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The Blood-Testis Barrier in Men of Diverse Fertility Status: An Ultrastructural Study

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Summary. The permeability of the blood-testis barrier was studied in 41 men of diverse fertility status by observing the distribution of lanthanum, an electron-opaque tracer administered during immersion fixation of testicular biopsies.

Evidence for barrier mechanisms operating at the specialised tight junctions between Sertoli cells was seen in all specimens. In approximately one half of the seminiferous tubules examined this barrier appeared to be less effective as seen by the penetration of lanthanum past the Sertoli cell tight junctions. This phenomenon was found to be significantly related to both the tubular spermatogenetic activity as measured by a scoring method and the presence of subcellular changes in the Sertoli cells. A depression of spermatogenesis was associated with an increase in the incidence of tubules with apparently defective tight junctions. In men with obstructive azoospermia there was no increase in the incidence of this phenomenon.

Key words: Male infertility – Blood-testis barrier – Sertoli cells – Lanthanum

Introduction

The concept of a blood-testis barrier, whereby the permeability of the seminiferous tubules to substances within the blood is regulated, has been well established (Setchell and Waites 1975).

Methods involving the intravascular or interstitial injection of intercellular electron-opaque tracers, such as horse-radish peroxidase and lanthanum, have shown that in laboratory animals these tracers do not penetrate past the tight junctions between neighbouring Sertoli cells of the seminiferous epithelium and that a further, though not as effective, barrier can be located at the site of the tight junctions between the peritubular myoid cells (Fawcett et al. 1970;

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Dym and Fawcett 1970). This has led to the description of two compartments within the seminiferous tubules. One is located basally and is postulated as having a degree of intercellular permeability to circulating substances. It contains spermatogonia and preleptotene spermatocytes. The other compartment is adluminal and is located above the Sertoli cell tight junctions. It consists of spermatocytes and differentiating spermatids which are believed to be shielded from substances circulating intercellularly (Dym and Fawcett 1970). The blood-testis barrier of the developing testis has been shown to form at the onset of spermatogenesis (Kormano 1967; Vitale et al. 1973). The current views on the significance of its existence are that it provides an environment within the seminiferous epithelium essential for the maintenance of spermatogenesis and at the same time provides the immunological isolation of antigenic haploid developing spermatozoa (Katsch 1960; Johnson 1970c).

The stability of the intra-epithelial component of the blood-testis barrier has been demonstrated in a variety of experimental conditions (Castro and Seiguer 1974; Gravis et al. 1977; Hagenäs et al. 1977, 1978; Osman et al. 1978). It was, therefore, of interest to investigate tubular barrier mechanisms in the human testis with particular reference to depressed spermatogenesis as often encountered in male infertility. In 1973, Neaves reported on the breakdown of the blood-testis barrier in rats following proximal ligation of the spermatogenetic ducts. Furthermore, Heidger (1974) found this to be true in dogs following distal ligation as in vasectomy. In view of these findings it was of interest to see whether the presence or site of obstruction had any effect on the blood-testis barrier in man.

Materials and Methods

A total of 47 biopsies (6 bilateral) were obtained from 41 men. The clinical assessment of fertility for each patient was not revealed until the end of both light and electron-microscopic evaluation of the material. The patients consisted of: 6 men undergoing vasectomy (no obstruction – controls); 5 men undergoing vasectomy reversal (distal obstruction); 3 men with congenital absence of the vas deferens (distal obstruction); 5 men found to have epididymal occlusion (proximal obstruction) and 22 men with oligozoospermia or azoospermia due to varying degrees of testicular failure.

Pilot studies (unpublished data) on normal rat testicular biopsies, of a similar size to those taken for this study and treated in an identical manner, showed the distribution of lanthanum to be restricted either to the basal compartment of the seminiferous tubules or to the tight junctions between myoid cells. Exceptions occurred in a small proportion of tubules where it is believed that the tracer penetrated through open ends of tubules and was observed in the adluminal region above the Sertoli cell tight junctions (c.f. Neaves 1973; Hagenäs et al. 1978) and in these few cases a close inspection of tight junctions revealed that their impermeability was maintained.

