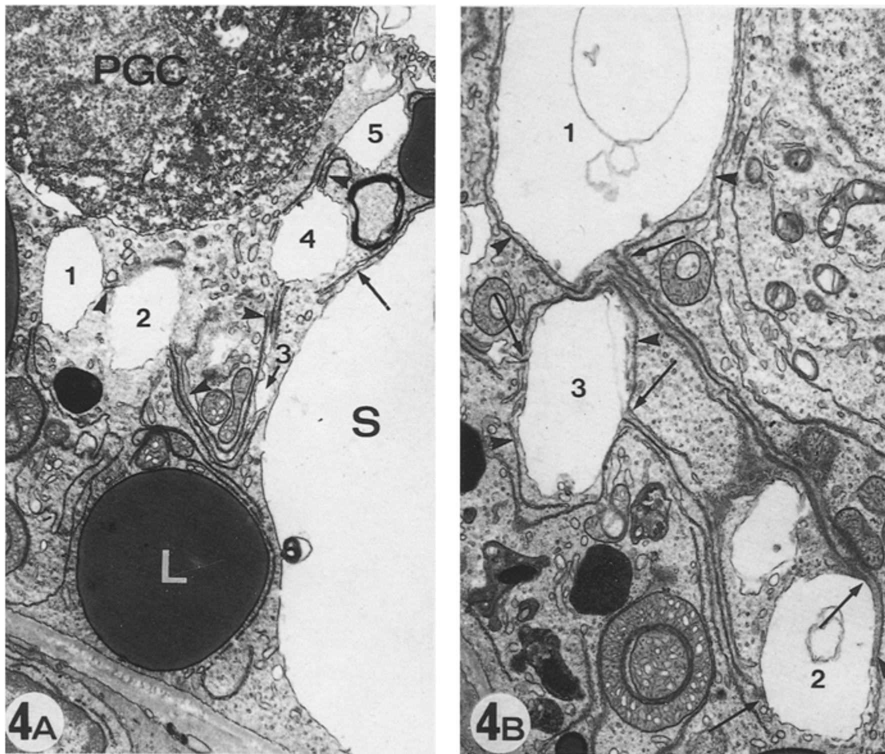


Fig. 4A, B. Ultrstructure of basal epithelial region 6 days post-EDS. **A** Five intercellular spaces are shown (*1–5*) separated by Sertoli cell junctional complexes (*arrowheads*). A pyknotic germ cell (*PGC*) and a lipid inclusion (*L*) are shown. Part of a large intercellular space (*S*) is associated with a membranous cisterna (*arrow*). ×7800. **B** Three intercellular spaces (*1–3*) interconnected by segments of a Sertoli cell junctional complex (*arrows*) whose accompanying membranous cisternae follow the contours of the spaces (*arrowheads*). ×8200

First, at 4 days after EDS treatment, at thin crescent-shaped space was occasionally observed adjacent to a pyknotic germ cell (Fig. 2a); secondly, 6 days post-EDS, circular spaces were associated with the periphery of pyknotic germ cells (Fig. 2b); and thirdly at 8 days after EDS treatment, small and large circular spaces often formed focal aggregations around the circumference of seminiferous tubules (Fig. 2c). However, pyknotic germ cells were not always associated with these spaces, and degenerating germ cells were not seen in several sections taken from more superficial or deeper levels of the tissue block. Ultrastructural examination of clusters of spaces showed those forming crescent-shaped cavities associated with degenerating germ cells together with many empty-looking spaces located between Sertoli cell nuclei or between germ cells and Sertoli cell nuclei (Fig. 3). However, at relatively low magnification (up to ×5000) it was not possible to distinguish if the spaces were intracellular or intercellular. At higher magnification of the Sertoli cells, spaces were seen as small focal dilations of the intercellular space between segments of the inter-Sertoli cell junctional complexes (Fig. 4a). In favourable sections intercellular spaces connected by segments of tight junctions were flanked by thin membranous cisternae indicating that the opposing junctional complexes had moved apart, creating dilated intercellular spaces (Fig. 4b).

