Effects of obstruction of the flow of seminiferous tubule fluid on the germinal epithelium in the rat

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Summary. Blocking the lumen of a single seminiferous tubule by introducing a plug of non-toxic latex produced a lesion in that tubule, but not in immediately adjacent tubules. The lesion extended for up to 50 mm from the end of the latex. Nearest to the block the tubule was completely aspermatogenic; further along the tubule, the lesion was less severe, involving disorganization and reduction in germ cell numbers, with the cells showing vacuolation, pycnosis and karyolysis. Binucleate and giant cells were common, and cells were often exfoliated into the lumen. The lesion tended to increase in length with longer times after introduction of the plug, but there appeared to be no preferential involvement of the shorter segment of the tubule between the block and the rete. The transition from damaged to healthy tubule was abrupt.

Introduction

The blood–testis barrier controls the composition of the seminiferous tubule fluid, which is thought to be important for the nurture of germ cells. Its composition probably reflects the conditions within the adluminal compartment of the germinal epithelium in which meiosis is completed (Setchell, 1970, 1978, 1980; Setchell & Waites, 1975). In addition, a possible involvement of fluid in the entrainment of the spermatogenic wave has been suggested (Perey, Clermont & Leblond, 1961) and flow of fluid may be important in the regulation of spermatogenesis.

Suoranta (1971) selectively damaged single seminiferous tubules of rats by touching the exposed testicular capsule for about two seconds with a hot tungsten wire. Marked degenerative changes occurred in the treated tubules, and in tubules in the vicinity of the assault, over the next 6 months. She noted that the lesion usually progressed along the shorter portion of the tubule between the lesion and the rete testis, while in the other direction the damage was confined to a much shorter distance, suggesting that the spread of the lesion is somehow involved with the flow of fluid. The results of preliminary experiments in which formalin or cadmium chloride were injected by micropuncture into a single seminiferous tubule of rats indicated that the lesion produced by these toxic substances progressed along the tubule to the rete testis but also affected nearby tubules (Setchell et al., 1978).

To study the role of fluid flow without causing necrosis to the epithelium, a non-toxic latex plug was introduced into single seminiferous tubules which were subsequently dissected free and examined in isolation.

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Materials and Methods

Nine male rats of the Porton-Wistar strain, weighing 250–300 g, were used. They were anaesthetized with pentobarbitone sodium (Sagatal: May & Baker, Dagenham, U.K.) and approximately 5 ml Latex-White-MR (Ballman, Ivy & Carter Ltd, Raynes Park, London, U.K.), stained with Monastral fast blue (Keego Brico Tetley Chemicals, London, U.K.), was injected by micropuncture (Tuck, Setchell, Waites & Young, 1970) into a single tubule in one testis. The tubule chosen was a superficial one on the lateral surface of the testis away from obvious blood vessels. Because of the complex arrangement of the tubules in the testis, it was not possible to tell in advance how near the end of the tubule the puncture was being made, but in the event all were more than about 10 mm from the rete, measured along the tubule. The latex hardens when exposed to aqueous fluids, forming a plug. The testes were then returned to the scrotum and the abdominal wall sutured.

Dissection of the tubules

Pairs of rats were killed 1, 7, 21 or 49 days later and the testes removed. Dissection of the tubules was aided by infusing trypsin (Sigma, Poole, Dorset, U.K.) solution (2.5 mg/ml in 0.9% (w/v) NaCl) into the testicular artery, or by decapsulating the testis and simply shaking the tubules free in a Petri dish containing 2.5 mg trypsin/ml 0.9% NaCl. After shaking for about 30 min, the decapsulated testes were immersed in a solution of trypsin inhibitor (Sigma; 2.5 mg/ml in 0.9% (w/v) NaCl). The enzyme treatment had no effect on the histology of the tubules, and tubule lengths of up to 15 cm could be dissected free in this way. The microinjection of a solution of Lissamine green (Gurr, London, U.K.) into the blocked tubule before dissection proved useful in identifying the same tubule over a considerable length, mostly along the whole length of the shorter route to the rete testis.

