VASECTOMY: MORPHOLOGICAL AND IMMUNOLOGICAL EFFECTS IN THE RAT.

© STUART WILLIAM McDONALD

A thesis submitted to the University of Glasgow for the degree of Doctor of Philosophy.

Department of Anatomy,
University of Glasgow.

February, 1989.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

CONTENTS

TABLES AND FIGURES

ACKNOWLEDGMENTS

I am indebted to Professor R. J. Scothorne for his advice and support during the preparation of this thesis. I am also most grateful to Mr N. K. Bennett, Miss M. Hughes and Mr A. Lockhart for technical help and Miss C. Morris for art work.

SUMMARY

This thesis examines morphological and immunological effects of vasectomy in inbred Albino Swiss rats. It comprises six separate studies.

1) The response of the regional testicular lymph node six and nine months after vasectomy.

This work extends a previous study and shows that, while the response to vasectomy of the regional testicular lymph node increased in magnitude up to and including 3 months after operation, it waned through 6 and 9 months. The findings indicate the involvement of the regional lymphatics and lymph nodes in the formation of circulating antisperm antibodies after vasectomy. The response of the testicular nodes varied between individual rats.

2) The lymphatic drainage of the epididymis and of the ductus deferens, with reference to the immune response to vasectomy.

The lymphatic drainage pattern of the unoperated reproductive tract is determined. Epididymal lymphatics always united with those of the testis. There is little previous work on lymphatics of the ductus deferens. The more cranial part of the scrotal ductus drained towards the inguinal canal and the iliac nodes. Lymphatics from the more caudal part of the ductus united with those of the epididymis. The results suggested a lymphatic watershed about the middle of the scrotal ductus.

The role of variations in the lymphatic drainage of the sperm granuloma, the site of release of spermatozoal antigens, in the variable responses of the testicular lymph node to vasectomy is investigated. Vasectomy did not interrupt the lymphatic drainage of the epididymis. While lymph from epididymal granulomas invariably reached the regional testicular node that from vasal granulomas may occasionally have failed to do so. Variations in the lymphatic drainage of vasal granulomas, but not epididymal ones, may have been partly responsible for the lack of response in certain testicular nodes reported previously.

3) <u>The response of the regional lymph node to epididymal sperm granulomas after vasectomy.</u>

The contribution of variations in the lymphatic drainage of vasal sperm granulomas to the variable nodal response to vasectomy is further analysed. By carrying out vasectomy at the junction of the ductus deferens with the epididymal duct, sperm granulomas were induced to form at the epididymis; at this site, their lymph always drains to the testicular node. In spite of the presence of epididymal granulomas in all rats 12 weeks after vasectomy, not all testicular nodes responded. It is concluded that variations in the lymphatic drainage of vasal granulomas had not been wholly responsible for the variations in the response of the testicular lymph node found previously and that additional unknown factors were involved.

4) On the mode of sperm autoantigen presentation to the regional lymph node of the testis after vasectomy.

Cytological smears were used to detect whole spermatozoa in the testicular lymph nodes of vasectomised rats. Very few spermatozoa were found; this contrasts with the large numbers reported, by others, in nodes from rams and boars. The basis of this species difference is unclear.

5) A quantitative study of the effect of vasectomy on spermatogenesis.

Quantitative studies of the cycle of the seminiferous epithelium and seminiferous tubular dimensions were performed on the testes of rats vasectomised for 6 months. The results indicate that, in functional tubules, there was no alteration in the cycle of the seminiferous epithelium, no tubular distension and no retention of spermatozoa.

6) The effect of testicular biopsy on the regional lymph node of the testis.

Testicular biopsy, like vasectomy, damages epithelial barriers of the reproductive tract. This study investigates whether the procedure produces lymph node changes similar to those seen after vasectomy.

The testicular lymph nodes of rats 1 and 3 months after testicular biopsy were indistinguishable in every way from those of sham-operated control animals. The

absence of lymph node changes following testicular biopsy is likely to reflect the minimal amount of spermatozoal extravasation and inflammation in the testis. The study offers some reassurance about the safety of testicular biopsy in the investigation of male infertility.

INTRODUCTION

VASECTOMY: MORPHOLOGICAL AND IMMUNOLOGICAL EFFECTS*

In the past twenty years much has been learned about the effects of vasectomy but several problems of biological and clinical importance remain. This literature review poses questions about the effects of vasectomy, with special reference to clinical problems and areas of controversy, and highlights the contribution of morphological and experimental studies to current knowledge.

Does vasectomy harm general health?

In the 1970s considerable concern was expressed that the formation of circulating sperm autoantibodies after vasectomy might precipitate autoimmune diseases. The worry following reports of increased immune-complex deposition in the kidneys of vasectomised rabbits and monkeys (Alexander & Tung, 1979a & b) was intensified by the finding of arterial damage in monkeys and humans (Clarkson & Alexander, 1980; Fahrenbach et al., 1980; Campbell et al., 1983) despite negative results from some groups (Linnet et al., 1982; Lauersen et al., 1983).

The future of vasectomy seems assured, however, by several papers indicating no increase in autoimmune diseases (Mathews et al., 1976; Bullock et al., 1977), blood coagulation disorders (Kisker et al., 1979) or

* A shorter version of this review has been published in Clinical Anatomy 1 187 - 195 (1988).

ischaemic heart disease (Goldacre et al., 1983; Petitti et al., 1983; Walker et al., 1983; Perrin et al., 1984; Rosenberg et al., 1986). Further work is required, however, to investigate suggestions that the peripheral rather than the coronary arteries might be affected (Campbell et al., 1983) and that vasectomy exacerbates arterial disease in diabetic monkeys (Bansal et al., 1986).

Does vasectomy cause any longterm problems?

Two specific problems may arise from vasectomy: low fertility after vasectomy reversal, and chronic local pain or discomfort.

1) Low fertility after vasectomy reversal

While about 90 percent of vasovasostomised patients regain spermatozoa in their ejaculates, less than 70 percent are fertile (Silber, 1978; Lee & McLoughlin, 1980; Martin, 1981; Weinerth, 1984; Jarow et al., 1985; Lee, 1986). There are four general reasons for this discrepancy.

a) Anatomical/ functional changes in the reproductive tract

Poor quality sperm reflects anatomical and/ or functional changes in the reproductive tract. Stricture at the vasovasostomy site is well documented and treatable by repeat operation (Silber, 1978; Owen & Kapila, 1984). The precise nature of other changes is poorly defined in man.

b) Antisperm antibodies

While some workers believe immunological causes of vasovasostomy failure to be rare (Silber, 1978; Thomas et al., 1981), there is increasing evidence that seminal plasma antisperm antibodies reduce fertility. They are reported in 15 to 35 percent of vasovasostomised patients in association with high titres of serum antisperm antibodies (Linnet et al., 1981; Fuchs & Alexander, 1983; Parslow et al., 1983; Requeda et al., 1983; Sutherland et al., 1984). Some workers consider the pre-reversal serum antibody titre to be a useful prognostic indicator of the success of vasovasostomy (Linnet et al., 1981; Fuchs & Alexander, 1983) but others refute this (Parslow et al., 1983; Weinerth, 1984).

c) Infection

Weinerth (1984) found increased numbers of leukocytes in the semen of some vasovasostomised patients and that antibiotic therapy improved fertility.

d) Infertile partner

Since many reversals of vasectomy are requested after remarriage, some of the disappointing results may not be related to the vasovasostomy itself, but rather may reflect infertility in the wife, or husband-wife incompatibility.

2) Chronic local pain or discomfort

Epididymal distension and sperm granuloma formation may cause temporary discomfort, but in some vasectomy

patients symptoms persist and may be intensified by ejaculation. A range of treatments has been advocated (Livingstone, 1971; Esho et al., 1973; Schmidt, 1979; Shapiro & Silber, 1979; Edwards & Errey, 1982; Selikowitz & Schned, 1985).

If the above problems are to be prevented or alleviated, information is required on the following sequelae of vasectomy: changes in the reproductive tract and autoimmunity to spermatozoa.

1) CHANGES IN THE REPRODUCTIVE TRACT

What is the effect of vasectomy on the testis?

There has been much debate about the effect of vasectomy on the endocrine and spermatogenic functions of the testis.

The hormonal effects of vasectomy have been reviewed by Richards (1981). There is general agreement that vasectomy probably produces no marked effect on the pituitary-testicular axis in any species. The results of some workers, however, have suggested there may be hormonal changes but that these are generally small and within the normal range; this controversy remains unsettled.

The effect of vasectomy on the seminiferous tubules shows species variations; the dog undergoes temporary depression of spermatogenesis (Grewal & Sachan, 1968; MacDougall et al., 1975), while guinea pigs suffer

autoimmune orchitis (Tung, 1979), although this has been disputed (Muir et al., 1976). Several groups report various testicular changes in humans: spermatogenic arrest, tubular dilatation, basement membrane thickening and interstitial fibrosis (Gupta et al., 1975; Fallon et al., 1978; Bigazzi et al., 1979; Jenkins et al., 1979; Choi & Reiner, 1983; Jarow et al., 1985; Mehrotra et al., 1985). It is possible that some testicular changes may be reversed by vasovasostomy (Jenkins et al., 1979; Mehrotra et al., 1985).

What are the mechanisms of the testicular changes?