Immediately after excision each human biopsy specimen was cut into two equal parts and placed respectively into a colloidal lanthanum fixative for electron microscopy and into Bouin's fixative for routine histological examination.

A 1% lanthanum silicate in 0.1 M cacodylate buffered 2% glutaraldehyde solution pH 7.5 was prepared by the method described by Neaves (1973). Tissues for electron microscopy were placed in this fixative overnight at 4° C. After several washes in lanthanum-free 0.1 M cacodylate buffer the peripheral tissue was gently excised in order to remove open cut ends of tubules and the central core diced into 1 mm^3 blocks and post-fixed in lanthanum-free veronal buffered 1% osmium tetroxide pH 7.5 for 1 h. These were then rapidly dehydrated through a graded series of acetone and resin (Taab Laboratories, Reading) embedded overnight. Blocks were polymerised at 100° C for 4 h. Sections for light microscopy, 1 μm thick, were cut on glass knives and stained

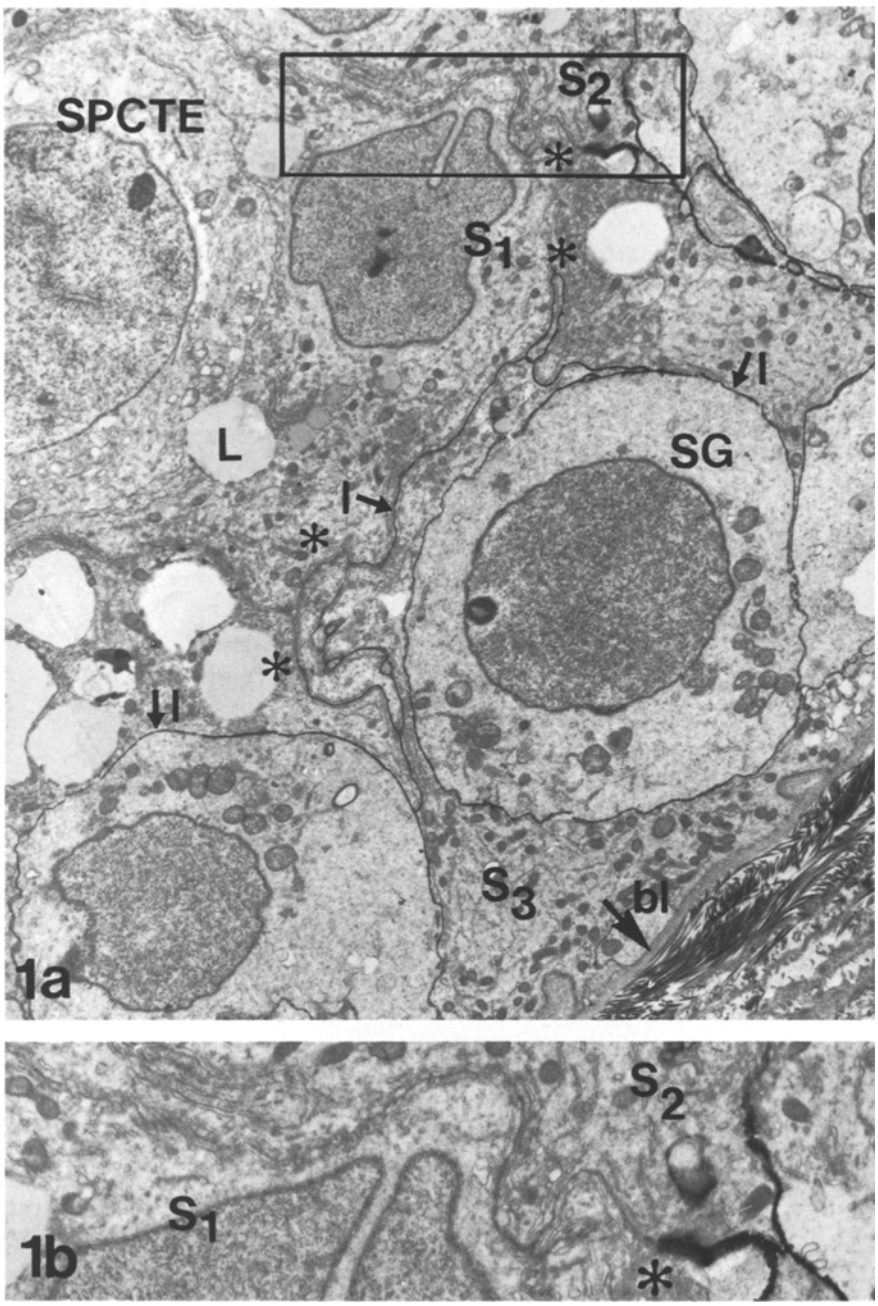

Fig. 1. a Basal region of seminiferous tubule. Sertoli cell junctional complexes (asterisk) are impermeable to lanthanum penetration. *bl*, basal lamina; *SG*, spermatogonium; *SPCTE*, spermatocyte; *l*, lanthanum; *S*, Sertoli cell; *L*, lipid. Magnification: ×5,000. **b** Detail of junctional complex from **a**. Note arrest of lanthanum at origin of junction (above asterisk). Magnification: ×15,000