Histological processing

The tubules were cut into two pieces, through the latex block and each section handled separately.

Each portion was coiled into a glass chamber (Perey et al., 1961) during fixation for 18 h in 5% glutaraldehyde : saturated aqueous picric acid : glacial acetic acid (25:75:1 by vol.) (Solcia, Vassalo & Capella, 1968). Other fixatives which were suitable for larger sections of testis were too severe for use with the isolated tubules. After transfer to 70% alcohol, the tubules were carefully removed from the chambers into Petri dishes and held flat by a ‘bridge’ of coverslips. Solutions were changed using a Pasteur pipette. The tubules were double-embedded in both celloidin and paraffin wax while still contained in the Petri dish. Sections (8 μm) were cut in the longitudinal plane and stained with haematoxylin and eosin. By this method, a considerable length of tubule was preserved on a single slide.

Evaluation

Reconstructions of the tubule were made using a low power (×2.5) microscope and drawing tube (Wild). Each tubule was divided into segments of 2.3 mm (5 cm on the drawing). Under high magnification, each segment was examined and the general appearance of the epithelium, the cell types present and the overall direction of the spermatogenic wave were noted.

One testis was fixed in its entirety in Bouin’s fluid, 14 days after the microinjection of latex plug into one tubule, and serially-sectioned in the transverse plane, in order to examine the response of adjacent, uninjected tubules. Checks were made with representative sections of other testes.
The latex formed an effective plug in the tubule lumen and resulted in the formation of a lesion in the tubule which spread along the tubule in both directions. Seen in serial sections of the whole testis, the effect produced by the latex blockage was not transmitted to adjacent tubules (Pl. 1, Fig. 1). In the immediate vicinity of the latex, margination of polymorphonuclear leucocytes in the blood vessels was apparent after 14 days, but this inflammatory reaction remained confined to the area of the plug.

Classification of lesion

The tubular lesion decreased in severity (see Pl. 1, Figs 2–6) as the distance from the latex plug increased. Tubules were classified into four categories: (i) aspermatogenic, (ii) severe damage, (iii) slight damage, and (iv) normal.

Aspermatogenesis. In the immediate vicinity of the latex there appeared to be no surviving germ cells. The tubule diameter was reduced (0.16 ± 0.03 mm) and the epithelium was extensively vacuolated, giving a ‘soap bubble’ appearance. Inclusions of latex were often present in the Sertoli cell cytoplasm, and occasional macrophages containing latex were found. The remaining cells were necrotic and unidentifiable. Often in this region the lumen was blocked by a plug of PAS-positive material and sperm debris.

Severe damage. Some germ cells were present, but in markedly reduced numbers (Pl. 1, Fig. 3). There was great variability in the first germ cell types encountered after the aspermatogenic zone, i.e. in the cell types surviving nearest to the plug. However, the most common types were round spermatids and early prophase spermatocytes (Pl. 1, Fig. 4). Most cells were abnormal, demonstrating acidophilia, pycnosis and karyolysis. The general appearance of the epithelium in this region was one of great disorganization, vacuolation and exfoliation of cells into the lumen, leading to the appearance of areas depleted of germ cells (Pl. 1, Figs 4, 5 and 6).

Spermatogonia were apparently unaffected 1 day after the introduction of the block but at later times were dense and hyperchromatic, especially at stages of transition from type A to intermediate, or from intermediate to type B, and were rarely seen in mitosis.

Newly formed preleptotene spermatocytes showed changes similar to those seen in the spermatogonia. Early prophase primary spermatocytes showed vacuolation and clumped chromatin as early as Day 1 but primary spermatocytes in late prophase seemed less vulnerable. In one tubule at Day 1, meiosis and the formation of secondary spermatocytes and spermatids appeared unaffected. By Day 7, primary spermatocytes in late prophase (pachytene onwards) seemed to be decreased in number. Early prophase primary spermatocytes were present, but near the latex they were ragged in appearance. Their condition improved with increasing distance from the latex plug, but all primary spermatocytes showed some degenerative changes. At later times, the number of spermatocytes was reduced and they showed extensive degeneration. Depletion of spermatocytes was especially common in areas containing late prophase spermatocytes approaching meiosis.