The aetiology of the testicular lesions in man is unclear, but animal experiments have revealed a number of possible mechanisms.

a) Non-specific

In the rat, many workers have reported normal testes after vasectomy (Oslund, 1924; Poynter, 1939; Smith, 1962; Flickinger, 1972b; Kwart & Coffey, 1973; Neaves, 1974; Hernandez-Jauregui & Olivera, 1975; Harris & Lipshultz, 1981). Some studies, however, have shown testicular atrophy in some individuals (Rumke & Titus, 1970; Thakur et al., 1972; Sackler et al., 1973; Kuwahara & Frick, 1975) which later workers believe to have been caused by non-specific side-effects of vasectomy, such as damage to blood supply, cryptorchidism or infection (Plaut, 1973; Heller & Rothchild, 1974; McGlynn & Erpino, 1974; Neaves, 1974).

b) Autoimmune orchitis

Autoimmune orchitis is reported to follow vasectomy in the guinea pig, mouse, monkey and rabbit (Alexander & Tung, 1977; Tung, 1979; Tung & Alexander, 1980; Anderson & Alexander, 1981). Most rats do not suffer immunological orchitis but certain strains may be susceptible (Neaves, 1978). Al-Saffar (1987) reports bilateral testicular atrophy in unilaterally vasectomised Albino Swiss rats; the bilateral nature of these changes suggests an immune aetiology, but no active autoimmune orchitis has yet been observed in rats. Whether immunological orchitis occurs in some species and strains but not in others is important as it implies that certain patients could be susceptible to the condition.

c) Mechanical effects

In unilaterally vasectomised rats, degeneration of the ipsilateral testis is often associated with a sperm granuloma in the caput epididymidis (McDonald & Scothorne, unpublished). Bedford (1976) reports similar findings in vasectomised hamsters and rabbits. The damage to the seminiferous epithelium is likely to result from increased intraluminal pressure (McDonald et al., in preparation). Whether similar degeneration occurs in man is unknown.

Does vasectomy affect the cycle of the seminiferous epithelium?

Few workers reporting normal testes after vasectomy have looked for subtle changes, such as disturbance of the

cycle of the seminiferous epithelium, which might be revealed only by detailed quantitative analysis of individual tubules.

Such studies suggest that spermatogenesis is altered in the mouse and rat (Croft & Bartke, 1976; Lamano-Carvalho et al., 1984) but is unaffected in the bull and monkey (Amann, 1962; Hadley & Dym, 1983). Lamano-Carvalho et al. (1984) and Miller et al. (1984) have suggested that spermatozoal retention occurs in the rat testis. Chapter 5 of this thesis presents the results of a more detailed analysis of the rat testis designed to detect alterations in the cycle of the seminiferous epithelium and retention of mature spermatozoa after vasectomy.

How does vasectomy affect the epididymis and ductus deferens?

Depending on the species, the continued production of sperm causes distension and/ or leakage of the epididymis and ductus deferens, distension usually preventing or delaying leakage. In all species extravasated spermatozoa form a sperm granuloma, a mass of spermatozoa surrounded by macrophages and chronic inflammatory cells, which is an important site of sperm destruction. The macrophages are epithelioid in type and phagocytose the spermatozoa (Bedford, 1976; Chapman & Heidger, 1979); occasional Langhans giant cells are present. The macrophage layer is surrounded in turn by a layer of vascular connective tissue rich in lymphocytes and plasma cells. Sperm granulomas are not specific to vasectomy but may follow

spermatozoal extravasation from any cause eg. trauma or infection (Steinberg & Straus, 1947; Friedman & Garske, 1949). Their histological features resemble tuberculous granulomas and they are believed to be induced by a lipid component of spermatozoa chemically similar to the mycolic acid of tubercle bacilli (Berg, 1954).

In rats, vasectomy produces minimal tract distension and granuloma formation occurs regularly within two weeks at the epididymis and/ or vasectomy site (Smith, 1962; Kennedy & Heidger, 1980; McDonald & Scothorne, 1986). Other species are more variable in their timing of granuloma formation. The hamster tract, like that of the rat, shows little distension and granulomas form within a few weeks of vasectomy (Johnson & Howards, 1975). In guinea pigs and monkeys, sperm granulomas often take weeks or months to form (Alexander, 1977; Galle & Friend, 1977; Chapman et al., 1978; Howards & Johnson, 1979). The rabbit tract shows gross distension after vasectomy; some individuals never develop granulomas and in others they appear only after several months have elapsed (Jones, 1973; Alexander & Tung, 1977; Alexander & Tung, 1979b). In man, the tract usually distends and sperm granulomas form after variable intervals. There is no firm agreement on the incidence of granuloma formation in man but it is probably between 30 and 60 percent (Alexander & Schmidt, 1977; Silber, 1978; Owen & Kapila, 1984; Lee, 1986).

Limited information is available on the mechanism of sperm granuloma formation. When granulomas form in the rat, the tract epithelium shows local thinning at the breach (Kennedy & Simon, 1974). Howards and Johnson (1979) have shown in the hamster that granuloma formation dissipates the intraluminal pressure resulting from continued sperm production. Multiple areas of epithelial thinning associated with the entry of neutrophils into the tubular lumen suggest a gradual seepage into the surrounding tissue rather than a sudden rupture. Neutrophils frequently invade the sperm in granulomas and migrate into communicating tubules (Bedford, 1976; Al-Saffar, 1987).

In rats, sperm granulomas tend to form in progressively more proximal regions of the tract (Al-Saffar, 1987). Presumably an enlarging granuloma compresses and blocks surrounding tubules or is limited in size by neighbouring structures so that, whatever the mechanism, it no longer relieves the intraluminal pressure and another, more proximal, granuloma forms.

The literature on the life history of sperm granulomas is confused as many workers have not related vasal and epididymal granulomas. Al-Saffar (1987) studied rats to 18 months after vasectomy and noted that solitary granulomas gradually enlarged throughout the period. When a second more proximal granuloma had formed, sperm entered

it preferentially and the original granuloma became smaller as phagocytosis continued.

After vasectomy does sperm phagocytosis occur only in granulomas?

In vasectomised men, monkeys and rabbits, macrophages in the epididymal lumen degrade spermatozoa (Phadke, 1964; Alexander, 1972; Alexander & Tung, 1977; Tung & Alexander, 1980). In contrast, intraluminal phagocytes are rare at sites remote from granulomas in rats and guinea pigs (Flickinger, 1972a; Alexander, 1973; Galle & Friend, 1977).

Several workers have performed ultrastructural studies of the rat epididymal epithelium to determine whether its cells phagocytose sperm following vasectomy. It is certain that the epithelium resorbs products of intraluminal sperm degradation or dissolution but it is likely to play, at most, a minor role in the phagocytosis of whole spermatozoa (Flickinger, 1972a; Alexander, 1973; Cooper & Hamilton, 1977).

In man the relative importance of intraluminal macrophages and sperm granulomas in sperm degradation after vasectomy is not established, but in rats the granuloma is undoubtedly the prime site.

What effects does vasectomy have on the innervation of the tract?

Vasectomy interrupts the sympathetic neurons which supply the cauda epididymidis and proximal ductus

deferens. The resulting alteration in smooth muscle function may be important in granuloma formation and in the poor results of vasovasostomy. Evans et al. (1972) found that epididymal sperm granulomas formed in rats after administration of guanethidine, which depletes the nerve endings of noradrenaline. In dogs, it has been shown that sparing of the nerves at vasectomy gave better sperm counts after vasovasostomy (Esk & Pabst, 1981).

2) AUTOIMMUNITY TO SPERMATOZOA

What is the nature of spermatozoal autoantigens?

Spermatozoa are autoantigenic because they possess unique chemical moieties and do not form until puberty, while immunological tolerance to other body tissues develops in fetal life. Autoantigens are not confined to mature spermatozoa, but also occur on spermatocytes and spermatids (Millette & Bellve, 1977; O'Rand & Romrell, 1977; Tung & Fritz, 1978). The first autoantigens are believed to appear on pachytene spermatocytes shortly after they penetrate the junctional complexes between Sertoli cells (O'Brien & Millette, 1984). Recent work by Yule et al. (1988) indicates that some autoantigens appear on preleptotene spermatocytes before they penetrate the blood-testis barrier. These workers suggest that, under normal conditions, the immunological privilege of the testis prevents autoimmune responses to these antigens. Ejaculated spermatozoa are additionally covered in "coating antibodies"; these are substances, such as lactoferrin, which are produced by the accessory glands of

the reproductive tract and adsorbed on to the surface of the spermatozoon (reviewed by Mancini, 1976).

Various components of spermatozoa are autoantigenic and these vary with the species. Several autoantigenic proteins and glycoproteins have been isolated from guinea pig spermatozoa (reviewed by Boettcher, 1977) and a sperm-specific form of lactate dehydrogenase has been shown to be autoantigenic in mice and rabbits but probably not in man (Kolk, 1979). The best documented human spermatozoal autoantigen is protamine, a basic arginine-rich compound found in spermatozoal nuclei (Samuel & Kolk, 1979). Antibodies against spermatozoal protamines have caused some concern because of the potential risk of cross-reaction with salmon protamines, used in insulin preparations and in the reversal of heparin-induced anticoagulation for cardiac catheterisation.

Why does an autoimmune response occur after vasectomy?

Spermatozoa are normally sequestered from the immune system by the blood-testis barrier, formed primarily by junctional complexes between Sertoli cells, and by similar complexes between the epithelial cells of the other regions of the tract (reviewed by Fawcett, 1979). The tightness of junctional complexes varies in different parts of the reproductive tract. Freeze-fracture studies have suggested that the complexes are best developed in the seminiferous tubules and weakest in the rete testis. Spermatogonia and preleptotene spermatocytes lie basal to

the blood-testis barrier; the developing germ cells pass through the barrier at the leptotene spermatocyte stage (Russell, 1977 & 1978). Vasectomy produces localized damage to these barriers.

In the rat, the sperm granuloma is assumed to be the main site of sperm antigen escape for three reasons: 1) it is a site of considerable sperm phagocytosis; 2) it is rich in lymphocytes and plasma cells; 3) the remainder of the reproductive tract appears normal.

In some species, however, there may be other sites where the barriers are damaged. A humoral response to escaping antigen may result in the deposition of immune-complexes at sites of antigen-antibody interaction. Such complexes have been found in the caput epididymidis in monkeys (Tung & Alexander, 1980) and in the testes of rabbits (Bigazzi, 1979), suggesting that leakage of antigens was occurring at these locations.