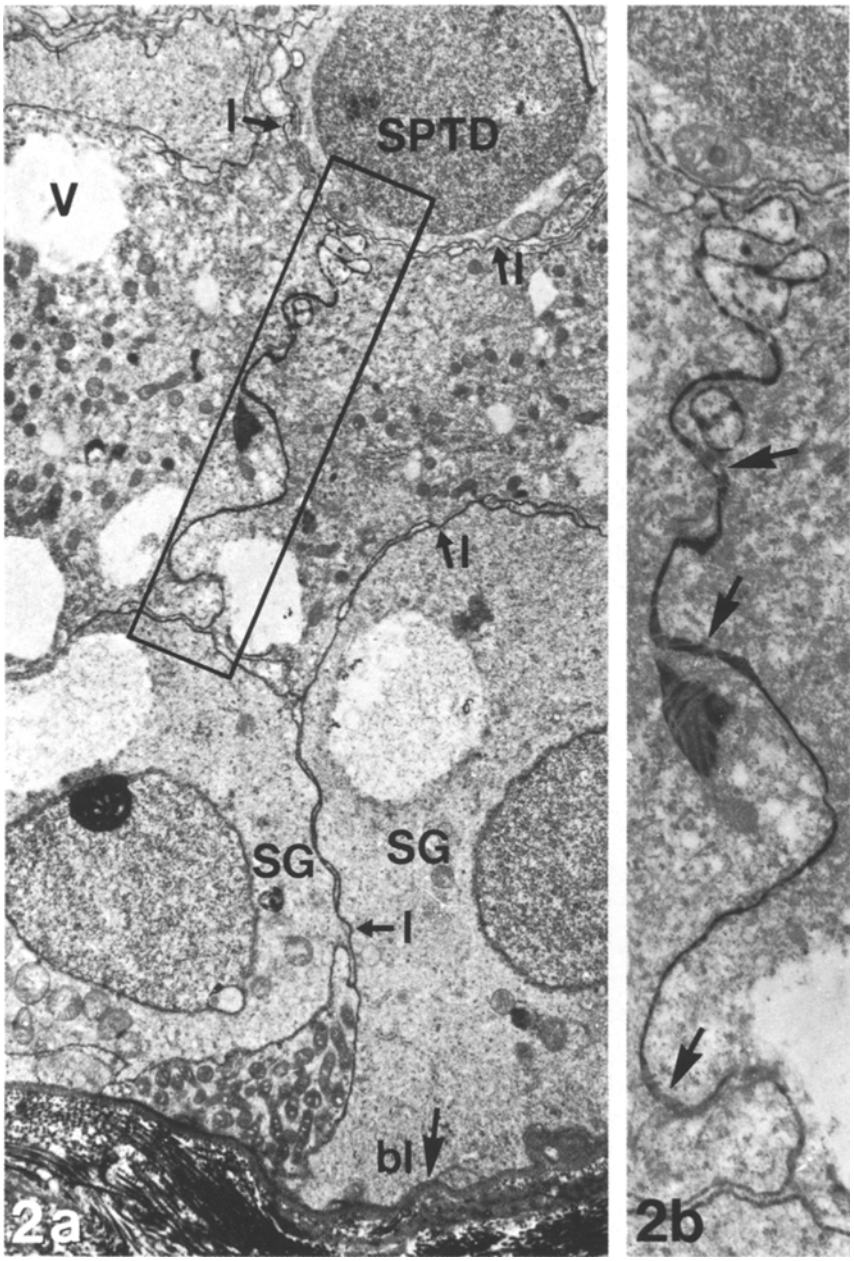

Fig. 2. a Portion of seminiferous tubule. Junctional complex (in box) is permeable to lanthanum which is also seen surrounding a maturing spermatid (*SPTD*). *V*, vacuole. Magnification: ×3,500. **b** Detail of junctional complex from **a**. 'Lakes' of lanthanum (arrows) can be seen in between regions of membrane fusion (light areas). Magnification: ×40,000

with 1% toluidine blue in 1% borax. Paraffin embedded, 5 μm thick, haematoxylin and eosin stained sections of the corresponding part of the biopsy were examined and a mean score of spermatogenesis, assessed by the method of Johnsen (1970), was evaluated for each biopsy. In this technique spermatogenesis is scored on a scale from 1–10. Tubules seen to have spermatozoa are given scores of 8, 9, or 10; tubules seen to have spermatids but no spermatozoa are given scores of 6 or 7; scores of 4 or 5 are given to tubules with spermatocytes; a score of 3 is assigned to tubules with spermatogonia; a score of 2 to tubules seen to have only Sertoli cells and finally a score of 1 is assigned to those seen to have no cells present. Tubules for electron-microscopic examination were selected to reflect the spermatogenetic distribution as observed light microscopically. Tubules with a score of 1 were not selected for electron microscopic evaluation.

Sections for electron microscopy, of a pale gold interference colour, were cut with diamond knives on an L.K.B. Ultrotome, stained with uranyl acetate and lead citrate and examined with an A.E.I. 6M electron microscope at an accelerating voltage of 80 KV.

Results

A total of 268 tubular cross sections were examined with the electron microscope, with an average of 5.7 tubules per biopsy specimen. In almost all the tubules lanthanum was observed to be outlining the spermatogonia due to the accumulation of the tracer in the intercellular regions below the tight junctions between Sertoli cells. The peritubular components, including the myoid cells, did not appear to offer any barrier to the entry of the tracer into the seminiferous tubules. In a small proportion of tubules, the seminiferous epithelium showed severe degenerative features and in all of them lanthanum accumulation was