Round spermatids as early as Day 1 but especially at Day 7 and later showed vacuolation of the nucleus, in which the chromatin was pushed to the periphery, giving a ‘signet-ring’ appearance. Binucleate or giant cells were common (Pl. 1, Figs 4, 5 and 6). On Day 1, mature spermatozoa were often found at the periphery of the tubules, their heads close to the basement membrane. They appeared to be trapped, prevented from migrating towards the lumen of the tubule where they would normally be found. Mature spermatids and spermatozoa were rare in tubules at Day 7, and spermatozoa were absent from the epithelium at later times, when cell ghosts, pycnotic spheres and degenerating giant cells were found in the lumen (Pl. 1, Fig. 5).

Slight damage. The transition from the severely damaged epithelium described above to more healthy tubule morphology was usually abrupt. In slightly damaged regions, epithelial
height was normal and a full complement of germ cells was present. The only signs of abnormality were isolated areas of vacuolation (Pl. 1, Fig. 7) and the presence of hyperchromatic or pycnotic spermatogonia and early leptotene primary spermatocytes. Late prophase spermatocytes occasionally exhibited disorganization of their chromosomes and karyolysis. In some tubules, there were areas of caseation and cellular disorder in an otherwise normal epithelium (Pl. 1, Fig. 8).

Changes in the extent of the lesion with time

The extent of the aspermatogenic region appeared to rise to a maximum at 21 days, and then to decrease again, although there was considerable variability between animals (Table 1). The extents of the severe and slight lesions was even more variable, but generally increased between 1 and 7 days and showed no consistent change thereafter. In the severely damaged region, the presence of early stages of spermatogenesis at 21 and 49 days indicated that some stages of spermatogenesis could continue in the face of a chronic damaging effect of the tubule blockage.

Direction of spread of lesion

As each tubule consists basically of a two-ended loop opening at both ends into the rete, two segments of tubule could be identified, namely a shorter and a longer route to the rete from the latex block. The respective lengths of the three types of lesion were not consistently greater in the

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PLATE 1

Fig. 1. A transverse section of a rat testis in which a latex block had been introduced into one tubule. The aspermatogenic portion of this tubule, cut twice, can be seen at the lower edge of the picture, but completely normal sections of other tubules are immediately adjacent. × 70.

Fig. 2. A reconstruction, made using a drawing tube, of a dissected seminiferous tubule in which a latex block had been introduced 21 days previously. The lettered circles indicate the regions from which Figs 3 to 6 were taken.

Fig. 3. The tubule at region A (Fig. 2) 17-2 mm from the end of the latex plug. There are only a few isolated germ cells, which are undergoing pycnosis or karyolysis. × 270.

Fig. 4. The tubule at region B (Fig. 2) 24 mm from the end of the latex plug. There are more germ cells than in region B, mostly leptotene spermatocytes and round spermatids. Some of the former show pycnosis or karyorrhexis. The chromatin of the latter is arranged at the periphery of the nucleus, giving a signet ring appearance and there are some binucleate cells. The epithelium is disordered and vacuolated and cells have been exfoliated into the lumen. × 270.

Fig. 5. The tubule at region C (Fig. 2) 33 mm from the end of the latex plug. The epithelium is low and disorganized. Two generations of spermatocytes, leptotene/zygotene and pachytenes are present, but show pycnosis with vacuolization and karyorrhexis respectively. The early spermatids have formed giant cells, with pyknotic nuclei or giant cell ghosts. × 270.

Fig. 6. The tubule at region D (Fig. 2) 40 mm from the end of the latex block. The epithelium is narrow, disorganized and vacuolated: the leptotene/zygotene spermatocytes are pycnotic and many of the pachytenes spermatocytes are exfoliated. The spermatids have formed exfoliated multinucleate giant cells, many of which are degenerating. × 220.