Vasectomy is not the only surgical procedure to damage the epithelial barrier of the reproductive tract. Testicular biopsy, by severing the seminiferous tubules, also risks an autoimmune response to spermatozoa. Chapter 6 of this thesis presents the results of a study of the effect of testicular biopsy on the regional lymph node in rats.

<u>Could epididymal intraepithelial lymphocytes be vehicles of antigen escape?</u>

The epididymal epithelium contains numerous lymphocytes which are mostly cytotoxic/ suppressor T-cells

(Ritchie et al., 1984). Rather than promoting immune responses to epididymal sperm, they may be active in preventing them. Preliminary studies in rats have suggested that their numbers are unchanged by vasectomy (Al-Saffar, 1987). The epididymal epithelium, of man and rat at least, also contains macrophages and monocytes; the role of these cells is unknown (Wang & Holstein, 1983; Al-Saffar, 1987).

What is the nature and timing of the autoimmune response to vasectomy in man?

Antisperm antibodies are usually detected by modifications of the sperm agglutinating technique of Kibrick et al. (1952) or of the sperm immobilizing technique of Isojima et al. (1968). Sperm immobilizing activity is almost always found in conjunction with high titres of sperm agglutinating antibodies and there is debate as to whether both tests detect the same antigens, the agglutinating technique being the more sensitive.

About 60 percent of vasectomised patients develop serum sperm agglutinating antibodies (Shulman et al., 1972; Samuel et al., 1975; Ansbacher et al., 1976) and about half of these individuals also have sperm immobilizing antibodies (Gupta et al., 1975; Ansbacher et al., 1976; Lucas & Rose, 1978). The timing of the response varies between individual patients; most develop the antibodies during the first year after operation, some as early as two weeks but others after several months (Shulman et al., 1972; Ansbacher, 1974; Samuel et al.,

1975). In some individuals the response is relatively transient and in others it may fluctuate. Some workers report that the response declines one to two years after vasectomy (Ansbacher et al., 1976) but most have shown that many patients have appreciable antisperm antibody titres for many years (Alexander et al., 1974; Gupta et al., 1975; Linnet & Hjort, 1977; Rumke & Hellema, 1979).

There is some evidence that antibodies develop more readily in younger men (Lucas & Rose, 1978) and in men with higher sperm counts (Linnet & Hjort, 1977). The relationship of sperm antibody formation with sperm granuloma development is unclear.

Few studies have been performed to determine whether cell-mediated immunity against spermatozoa occurs in man and animals. Guinea pigs, rabbits and monkeys are believed to show autoimmune orchitis, which suggests that cell-mediated immunity occurs in these species (Alexander & Tung, 1977; Tung, 1979; Tung & Alexander, 1980; Anderson & Alexander, 1981). Because of technical difficulties few studies of cell-mediated immunity have been performed and the results are conflicting (Brannen et al., 1974; Nagarkatti & Rao, 1976; Muir et al., 1977; Tumboh-Oeri & Roberts, 1978 & 1979).

Where is the immune response to vasectomy elicited?

Having escaped from the reproductive tract, sperm antigen could travel in lymphatics to the regional lymph nodes and/ or via the bloodstream to the spleen.

Experiments on vasectomised rats have shown clearly that humoral responses occur in the regional nodes (McDonald & Scothorne, 1986), but comparable changes have not been detected in the spleen (Al-Saffar, 1987). The study on the regional lymph nodes (McDonald & Scothorne, 1986) examined histological changes up to 3 months after vasectomy; Chapter 1 of this thesis follows the progression of the response to 6 and 9 months after operation.

Following vasectomy, in sheep and pigs, whole spermatozoa enter testicular lymphatics and reach the regional lymph nodes (Ball & Setchell, 1983). In the nodes, spermatozoa were present in such large numbers that they elicited the formation of granulomas. Large numbers of spermatozoa have also been reported in a para-aortic lymph node from a vasectomised patient undergoing a staging laparotomy for Hodgkin's disease (Ball et al., 1982). Chapter 4 of this thesis investigates whether whole spermatozoa reach the regional lymph nodes in vasectomised rats.

Why do some vasectomy patients fail to develop antisperm antibodies?

Serum antisperm antibodies are detectable in only about 60 percent of vasectomy patients (Shulman et al., 1972; Samuel et al., 1975; Ansbacher et al., 1976). There has been unresolved speculation that in the other patients antibodies form immune-complexes that are undetectable by conventional assays (Alexander & Anderson, 1979; Lenzi et al., 1985).

When the histology of the regional testicular lymph nodes of vasectomised rats was compared with nodes from sham-operated control animals, not all of them showed changes (McDonald & Scothorne, 1986). One explanation for the lack of response in certain individuals might be that the testicular node did not receive lymph from the sperm granuloma, the presumed site of entry of antigen into lymphatics, either because lymph from the granuloma drained to another node or because lymphatics were interrupted by vasectomy. These possibilities are investigated in Chapter 2 of this thesis and may have been partly responsible for the variable response of the testicular node. Chapter 3 shows that even when the testicular nodes were known to be receiving lymph from granulomas, their response was still variable. This supports the view that not all rats of an inbred strain and not all patients develop antisperm antibodies (Bigazzi et al., 1977). It must be added that until an anatomical basis for the lack of response of the lymph nodes of certain animals is excluded, the variability of the antibody response in the human population cannot be dismissed as being due to "genetic predisposition", in spite of evidence from human and animal studies that there is a link between phenotype and immune responses to spermatozoa (Bigazzi et al., 1977; Law et al., 1979).

Vasectomy presents clinical problems which are difficult to investigate in patients. Although much remains to be explained, morphological studies on experimental animals have played an important role in elucidating the effects of the operation. They also indicate areas of concern where future research is required in man.

CHAPTER 1

The response of the regional testicular lymph node six and nine months after vasectomy.*

SUMMARY

The testicular lymph nodes of Albino Swiss rats were examined 6 and 9 months after vasectomy and sham operation. The histology of the nodes was assessed for evidence of their involvement in the immune response against spermatozoa which follows vasectomy. The volumes of the nodes and their cortical nodule content were assessed quantitatively. Some of the nodes were enlarged at 6 and 9 months while the cortical nodule content was elevated in the 6 months group only. The nodes showed histological changes in cortical nodules and medullary cords at 6 months but only in the cords at 9 months. Changes in the cords included an increase in the number of plasma cells and a decrease in haemosiderin and giant cells. The results suggest a waning humoral response beyond 3 months after vasectomy in the rat.

INTRODUCTION

In a previous paper (McDonald & Scothorne, 1986) histological changes were described in the first regional lymph node of the rat testis at intervals up to 3 months after left unilateral vasectomy. These changes - enlargement of the node and enhanced development of

* This study has been accepted for publication in Journal of Anatomy.

cortical nodules and medullary cords - increased in magnitude up to the 3 months interval. They were consistent with the production of circulating antisperm antibodies, which is known to occur in rats after vasectomy (Rumke & Titus, 1970; Bigazzi et al., 1977). At all intervals after vasectomy some nodes showed no response. This study examines the response in the node at 6 and 9 months after operation.

MATERIALS AND METHODS

Young adult male Albino Swiss rats, from an inbred colony maintained in the Department and aged 8 to 17 weeks, underwent left unilateral vasectomy under ether anaesthesia and with sterile precautions. The ductus deferens was exposed and doubly ligated with silk; its blood vessels were included in the ligatures. A 4 mm length of the ductus was then excised between the ligatures. The excision was made about 1 cm above the junction of the ductus deferens and the epididymis. Control animals underwent sham operation in which two loose ligatures were passed around the ductus deferens which was not transected. The cremaster muscle and skin were closed with catgut and silk sutures respectively and the wound protected by a plastic dressing.

Animals were weighed and killed by an overdose of ether at 6 and 9 months after operation. Immediately after death the pattern of extrinsic lymphatic drainage of the left testis was determined for each animal by injecting a

small quantity of a 0.5 percent solution of pontamine sky blue, a water soluble dye, beneath the tunica albuginea. Material was used only from those rats in which lymph from the left testis was found to drain directly to the left renal node, without previous interruption by another node. The left renal node has been defined by Tilney (1971). This pattern of drainage was found in 7 out of 9 vasectomised rats and 5 out of 10 controls at 6 months, and in 5 out of 9 vasectomised and 9 out of 10 control animals at 9 months after operation.

The left renal nodes were excised, fixed in Bouin's fluid, processed and embedded in wax, serially sectioned at 5 μm and stained with toluidine blue and eosin.

Sections were assessed for signs of an immune response; the criteria used were the size of the node, the number, size and cell content of cortical nodules, and the width and cell content of the medullary cords. Two of these parameters were quantitated:

i) the total volume of each node was calculated from the sum of the areas of every tenth section, measured by means of a MOP-AMO2 electronic planimeter (Kontron, Munich.).

ii) the numbers of cross-sectional profiles of cortical nodules appearing in every tenth section were totalled. This procedure was adopted because it reflected both the number and size of the cortical nodules. A cortical nodule was defined as a circular or oval area in the diffuse cortical tissue containing medium and large lymphocytes (lymphoblasts) and tingible-body macrophages.

Left and right testes and epididymides were inspected for abnormalities and the position of any sperm granulomas was noted. The testes were excised, trimmed of fat and weighed. They were then fixed in Bouin's fluid, processed for wax histology and retained for the study reported in Chapter 5.

RESULTS

At sacrifice, rats in the 6 months group ranged in weight from 290 g to 400 g and, in the 9 months group, from 310 g to 410 g.