Testis of Sertoli cell-only rats

The seminiferous tubules of SCO testes contained basally-located Sertoli cell nuclei with the cytoplasm extending towards the centre of the tubules often meeting the cytoplasm from Sertoli cells on the opposite side of the seminiferous tubule. Clear spaces or vacuoles were usually confined to the more central region of the tubules (Fig. 5a). Following the withdrawal of testosterone after administration of EDS, the number of spaces was increased in all tubules and they were located at all levels within the seminiferous epithelium (Fig. 5b). At the ultrastructural level, many of the spaces regardless of their size, were identified as intercellular dilations of the inter-Sertoli cell junctional complexes with as many as 10 or more forming a continuous chain separated by short segments of intact tight junctions (Fig. 5c, d).

Quantitative analysis of spaces

These data are presented in Fig. 6 and show the mean volume of intercellular spaces within cross-sectioned seminiferous tubules. In 6-day-hypophysectomized animals, the volume of vacuoles in stage VII–VIII tubules was significantly ($P<0.001$) increased compared to control animals. In all EDS-treated groups, vacuoles were significantly ($P<0.05$) greater in their total volume per tubule compared to hypophysectomized rats. When SCO rats were treated with EDS, the volume of vacuoles measured within tubules was significantly ($P<0.05$) increased compared to their occurrence in SCO testes.

Discussion

The appearance of intraepithelial spaces referred to as vacuoles which accompany experimental disruption of spermatogenesis has long been recognized in laboratory species and is commonly noted in abnormalities of the