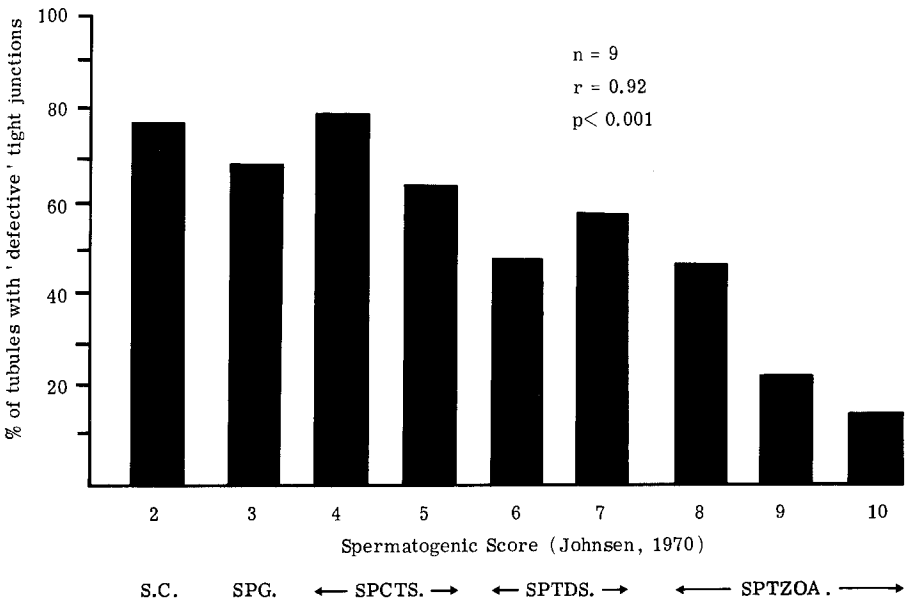

Fig. 3. Incidence of tubular permeability to lanthanum in relation to spermatogenic status (268 tubules examined)