The experiments were originally performed on the assumption that the route of lymph drainage demonstrated by the injection of dye into the testis reflected accurately, in each animal, the lymphatic drainage of the sperm granuloma. This assumption was borne out by a detailed study of the regional lymphatics (Chapter 2); this showed that the regional testicular lymph node always received lymph from granulomas in the epididymis but that it might occasionally fail to do so from granulomas at the vasectomy site. In the present study, all rats showed granulomas at the time of sacrifice; of seven rats 6 months after vasectomy, two had a vasal granuloma only, one had an epididymal granuloma only, while four had granulomas at both the epididymis and the vasectomy site. Of five rats 9 months after vasectomy, one had a vasal granuloma only, one an epididymal one only, while the other three had them at both sites.

Histology of the regional testicular lymph nodes.

Sham-operated rats.

Six and 9 months after sham operation, the testicular lymph nodes had similar histological appearances. The general features of the left testicular (renal) node have been described by Kazeem et al. (1982) who reported it to be hypoactive and of the haemolymph variety. The nodes from sham-operated rats showed similar features: cortical nodule development remained minimal (Fig. 1.2), the paracortex was quiescent and contained only occasional lymphoblasts, the medullary cords were poor in plasma cells (Fig. 1.3 & 1.8). When compared with control nodes from the previous study up to 12 weeks after operation (McDonald & Scothorne, 1986), those at 6 and 9 months showed slightly wider medullary cords containing larger deposits of haemosiderin and associated giant cells, a feature of haemolymph nodes (Fig. 1.1, 1.3, 1.7 & 1.8). These small differences were attributed to the greater age at sacrifice of the rats in the present study.

Vasectomised rats.

6 months.

In the nodes from rats vasectomised 6 months previously, the response was variable. The relative amounts of cortex and medulla were similar to those of controls. In several of the nodes, cortical nodules were obviously larger and more numerous (Fig. 1.4 & 1.5), but in two specimens they were similar in size-range and cellular content to those of the sham-operated controls.

Enlarged nodules were rich in medium and large lymphocytes (lymphoblasts) and also contained tingible-body macrophages and reticular cells; mitotic figures were frequent. Some of the cortical nodules showed light and dark poles, a feature of active nodules (Fig. 1.5). They did not, however, regularly possess a corona of small lymphocytes, another feature of active nodules which had been seen in the material 3 months after vasectomy. It must be stressed that all cortical nodules were active; there were no effete nodules, so-called "reaction centres" such as those described by Conway (1937). As in controls, the paracortex in all the vasectomised rats contained only modest numbers of lymphoblasts. Some nodes 6 months after vasectomy showed slightly thicker medullary cords than those after sham operation (Fig. 1.1, 1.3, 1.4 & 1.6) while others were similar to the better developed of the controls. Although the cords from vasectomised rats did not differ greatly in size from controls, they were generally richer in plasma cells, but markedly poorer in haemosiderin and giant cells (Fig. 1.3 & 1.6). However, the medullary cords of one node 6 months after vasectomy were indistinguishable from those of sham-operated rats. None of the nodes 6 months after vasectomy, however, had medullary cords as large as some of those seen after 3 months (McDonald & Scothorne, 1986).

9 months.

The histological appearance of the nodes 9 months after vasectomy was rather more uniform than that at 6 months. All nodes showed a quiescent cortex and paracortex with cortical nodules indistinguishable in number and size from controls (Fig. 1.7 & 1.9). In all nodes, the cords were richer in plasma cells and poorer in haemosiderin and giant cells than those in control nodes (Fig. 1.8 & 1.10).

Quantitative observations on regional testicular lymph nodes.

Two features of the lymph nodes were assessed quantitatively: the volume of the node and the total number of cortical nodule profiles seen in every tenth section of a complete series. The results are shown in Tables 1.1 and 1.2.

When the data was subjected to two-tailed Student's t-test to compare the two parameters at both 6 and 9 months after vasectomy with their corresponding controls, only the mean nodal volume at 9 months after vasectomy was found to be significantly increased ($p < 0.001$). Although the mean nodal volume at 6 months and the mean number of cortical nodule profiles at 6 and 9 months after vasectomy were all elevated, the variances were high and the differences in the means not statistically significant. In some vasectomised animals, however, the values exceeded the mean control value by

more than 3 standard deviations. They are underlined in Tables 1.1 and 1.2: at 6 months, 4 out of 7 nodes were larger than the control mean by 3 standard deviations, and at 9 months 4 out of 5 showed comparable enlargement. In terms of the number of cortical nodule profiles, 5 out of 7 nodes 6 months after vasectomy similarly exceeded the mean control value, but only one did so at 9 months.

DISCUSSION

Previous work (McDonald & Scothorne, 1986) described the response of the regional lymph node of the testis up to 3 months after left unilateral vasectomy and sham operation; the present study extends the series to 6 and 9 months. The histology of the nodes at 6 and 9 months indicates a waning response. Although some nodes remained enlarged at both 6 and 9 months, the cortical nodule activity fell between these two intervals while the plasma cell content of the medullary cords was similar at 6 and 9 months but less than that at 3 months. The regressive changes need not necessarily imply a fall in antibody production but they do, in a general way, correspond to the gradual reduction of circulating antisperm antibodies beyond 3 months after vasectomy noted, in certain other strains of rat, by Bigazzi et al. (1977).

Some of the variation between the nodes in the 6 months group may be related to differences in the lymphatic drainage of vasal granulomas (Chapter 2). Alternatively some of the variation between individuals may reflect the variability in the circulating antibody

response found by Bigazzi et al. (1977), who reported that not all rats from the same inbred strain produced serum antisperm antibodies following vasectomy. In only one rat 6 months after vasectomy was granuloma formation confined to the epididymis. The study to be described in Chapter 2 showed that the testicular node of this rat would receive all the lymph-borne antigen from the granuloma and, indeed, the node showed morphological changes indicative of an immune response when compared with controls. Of the 2 rats 6 months after vasectomy whose testicular lymph nodes were similar to controls in both quantitative studies, one had a vasal granuloma only and the other had both vasal and epididymal granulomas. The lack of response in the testicular node of the rat with the vasal granuloma only (Rat 97) could be explained by lymph from the granuloma draining to another node. The lack of response in the testicular node of the rat with vasal and epididymal granulomas (Rat 94) could be explained by the vasal granuloma draining to another node and to insufficient spermatozoal antigen reaching the testicular node from the epididymal granuloma to produce sufficient morphological changes to distinguish it from controls.

The reason for the reduction in haemosiderin content of the medullary cords of those nodes showing a response to vasectomy is unclear but it may indicate increased macrophage activity and turnover after vasectomy.

The study concludes that the histological response of the regional testicular lymph node to vasectomy, although variable, persists up to at least 9 months after operation but that it wanes beyond 3 months, despite the continued presence of sperm granulomas.

TABLE 1.1.

VOLUMES OF TESTICULAR LYMPH NODES 6 AND 9 MONTHS AFTER VASECTOMY AND SHAM OPERATION

VASECTOMY		SHAM OPERATION	
Rat No.	Vol. of Testicular Lymph Node (mm^3)	Rat No.	Vol. of Testicular Lymph Node (mm^3)
6 months			
92	<u>6.96</u>	53	4.63
94	2.34	67	3.17
95	5.52	68	3.25
96	<u>12.50</u>	69	4.60
97	2.22	71	4.17
98	<u>10.57</u>		
99	<u>8.07</u>		
	Mean = 6.88		Mean = 3.96
	S.D. = 3.89		S.D. = 0.71
9 months			
100	<u>5.34</u>	55	3.35
102	<u>6.19</u>	56	2.07
114	4.22	57	3.05
115	<u>7.42</u>	58	3.70
117	<u>6.06</u>	59	3.59
		60	3.80
		61	2.65
		62	3.57
		63	3.11
	Mean = 5.85		Mean = 3.21
	S.D. = 1.18		S.D. = 0.56

Numbers underlined are those which exceed the mean for sham-operated controls, at the same time interval, by more than 3 x S.D.

TABLE 1.2.

TOTAL NUMBER OF CORTICAL NODULE PROFILES

IN EVERY TENTH SECTION OF THE TESTICULAR LYMPH NODE

VASECTOMY		SHAM OPERATION	
Rat No.	No. Profiles	Rat No.	No. Profiles
6 months			
92	349	53	42
94	32	67	1
95	77	68	10
96	314	69	7
97	1	71	2
98	572		
99	155		
	Mean = 214.3		Mean = 12.4
	S.D. = 207.0		S.D. = 16.9
9 months			
100	3	55	2
102	7	56	2
114	6	57	2
115	23	58	12
117	3	59	0
		60	2
		61	0
		62	3
		63	4
	Mean = 8.4		Mean = 3.0
	S.D. = 8.4		S.D. = 3.6

Numbers underlined are those which exceed the mean for sham-operated controls, at the same time interval, by more than 3 x S.D.

Fig. 1.1. Testicular node 6 months after sham operation. The cortex and medullary cords are unreactive.
Toluidine blue & eosin. X 100.

Fig. 1.2. Small cortical nodule (arrow) typical of those found in the testicular node 6 months after sham operation.
Toluidine blue & eosin. X 250.

Fig. 1.3. Medullary cords typical of those found 6 months after sham operation. They are rich in haemosiderin and giant cells (arrow) but poor in plasma cells.
Toluidine blue & eosin. X 250.

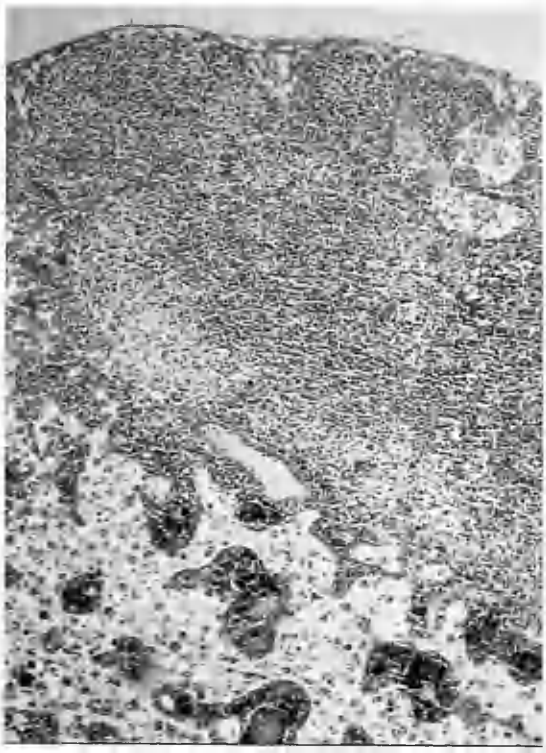

Fig. 1.1.