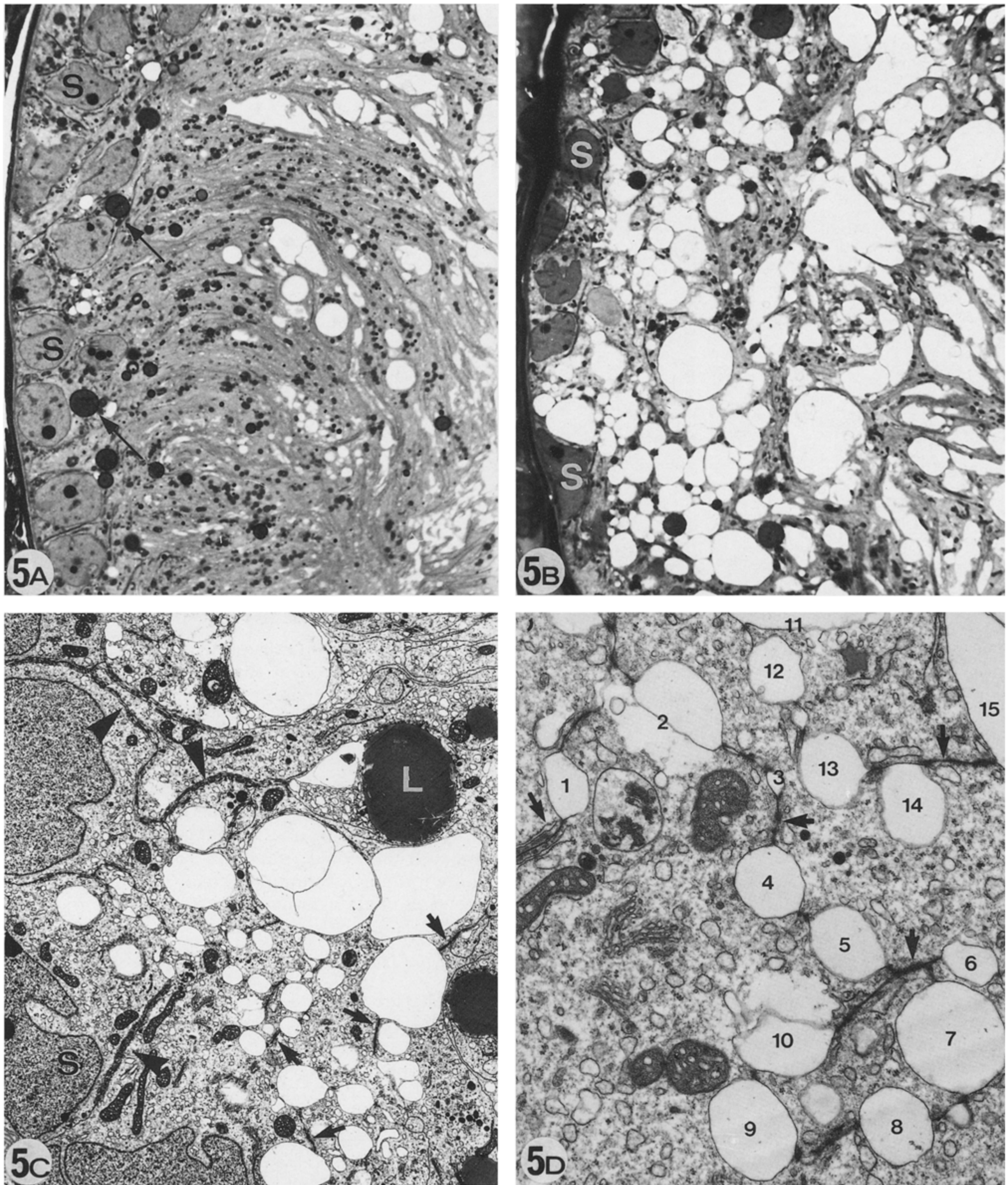


Fig. 5A–D. Seminiferous tubules in Sertoli cell-only testes from adult rats. **A** Untreated control testis showing Sertoli cell nuclei (*S*) and tissue spaces predominantly in the apical aspect of the epithelium. Lipid inclusions also shown (*arrows*). ×900. **B** 6 days after EDS showing many spaces throughout the depth of the epithelium. Sertoli cell nuclei are indicated (*S*). ×900. **C** Ultrastructure of Sertoli cell-only testes from adult rats 6 days after EDS, showing numerous intercellular spaces intervening between electron-dense lines (*arrows*) representing Sertoli cell junctional complexes. Intact Sertoli cell junctional complexes (*arrowheads*), Sertoli cell nuclei (*S*) and lipid inclusions (*L*) are shown. ×4000. **D** Fifteen focal extracellular dilations (*labelled 1–15*) forming intercellular spaces separated by Sertoli cell junctional complexes (*arrows*) ×14400

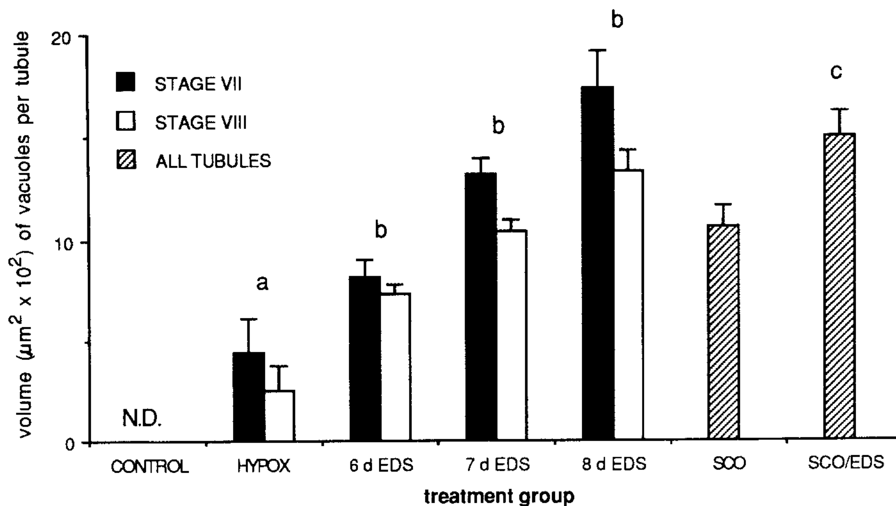


Fig. 6. Volume of intercellular spaces per cross-sectioned seminiferous tubule in control rats and in various treatment groups. Data represent means ± SD (*ND* = not detected). *a* $P<0.001$ compared to control; *b* $P<0.05$ compared to hypox; *c* $P<0.05$ compared to SCO