Fig. 7. Portion of a different tubule 21-4 mm from the end of the latex plug which had been introduced 7 weeks previously. The epithelial height is normal and all generations of germ cells are present. Vacuolation of the epithelium is evident and the nuclei of the early spermatocytes are hyperchromatic. × 270.

Fig. 8. Portion of another tubule, 14 mm from the end of a latex plug which had been introduced 21 days previously. The epithelium is normal except for an isolated area of caseation. In the vicinity of this area, the pachytenes spermatocytes show karyorrhexis and lysis. × 270.
part of the tubule which represented the shorter route to the rete testis, although again there was considerable variability between animals. Of the tubules examined, exactly half showed longer lesions in the longer route to the rete and there was no significant correlation between duration of the experiment (up to 7 weeks) and the ratio of the extent of the severe damage spreading along the shorter and longer route to the rete testis (Table 1).

Table 1. Lengths (in mm) of seminiferous tubules (each line gives value for a tubule from 1 rat) showing various forms of lesion after introduction of a plug of latex

<table>
<thead>
<tr>
<th>Days after introduction of latex</th>
<th>Class of lesion</th>
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<tbody>
<tr>
<td></td>
<td>Aspermatogenic</td>
<td>Severe</td>
<td>Slight</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shorter route to rete</td>
<td>Longer route to rete</td>
<td>Shorter route to rete</td>
<td>Longer route to rete</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>6.8</td>
<td>4.5</td>
</tr>
<tr>
<td>7</td>
<td>2.3</td>
<td>2.3</td>
<td>9.1</td>
<td>13.6</td>
</tr>
<tr>
<td>21</td>
<td>0.5</td>
<td>4.5</td>
<td>4.5</td>
<td>45.4</td>
</tr>
<tr>
<td>49</td>
<td>10.9</td>
<td>4.0</td>
<td>22.7</td>
<td>6.8</td>
</tr>
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Discussion

These experiments do not confirm Suoranta's (1971) observations that damage in a single seminiferous tubule spreads preferentially along the shorter route to the rete testis, but do demonstrate clearly that a block to the flow of luminal fluid has a drastic effect on the germinal epithelium.

This lesion should be compared with other forms of testicular damage. A threshold dose of X-irradiation first kills dividing spermatogonia although the later stages of spermatogenesis are subsequently affected by 'maturation depletion' (Dym & Clermont, 1970). Local heating of the testis seems to affect firstly the pachytenic spermatocytes, and again 'maturation depletion' follows (Waites & Ortavant, 1968). There is some debate about the effects of local ischaemia; Steinberger & Tjioe (1969) believed that they found specific damage to dividing spermatogonia whereas Oettlé & Harrison (1952) felt that early spermatids, late prophase spermatocytes and prophase spermatogonia were all affected in their experiments. More severe exposure to ischaemia (Steinberger & Tjioe, 1969), heat (Steinberger & Dixon, 1959; Waites & Ortavant, 1968; Collins & Lacy, 1969) or X-irradiation (Shaver, 1953; Oakberg, 1955) led to a more generalized destruction of the epithelium, injured cells often differentiating to give rise to abnormal cells after division (Shaver, 1953). Exfoliation of germ cells and the formation of giant cells occur after several different treatments, and pycnosis and karyolysis of the spermatogonia and prophase spermatocytes are common early signs of death of these cells.

In the present study, designed to follow the length of the degenerative lesion rather than its nature, detailed observations of cell numbers were not made, but the initial effect of the blockage does not seem to be specific. As the impaired late prophase spermatocytes do not appear until 7 days after introduction of the blockage, this effect may be due to a direct effect upon these cells, or to a latent effect produced by damage to spermatogonia or early spermatocytes.