Fig. 1.2.

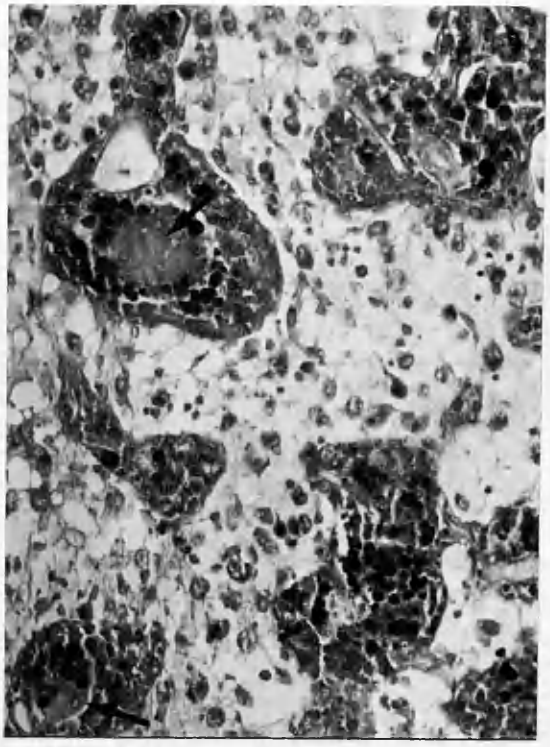

Fig. 1.3.

Fig. 1.4. Testicular node 6 months after vasectomy showing cortical nodule and medullary cord development. Toluidine blue & eosin. X 100.

Fig. 1.5. Cortical nodule typical of those seen in responding nodes 6 months after vasectomy. Light (L) and dark (D) poles have formed.
Toluidine blue & eosin. X 250.

Fig. 1.6. Medullary cords typical of those found 6 months after vasectomy. They contain many plasma cells but are poor in haemosiderin and giant cells.
Toluidine blue & eosin. X 250.

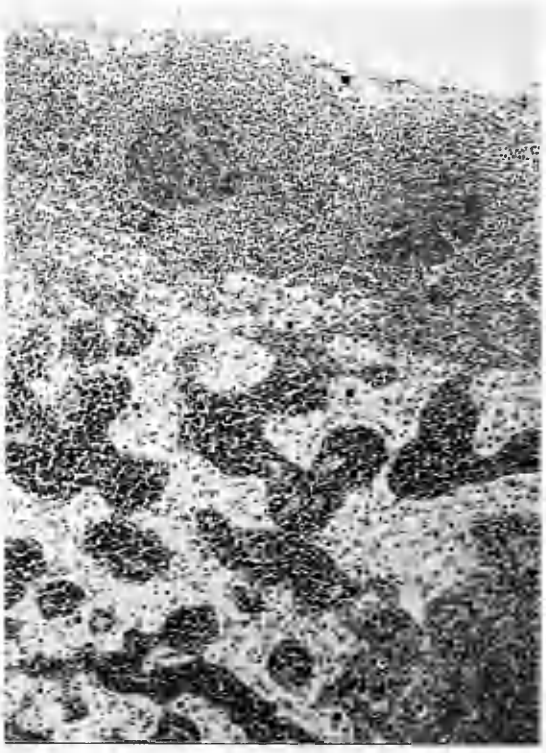

Fig. 1.4.

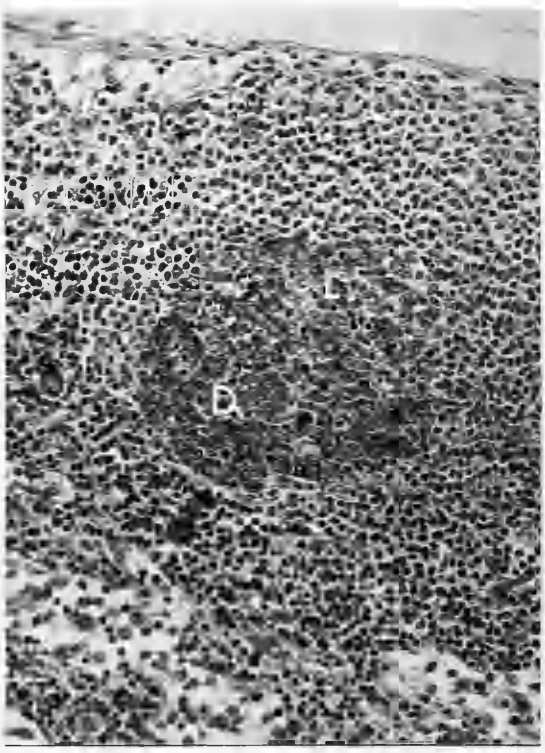

Fig. 1.5.

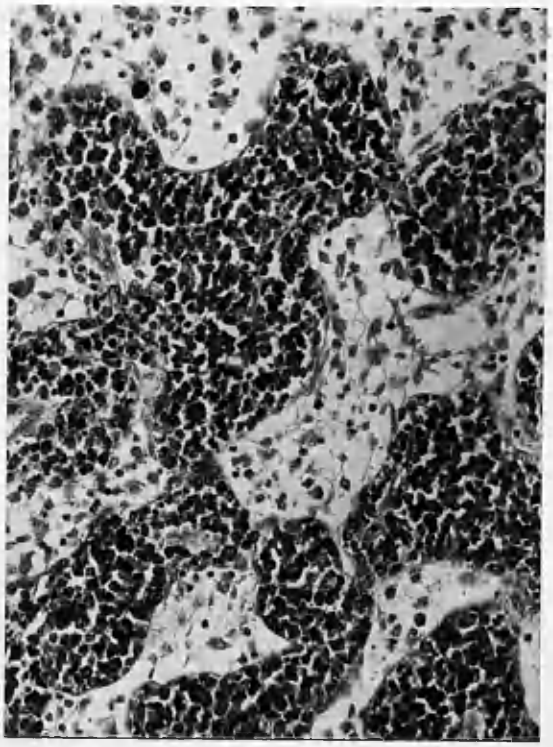

Fig. 1.6.

Fig. 1.7. Testicular node 9 months after sham operation. The cortex and medulla are unreactive.
Toluidine blue & eosin. X 100.

Fig. 1.8. Medullary cords typical of those found 9 months after sham operation. They are rich in haemosiderin and giant cells (arrow) but poor in plasma cells.
Toluidine blue & eosin. X 250.

Fig. 1.9. Testicular node 9 months after vasectomy showing an unreactive cortex.
Toluidine blue & eosin. X 100.

Fig. 1.10. Medullary cords typical of those found 9 months after vasectomy. They have many plasma cells but are poor in haemosiderin and giant cells.
Toluidine blue & eosin. X 250.

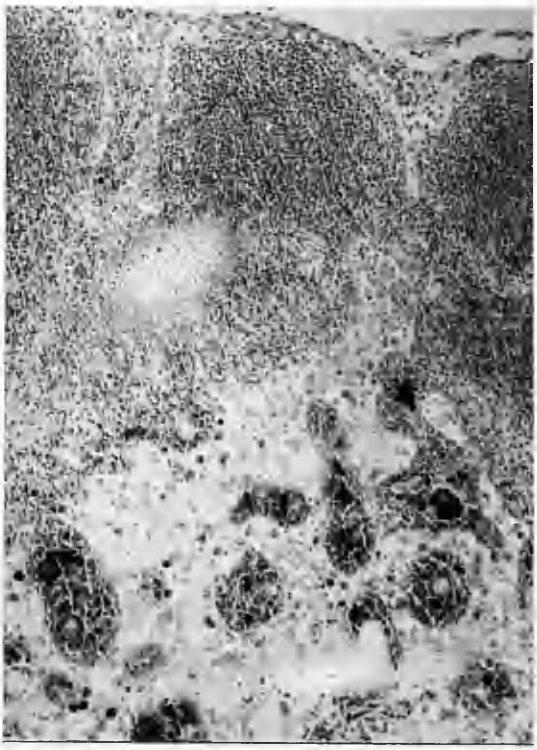

Fig. 1.7.

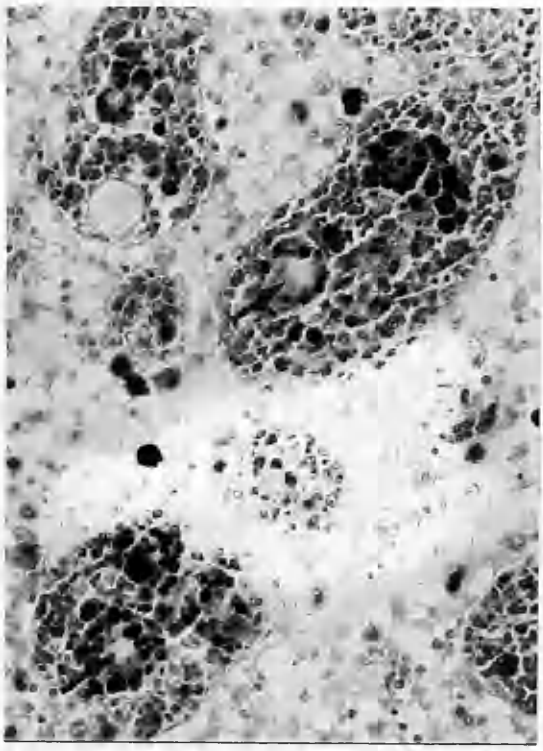

Fig. 1.8.