human testis (see reviews by de Kretser and Kerr 1988; Schulze 1984). Because these spaces occur in response to a wide variety of insults and/or endocrine disorders, it has been considered as a non-specific response of the Sertoli cells to injury (Fawcett 1975, 1977). In the present study it is shown that in the normal testis, the impairment of germ cell development which follows partial or complete withdrawal of testosterone is accompanied by the formation of intercellular spaces specifically at stages VII–VIII of the spermatogenic cycle. When normal adult rats were hypophysectomized or treated with EDS, a variety of spaces were formed approximately at the level of the nuclei of Sertoli cells. Tissue spaces visible by light microscopy could be classified into four categories: (1) small circular spaces ≤2–3 µm positioned at the base of the epithelium; (2) larger spaces, 5–15 µm, at the level of Sertoli cell nuclei; (3) crescent-shaped spaces associated with pyknotic spermatocytes; and (4) irregularly-shaped intercellular spaces between the Sertoli and/or germ cells. Since the germ cells at stages VII–VIII are acutely dependent upon an adequate supply of testosterone (Bartlett et al. 1986; Clermont and Morgentaler 1955; Russell and Clermont 1977) it is not surprising to encounter intercellular or extracellular spaces in the androgen-deprived seminiferous epithelium, arising from shrinkage, autolysis and phagocytosis of degenerating germ cells by the Sertoli cells. Degenerative changes in germ cells account for the appearance of the latter two of the four above categories. The first two categories of spaces represent both small and large expanding spaces arising from many focal dilations of opposing membranes of the inter-Sertoli cell junctional complexes. When these spaces were examined by light microscopy the position and size of the larger examples (similar to the dimensions of nearby germ cell nuclei) suggested to us that they were cavities left behind after the shrinkage of degenerating germ cells followed by their disappearance resulting from autolysis, and phagocytosis by the Sertoli cells. Ultrastructural analysis showed that these spaces were in fact large dilations of the intercellular space associated with the region of inter-Sertoli cell junctional complexes. There are other reasons for suggesting that these cavities did not arise from pyknotic germ cells (1) they did not contain cellular debris (2) lysosomes were not observed and (3) their numbers were far greater than the numbers of germ cells known to be positioned at the level of the Sertoli cell nulcei. Other studies of the rat testis at 6 days post-hypophysectomy have suggested that spaces were within the Sertoli cell cytoplasm (Ghosh et al. 1991) i.e., true vacuoles, yet, in contrast, it has also been reported that with the exception of increases in secondary lysosomes, the ultrastructure of all other components of the Sertoli cells at stage VII was unchanged compared to normal (Ghosh et al. 1992). The reasons for these different conclusions are not clear.

It could be argued that the effects of androgen withdrawal upon the junctional complexes was a secondary response of the Sertoli cells to the presence of degenerating germ cells but two observations make this suggestion untenable. First, focal dilations occurred along the junctional complexes at locations which were distant from pyknotic germ cells and secondly, identical dilations forming many intercellular spaces were seen when androgen was withdrawn from SCO testes, which do not contain germ cells. The possibility that EDS alone may have induced vacuole formation can be discounted since concurrent or intermittent testosterone administration prevents the appearance of intercellular spaces (Sharpe et al. 1988a, b; 1992). Dilation of the intercellular spaces after EDS treatment appears to be a specific consequence of testosterone withdrawal as opposed to cell degeneration or toxicity since only stages VII–VIII are affected and these are the most sensitive to testosterone withdrawal; their formation is prevented by exogenous androgen treatment and the same spaces are formed at stages VII–VIII in hypophysectomized rats. From these results and from the experiments presented here it can be concluded that the formation of intercellular spaces is a direct effect of androgen withdrawal upon the Sertoli cells, specifically altering the structural integrity of the inter-Sertoli cell junctional complexes. However a reduced supply of testosterone is not the only factor which promotes focal expansions in the region of these junc-

tions. The same response occurs in situations of spermatogenic damage induced by experimental cryptorchidism, vasectomy, efferent duct ligation, hCG treatment, vitamin A deficiency, or administration of gossypol, hydroxyurea or epinephrine (Bergh 1983; Flickinger 1981; Gravis et al. 1977; Hoffer 1983; Kerr et al. 1979; Kerr and Sharpe 1989; Rich et al. 1979; Ross and Dobler 1975). These observations raise the question of what mechanism(s) can account for a common morphological alteration of the Sertoli cells in response to seemingly unrelated insults to the seminiferous epithelium, even in the absence of the germ cells?