The present lesion does not seem to result from maturation depletion alone, as it takes at least 30 days in the rat to deplete the epithelium completely in this way after damage to the spermatogonia (Shaver, 1953; Dym & Clermont, 1970). The appearance of a short aspermatogenic region at 7 days and a sizeable one at 21 days indicates that the blockage to
fluid flow exerts a more generalized influence on the spermatogenic epithelium. The presence of a short aspermatogenic region and a long portion of severely damaged epithelium at 49 days suggests that the effect on the cells is prolonged. The ‘trapping’ of spermatozoa near the basement membrane, the sloughing of germ cells into the lumen and the widespread disorder and vacuolation observed throughout this study imply that Sertoli cell function may be impaired.

What then is the likely cause of the lesion? It seems unlikely that it is due to alterations in blood supply to the tubules which consists of an extensive network of capillaries running parallel with and over the seminiferous tubules in a ladder-like fashion (see Setchell & Waites, 1975) and which is capable of maintaining an adequate blood supply to the tubules even after experimental occlusion of some of the arterioles by microspheres (Suoranta, 1971). Although there were a few macrophages at the site of the latex and evidence of mild, chronic inflammation in the vicinity of the point of injection, these changes were not considered to be indicative of a significant immune response. This is in accordance with the results of Levine & Sowinsky (1970) who used a soldering iron to produce focal injury.

One curious feature of the lesion after blockage of the tubule is the abrupt transition from severely damaged to nearly normal tubules. A similar sharp transition was also found in tubules of X-irradiated rats (Hall & Hupp, 1970). The rate of spread of the lesion after blockage of the tubule is slower than that produced by deliberate injury to the epithelium. After touching a tubule with a hot needle, Suoranta (1971) reported a progression rate of 150 mm in 16 days. In addition, the latex-blocked tubules showed some signs of regeneration, not apparent in the ‘burned’ tubules indicating that the latter treatment produced a more severe effect on the tubules than that which results from obstructing fluid flow.

Although the chemical toxicity of the latex itself cannot be ruled out entirely our unpublished observations indicated that the effect of cadmium chloride or formalin, both frankly toxic, on the tubules is different from that of latex. After cadmium chloride or formalin administration, necrosis is rapid and coagulative. The necrotizing effect spreads in a radius to affect adjacent tubules or half tubules nearby. Furthermore, after cadmium injections there is a massive immune response, an accumulation of polymorphonuclear leucocytes in the interstitium after 2 days and infiltration by mononuclear cells by 16 days.

The cause of the latex lesion therefore seems to be mediated largely by the prevention of the flow of fluid in the tubule. It is difficult to find an explanation for the formation of a progressive lesion after obstructing fluid flow. There was no obvious distension of the tubule from accumulation of fluid near the plug, as is seen after efferent duct ligature (Setchell, 1970), so it must be concluded that secreted fluid flowed away from the plug in both directions. The fluid is probably very important for spermatogenesis as it provides the milieu in which the meiotic divisions are completed and in which sperm maturation takes place (Setchell & Waites, 1975; Setchell, 1980). The constant ebb and flow motion of the fluid in the tubules (Setchell et al., 1978; Hinton, 1979) makes it possible that adjacent areas of tubule may influence each other by the secretion of metabolites or messengers in a complex way. It is known that the presence of the germ cells themselves may alter the composition of the fluid. In busulphan-treated or prenatally X-irradiated rats in which some of the tubules are devoid of germ cells, the fluid in these tubules is serum-like in composition (Levine & Marsh, 1975; Setchell et al., 1978). The prevention of flow in the tubules may lead to local build-up of metabolites and/or shortage of some essential substrates in the tubule lumen and to pathological changes in differentiating germ cells. These degenerative changes may then lead to an alteration of the composition of the secreted fluid and so the lesion would progress.

At this stage there can be no satisfactory single explanation for the lesion but it is clear that uninterrupted fluid flow is essential for the maintenance of a healthy germinal epithelium, emphasizing again the importance of this unique fluid for spermatogenesis. It will be important to determine whether a similar lesion occurs in tubules which have been severed, and thereby presumably obstructed, during testicular biopsy.
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