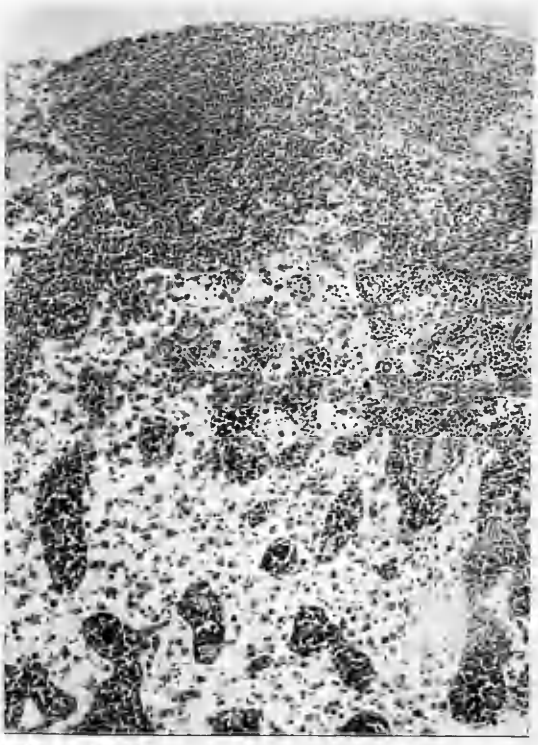

Fig. 1.9.

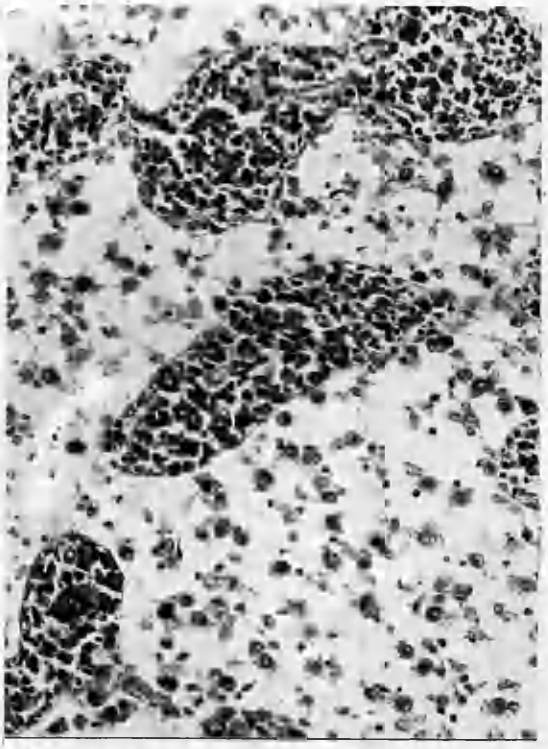

Fig. 1.10.

CHAPTER 2

The lymphatic drainage of the epididymis and of the ductus deferens, with reference to the immune response to vasectomy.*

SUMMARY

The lymphatic drainage of the testis, epididymis and ductus deferens was determined in unoperated and in unilaterally vasectomised Albino Swiss rats. In the vasectomised animals, the lymphatic drainage of epididymal and vasal sperm granulomas was also investigated.

The normal epididymis, and sperm granulomas which develop in it after vasectomy, drains to the regional testicular lymph node via the inferior epididymal trunk; vasectomy does not interfere with this route.

There is a lymphatic watershed within the middle one-third of the scrotal ductus deferens; lymph may drain caudally, to enter the inferior epididymal trunk and /or rostrally to the iliac node. Lymphatics draining granulomas at the vasectomy site may, therefore, be interrupted by vasectomy. This would contribute to, but does not fully explain, the variable immune response of the regional testicular node following vasectomy.

INTRODUCTION

In a previous paper (McDonald & Scothorne, 1986) and in Chapter 1, histological changes were described in the

* This study has been published in Journal of Anatomy 158 57 - 64 (1988).

first regional testicular lymph node of rats after left unilateral vasectomy. These changes showed that this node contributes to the humoral immune response which commonly follows vasectomy in rats (Rumke & Titus, 1970; Bigazzi et al., 1977) and in other species including man (Shulman et al., 1972; Samuel et al., 1975). The changes in the node were variable, however, and some nodes showed no structural response.

Within a week or two after vasectomy in the rat, the excurrent duct system always ruptures, either in the epididymis or at the vasectomy site, and commonly in both situations. The extravasated spermatozoa stimulate a chronic inflammatory lesion, the sperm granuloma, in which many spermatozoa are phagocytosed by macrophages (Bedford, 1976). The sperm granuloma is assumed to be the principal site of antigen release, and the involvement of the regional lymph node indicates that regional lymphatics provide one route of access of sperm autoantigens to the immune system.

The present study was designed to answer two questions:

(1) Does vasectomy interrupt the lymphatics which drain the sites of granuloma formation?

(2) Does lymph from sperm granulomas drain to nodes other than the regional testicular node?

Either of these possibilities, if true, might explain at least some of the variability of the histological

response of the testicular node to vasectomy. There may, of course, be other explanations for this variability since not all rats, even of a particular inbred strain, produce antibodies after vasectomy (Bigazzi et al., 1977).

The lymphatic drainage pattern of the testis, epididymis and scrotal part of the ductus deferens was therefore investigated in unoperated and in vasectomised animals. This has been determined previously for the normal testis and epididymis in rats (Kazeem, 1979; Perez-Clavier et al., 1982), but there is little detailed information on the lymphatic drainage of the ductus deferens, and none on the effects of vasectomy on drainage routes.

MATERIALS AND METHODS

Young adult Albino Swiss rats from an inbred colony maintained in the Department were used.

Left unilateral vasectomies were performed as described in Chapter 1, with sterile precautions. Anaesthesia was produced by intraperitoneal injection of pentobarbitone sodium, supplemented by inhalation of ether.

The rats were killed by an overdose of ether at intervals of three to six weeks after operation. In both vasectomised and unoperated rats, the left scrotal cavity was opened and lymphatics of the testis, epididymis and ductus deferens demonstrated by injection of India ink beneath the serosa through a 30G needle. In some cases, ink was injected directly into the granuloma or the lumen

of the ductus, using gentle pressure through a 27G needle. The ink was diluted 1 in 3 with water, and filtered before use. After injection, the site was gently massaged to encourage filling of lymphatics. The drainage pattern was studied using a binocular microscope and recorded by a camera lucida drawing in a total of 66 animals, 26 of them unoperated controls, and 40 vasectomised.

RESULTS

A. Unoperated rats

The general layout of the rat male reproductive tract is shown in Fig. 2.1. In each of the 26 unoperated control animals, ink was injected at one or more of the sites marked with an asterisk in Fig. 2.2, which summarizes the total number of successful injections made at each site, the route(s) of lymphatic drainage from each site and the frequency with which each route was demonstrated.

(a) The testis was injected in each animal; all showed ink-filled lymphatic vessels which accompanied the testicular blood vessels into the inguinal canal.

(b) The epididymis was injected, in each animal, at one or more sites: 7 injections were made into the caput, 8 into the corpus and 17 into the cauda epididymidis, a total of 32 injections in 26 animals. All 17 injections of the cauda, including some adjacent to the junction with the ductus, entered lymphatics which formed the inferior epididymal lymphatic trunk as defined by Perez-Clavier et al. (1982). This accompanied the inferior epididymal blood

vessels cranially and, in every case, received lymphatics draining the caput and corpus. Lymphatics from the cauda never drained along the ductus deferens.

(c) The ductus deferens. A total of 21 ink injections were made beneath the serosa of the scrotal portion of the ductus deferens. The exact position of each is shown in Fig. 2.2; the overall pattern is described here.

Ink injected into the caudalmost part of the ductus consistently followed one, or both, of two lymphatic routes to the inferior epididymal lymphatic trunk: caudally along the ductus to its junction with the cauda or across the mesorchium between ductus and epididymis.

Lymphatics of the cranialmost part of the scrotal ductus consistently followed it into the inguinal canal, usually reaching the left iliac node, as defined by Tilney (1971), but sometimes joining the testicular lymphatics to pass to the testicular node.

Between the two extremities of the scrotal ductus the regional lymphatics either crossed the mesorchium to the inferior epididymal trunk or passed cranially to the inguinal canal. The former route was more common in caudal regions, the latter in cranial ones. The pattern suggested the presence of a lymphatic watershed at about the middle of the scrotal ductus rather than a continuous lymphatic channel along its length. This was further supported by the observation that when each end of the scrotal ductus was injected, in 3 of these rats, the two

sites drained independently, with no communicating lymphatic.

B. Vasectomised rats

i. Epididymal granulomas

In each of 12 animals, ink injected into granulomas in the corpus and cauda epididymidis entered lymphatics which drained into the inferior epididymal trunk and thence to the testicular node.

ii. Vasal granulomas

Lymphatics from vasal granulomas accompanied, in a general way, the supplying blood vessels, which came from up to four sources (Fig. 2.3): always, from vessels of the ductus deferens caudal to the vasectomy site; usually, from branches of the inferior epididymal vessels; and, more variably, from vessels of the ductus cranial to the vasectomy site and/or from the cremasteric vessels. Lymphatics draining the vasal granuloma were found to accompany one or more of these vessels (summarized in Fig. 2.4). From the caudal sector of the granuloma, lymphatics consistently drained into the inferior epididymal trunk.

Frequently the blood vessels of the cranial ductus regenerated across the vasectomy site and in part supplied the granuloma. In 2 such cases, lymphatics from the cranial sector of the granuloma were found to accompany the vessels of the ductus cranially. Lymphatics from 3 vasal granulomas, which were adherent to the cremaster

muscle and partly supplied by the cremasteric blood vessels, drained by this route to the left iliac node(s).