It is known that the seminiferous tubules secrete the luminal fluid in which the spermatozoa are conveyed towards the rete testis (Setchell 1980; Waites and Gladwell 1982) the Sertoli cells being the source of this seminiferous tubule fluid (STF) created by moving water from their base towards the tubule lumen (Fawcett 1977). By virtue of their basal position within the seminiferous epithelium, the inter-Sertoli cell tight junctions maintain intercellular occlusion of membranes at the base of the cell whereas the intercellular spaces towards the lumen remain open (Dym and Fawcett 1970). The maintenance of normal Sertoli cell function is a complex interplay between an adequate supply of testosterone (Sharpe et al. 1990) for which receptors exist within the Sertoli cells but not within the germ cells (Tindall et al. 1977) and intercellular communications with the germ cells, every one of which is either partly or completely surrounded by ramifications of Sertoli cell cytoplasm. Since the intercellular clefts between the Sertoli cells and the germ cells are occupied by STF produced by the Sertoli cells, then the focal dilations of the membranes of the inter-Sertoli cell junctional complexes must be filled with STF. These fluid filled spaces have never been observed in the normal seminiferous epithelium, which suggests an abnormality of STF secretion in situations of germ cell impairment and/or androgen withdrawal. In the four cases in which the formation of intercellular spaces has been most carefully studied (hypophysectomy, EDS treatment, SCO testes and experimental cryptorchidism), STF production is significantly reduced compared to the normal testis (Rich and de Kretser 1977; Jegou et al. 1983a, b; O'Leary et al. 1987) and in each case, testosterone production is also significantly reduced (de Kretser 1987). Although the weight of structural and physiological evidence points to the acute sensitivity of the inter-Sertoli cell junctional complexes in response to alterations of the intratesticular environment, it is important to emphasize that the junctional complexes are structurally altered but the permeability of the blood-testis barrier is not breached, since the junctional complexes continue to exclude the intercellular penetration of electron-dense tracer molecules (Hagenas et al. 1977, 1978). An explanation of the mechanisms causing focal intercellular dilations is dependent upon an understanding of the macromolecular composition of the junctional complexes which constitute the blood-testis barrier.

Ectoplasmic specializations (ES) flank the sites where adjacent Sertoli cell plasma membranes are extensively fused. Each ES consists of dense mats of hexagonally-arranged actin filaments in a paracrystalline array, located between the tight junctions and a parallel cistern of endoplasmic reticulum. Immunohistochemical and immunofluorescent studies of ES of Sertoli cells have detected vinculin, fimbrin and Z0-1, proteins involved with membrane adhesion and tight junctions (Grove et al. 1990). The absence of myosin and the inability of Sertoli cell cultures to exhibit contraction indicates that the Sertoli cell tight junctions and associated ES are structural, stabilizing membrane specializations rather than fulfilling a contractile role (Vogl and Soucy 1985). It therefore seems unlikely that disruption of spermatogenesis or androgen withdrawal causes active retraction of opposing cell membranes to form enlarged intercellular spaces. An alternative explanation would involve a breakdown of cell adhesion molecules embedded within segments of the plasma membranes together with excessive fluid secretion into these focal sites, possibly generated by the plasma membrane enzyme Na^+K^+-ATPase which regulates ion pumping and is believed to be involved in fluid movement across the Sertoli cells (Byers and Graham 1990). This enzyme is chiefly associated with apicolateral Sertoli cell membranes, i.e. towards the lumen where STF is secreted, but it has also been detected at the level of the basal junctional complexes. It seems possible therefore that the reduction in STF production which accompanies seminiferous tubule disruption is a reflection of alterations in the fluid transporting properties of the Sertoli cell in which STF secretion is directed basolaterally rather than apically.

The results of this study add to the growing body of evidence that impairment of seminiferous tubular function not only interferes with germ cell development but also disrupts the structure and function of the Sertoli cells. It seems likely that it is the Sertoli cells, rather than the germ cells, which first respond to androgen withdrawal because the Sertoli cells are the exclusive site for androgen receptors within the seminiferous epithelium (Grootegoed et al. 1977; Sar et al. 1990). Future studies of the morphological expression of Sertoli cell disruption may provide important information on how androgens support the process of spermatogenesis.

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