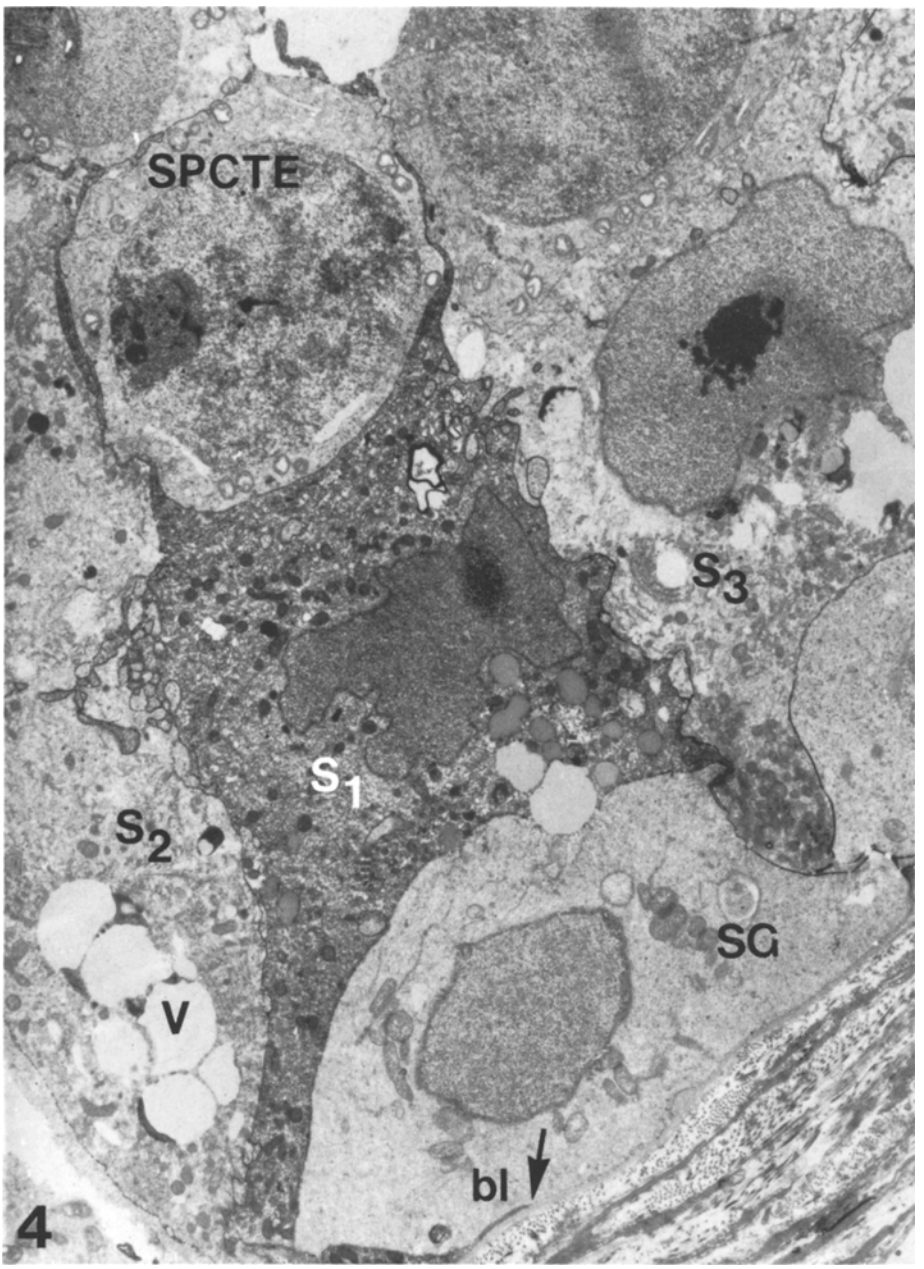

Fig. 4. Portion of seminiferous tubule with 'abnormal' Sertoli cells (*S1*). Note increased vacuolation (*S2* and *S3*)

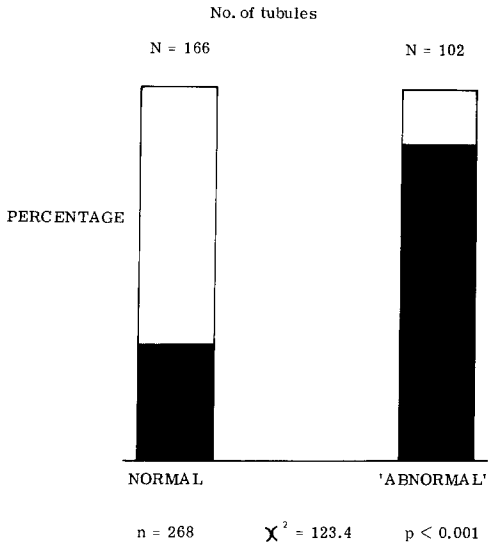

Fig. 5. Relationship between lanthanum permeability and ultrastructural morphology of Sertoli cells. ■ permeable, □ impermeable

absent. In a few other tubules showing evidence of poor fixation, lanthanum was observed both basally and luminally, (c.f. Castro and Seiguer 1974) and the specimens were excluded from the study.

In one half (49%) of the tubules lanthanum was totally excluded from the adluminal compartment by the tight junctions between Sertoli cells (Fig. 1a, b). In the other half (51%) lanthanum was present in varying degrees in the intercellular spaces above these junctions (Fig. 2a, b), a feature which will hereafter be described as being due to 'defective' tight junctions. For comparative purposes, all tubules were classified as being either positive or negative; those showing even the most discrete evidence of defective junctions being classified as positive. Each of the 268 tubules studied was allocated to 1 of 9 groups (as described for the Johnsen score) based on the appearances seen in the testicular biopsy score count under the light microscope. The respective percentage of tubules showing evidence of defective junctions in each of the 9 score groups is shown in Fig. 3.

A linear regression correlation analysis between the spermatogenetic score and percentage of tubules with defective tight junctions gave a coefficient of 0.92 which is highly significant ($P < 0.001$) (Tables from Clarke 1969).

During the course of the investigation it became apparent that a number of tubules showed subcellular features in the Sertoli cells which differed from those described as the normal in human seminiferous epithelium (Bawa 1963; Nagano 1966; de Kretser 1968; Schulze 1974; Schulze et al. 1976). These changes, observed in 32% of the tubules, consisted of an increase in vacuolation; condensation of the cytoplasm with altered mitochondrial morphology (condensed with small electron-dense granules), as well as increased nuclear invagination and loss of homogeneity of the nucleoplasm (Fig. 4).