The vasal granulomas were very friable and their lymphatics were difficult to inject. Indeed, lymphatics were not found in 8 of the 25 vasal granulomas studied, and in only 4 was more than one lymphatic route demonstrated. On 6 granulomas, a lymphatic plexus was observed in the vicinity of a supplying blood vessel; no injection of lymphatics was successful at a site on a vasal granuloma remote from the blood vessels. Although this failure to reveal lymphatics was probably technical, the possibility that they were indeed absent cannot be excluded. The resolution of this problem requires further study, by different methods.

iii) Excurrent duct system of vasectomised rats (Fig. 2.5)

After the sperm granulomas had been injected, the vasectomised specimens were used to investigate further the lymphatic drainage of the epididymis and ductus deferens.

In 4 animals, lymphatics of the cauda were injected at its junction with the ductus deferens. They all drained into the inferior epididymal trunk; none drained along the ductus.

In the rat, the ductus deferens is attached to the cremaster muscle by the mesorchium. After anastomosing with the vessels of the ductus, the inferior epididymal blood vessels cross the mesorchium to anastomose with the cremasteric vessels (Fig. 2.3). In unoperated controls,

no lymphatics followed this route. In one of the vasectomised animals, a lymphatic passed from the cauda in this direction, but ink could be traced along it for only about 0.5 cm, when it was apparently arrested by a valve. In another 4 rats, a lymphatic passed from the cauda towards the cremaster, but soon looped back to the cauda (Fig. 2.5). In one it joined the inferior epididymal trunk; in the other three the returning lymphatic was obscured by the ink at the injection site but, as no other filled lymphatics were present, it was assumed that it joined the inferior epididymal trunk, which had already received ink directly from the injection site.

To determine whether lymphatics on the cremaster muscle drained towards the cauda, 2 injections were made into the cremaster muscle about 1 cm from the attachment of the caudal free edge of the mesorchium. In one rat an injected lymphatic accompanied the cremasteric vessels away from the cauda, in the other it ran in the mesorchium to the cauda.

These injections of the cauda and the cremaster muscle confirmed the finding, in unoperated rats, that lymph from the cauda always passed only to the inferior epididymal trunk and also indicated that lymph may drain from the cremaster to the cauda but not in the opposite direction.

In 11 rats, lymphatics of the ductus caudal to the vasectomy site joined the inferior epididymal trunk either

by crossing the mesorchium or, after following a looped course downwards along the part of the mesorchium between the ductus and the cremaster muscle (Fig. 2.5).

No ductal lymphatics drained to the cremaster muscle; all those followed from the ductus caudal to the vasectomy site reached the inferior epididymal trunk. It must also be stressed that the direction of flow was always from the ductus to the inferior epididymal trunk.

In 6 animals, lymphatics of the ductus cranial to the vasectomy site passed to the inguinal canal. In one of these, however, that part of the cranial ductus immediately adjacent to the vasectomy site drained independently across the upper mesorchium to the inferior epididymal trunk (Fig. 2.5), another indication of a lymphatic watershed on the scrotal ductus.

The impression that there is no continuous lymphatic channel running the whole length of the scrotal ductus is further supported by the observation that in 3 rats a lymphatic of the ductus arose by at least two tributaries, one of which ran cranially to reach the common vessel while the other ran in the opposite direction, and that the confluence occurred on the mesorchium about 1 mm from the ductus rather than on the surface of the ductus itself.

DISCUSSION

The results provide a detailed and definitive account of the lymphatic drainage of the epididymis and ductus deferens, in normal and vasectomised rats.

Lymph from the cauda epididymidis consistently drained to the inferior epididymal trunk, which received lymphatics of the caput and corpus and joined testicular lymphatics. These findings confirm those of Perez-Clavier et al. (1982). Unlike these workers, however, in this study no case was found in which the epididymis drained towards the ductus. This is of particular importance in the present context because such a route would be interrupted by vasectomy with consequent reduction of uptake of sperm autoantigens from a caudal granuloma. In spite of looking specifically for such an arrangement, lymphatics were always found to drain from the caudal part of the ductus towards the epididymis and never in the opposite direction. Furthermore there was no evidence that the epididymis drained to the cremasteric lymphatics. In brief, therefore, lymphatic injections of the epididymis have shown that its lymph drains solely by a route which unites with that from the testis. Vasectomy, therefore, does not interrupt the lymphatic drainage of epididymal granulomas and their injection has shown that their lymph always reaches the testicular node.

A lymphatic watershed was found at about the middle of the scrotal ductus; the caudalmost part of the ductus was drained by lymphatics which always joined the inferior epididymal trunk to reach the regional testicular node. Lymphatics of the cranialmost part all drained cranially towards the urinary bladder and usually reached the left

iliac node(s) but sometimes left the ductus in the inguinal canal and joined the testicular lymphatics. The position of the watershed was rather variable, but it usually lay within the middle third of the scrotal ductus, near the site of vasectomy in this and the previous studies (McDonald & Scothorne, 1986 & Chapter 1). It is, therefore, possible that, in some cases, the operation interrupted the lymphatic drainage of the site of vasal granuloma formation, although it must be emphasized that lymphatics of the caudal sector of a vasal granuloma would be unaffected. The two questions posed in the Introduction can, therefore, be answered:

1) Vasectomy does not interrupt lymphatics draining sperm granulomas which develop in the epididymis.

2) Epididymal granulomas invariably drain to the regional testicular node, which also receives lymph from many vasal granulomas, via the inferior epididymal trunk. Vasal granulomas also drain to the left iliac node via the lymphatics of the distal ductus or of the cremaster muscle. However some vasal granulomas may lack lymphatics, or their lymphatics may be interrupted by vasectomy.

The variable response of the regional testicular lymph node to vasectomy cannot therefore be attributed to variability of lymphatic drainage of the epididymis and to consequently variable access of sperm autoantigens to the node; it may however, be affected by variations in the lymphatic drainage of the vasal granuloma.

Fig. 2.1 - 2.5. The testis, epididymis and ductus deferens have been reflected to expose their lateral aspects. In Fig. 2.2, 2.4 and 2.5 each injection site is marked with an asterisk and the numbers in the boxes show the number of successful injections made into each site. The figure beside each lymphatic route indicates the frequency with which that route was demonstrated (see text).

Fig. 2.1. General layout of the unoperated rat, left reproductive tract.

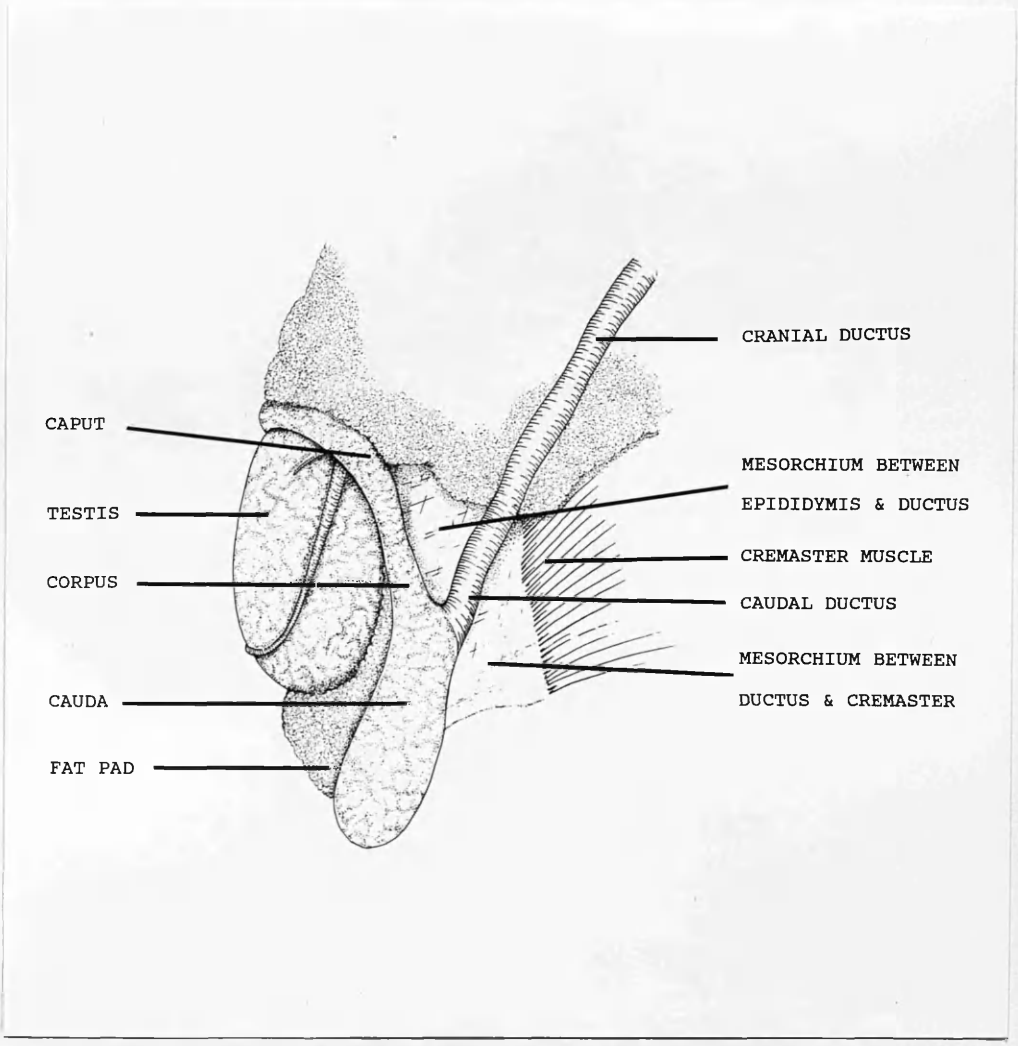
CRANIAL DUCTUS
CAPUT
MESORCHIUM BETWEEN
EPIDIDYMIS & DUCTUS
TESTIS
CREMASTER MUSCLE
CORPUS
CAUDAL DUCTUS
MESORCHIUM BETWEEN
DUCTUS & CREMASTER
CAUDA
FAT PAD

Fig. 2.1.