Table 1. Incidence of tubular permeability to lanthanum in men with 'normal' spermatogenesis but diverse patency of the ductal system

Clinical group	*N*	Site of obstruction	Mean spermatogenic score	% of tubules with 'defective' tight junctions
Vasectomy	6	None	8.3	45
Vasectomy Reversal/Vasal-aplasia	8	Vasal	8.0	46
Obstructive Azoospermia	5	Epididymal	8.1	42

Parenchymatous degenerative changes were also seen occasionally in other elements of the germinal epithelium (Fig. 2). A correlation analysis between spermatogenetic score and percentage of tubules showing these changes failed to demonstrate any significant relationship between the two, though an analysis between them and the presence of defective Sertoli cell tight junctions was highly significant ($P < 0.001$) (Fig. 5).

In the 19 men with active spermatogenesis, but diverse patency of the ductal system (Table 1), both mean spermatogenetic scores and the incidence of tubular permeability to lanthanum were similar.

The remaining (22) men were not amenable to classification into clearly definable groups. They were found to have defective spermatogenesis of varying aetiology as in hypospermatogenesis, spermatogenetic arrest, tubular hyalinisation, mixed Sertoli-cell-only and varicocele. In this heterogeneous 'group' there was an associated increase in the incidence of tubular permeability to the tracer ($\simeq 60\%$) as might be expected from the results obtained for the analysis of the individual seminiferous tubules.

Discussion

The accumulation of tracer particles, in a concentration which makes this clearly visible with the electron microscope, was almost exclusively seen in the interstitital clefts of the basal compartment of the seminiferous tubules. This suggests that, in the human, tubular barrier mechanisms are operating and that this phenomenon occurs in all tubules irrespective of the status of fertility of the patient and the observed 'leakage' of the tracer into the adluminal compartment. The barrier was found to be located at the site of the specialised tight junctions between Sertoli cells. An additional barrier, at the level of the peritubular myoid cells, as has been observed in laboratory rodents (Dym et al. 1970) was not encountered. It is concluded that the blood-testis barrier in man is more akin to that of the monkey where the anatomical elements of barrier mechanisms are believed to be exclusively attributable to the Sertoli cell tight junctions (Dym 1973).

The penetration of lanthanum past the Sertoli cell tight junctions into the adluminal intercellular spaces of the seminiferous tubules was seen significantly more often in those tubules with a low spermatogenetic score and suggests that in these tubules barrier mechanisms are less effective. The significant correlation between the presence of defective tight junctions and subcellular changes

of the Sertoli cells suggests that pathological alterations of these cells may be associated with the observed defective barrier mechanisms.

The implication of these findings is that a common factor might be responsible for both the depressed spermatogenesis and the defective barrier mechanisms. Alternatively, the defective barrier mechanisms may either allow an alteration of the environment in such a manner as to interfere with spermatogenesis or enable antigenic spermatozoal components, which are normally shielded behind the barrier by the Sertoli cell tight junctions, to enter the circulatory system. If the latter were the case, autoantibodies might reach the inner regions of the seminiferous tubules.

In this context it is of interest to note that both tissue damage and a degree of increased tubular permeability to acriflavine were observed by Johnson (1970b) in guinea-pigs iso-immunised with testicular extract. However, in none of the biopsies examined in this study was there any evidence of an increase in immunocompetent cells. Intratubular macrophages were also not observed.

The question as to whether duct obstruction causes a breakdown of the blood-testis barrier is of clinical interest in view of the possibility that the lanthanum technique might be used to detect sub-clinical obstructive lesions in cases of idiopathic oligozoospermia. However, it was found that neither the presence nor the site of obstruction appeared to affect the incidence of permeability to the tracer and this precludes its use in this context.

Finally, the observation that impaired spermatogenesis was associated with an appreciable increase in the incidence of tubular permeability and that this, in turn, was associated with abnormalities of the Sertoli cells is further evidence of the important role that these cells play in maintaining normal spermatogenesis.

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