Fig. 2.2. Summary of lymphatic drainage of left testis, epididymis and ductus deferens in unoperated control animals.

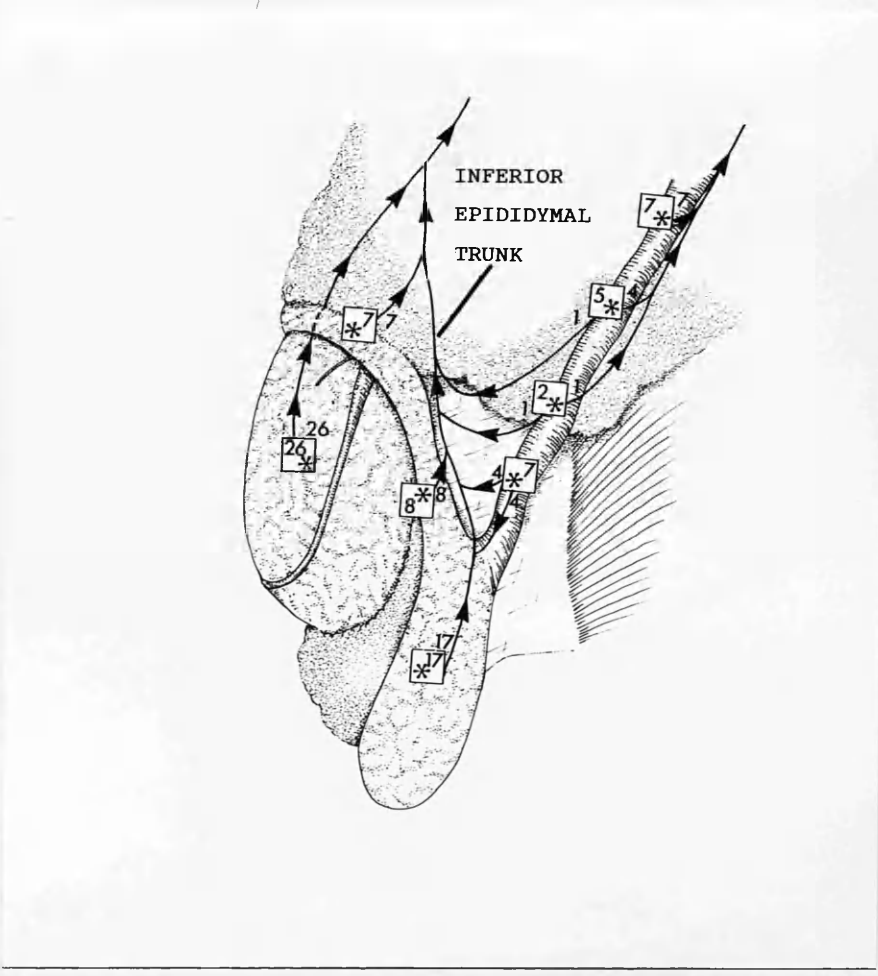

Fig. 2.2.

Fig. 2.3. The left male reproductive tract after vasectomy. A sperm granuloma (G) has formed at the vasectomy site. The ligature (L) at the cranial cut end of the ductus is visible. The blood vessels of the region are shown (see text).

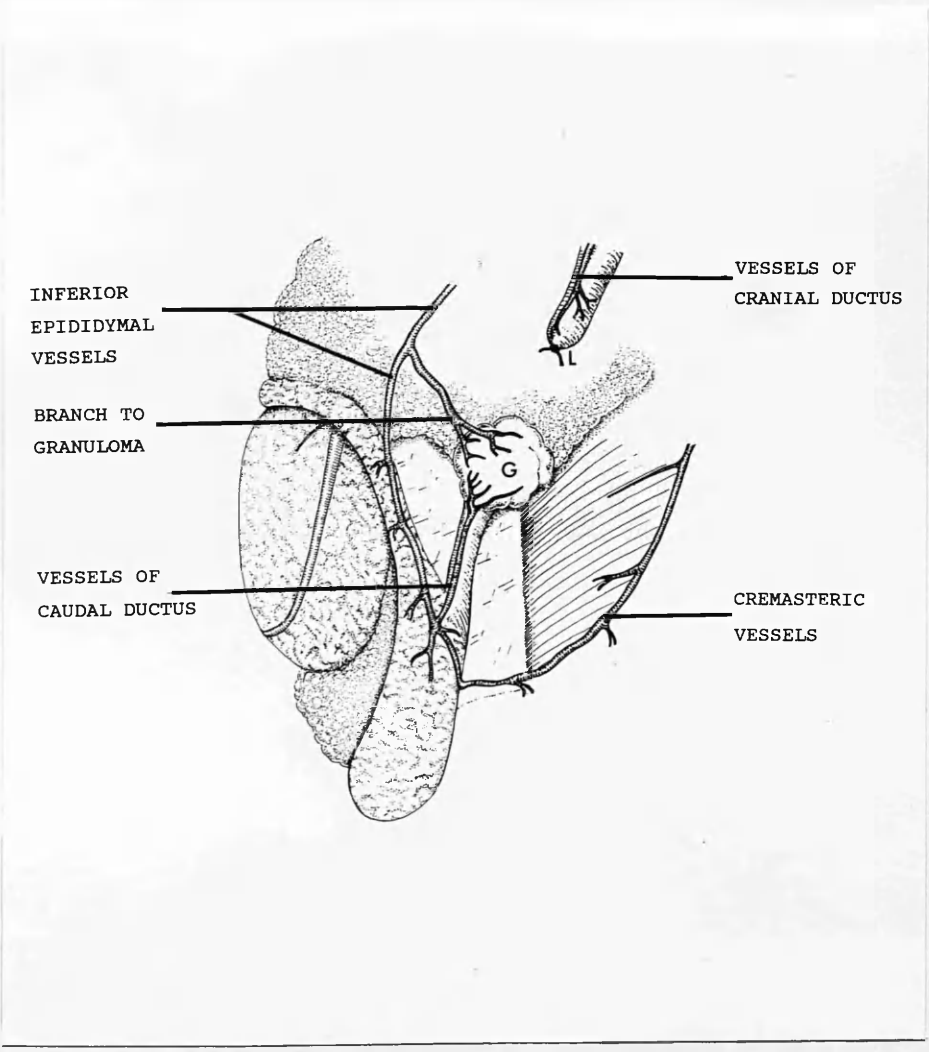
INFERIOR
EPIDIDYMAL
VESSELS
BRANCH TO
GRANULOMA
VESSELS OF
CAUDAL DUCTUS
VESSELS OF
CRANIAL DUCTUS
CREMASTERIC
VESSELS
G

Fig. 2.3.

Fig. 2.4. Summary of lymphatic drainage of vasal sperm granulomas.

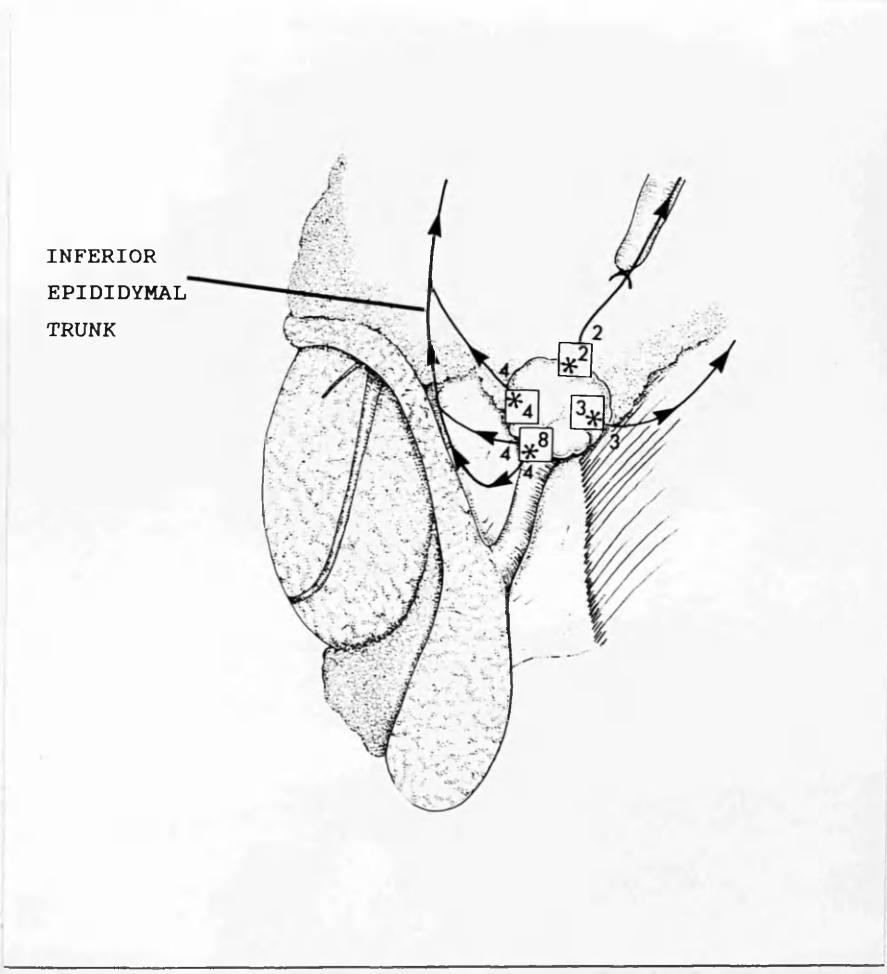

Fig. 2.4.

Fig. 2.5. Summary of lymphatic drainage of the epididymis and ductus deferens of vasectomised rats.

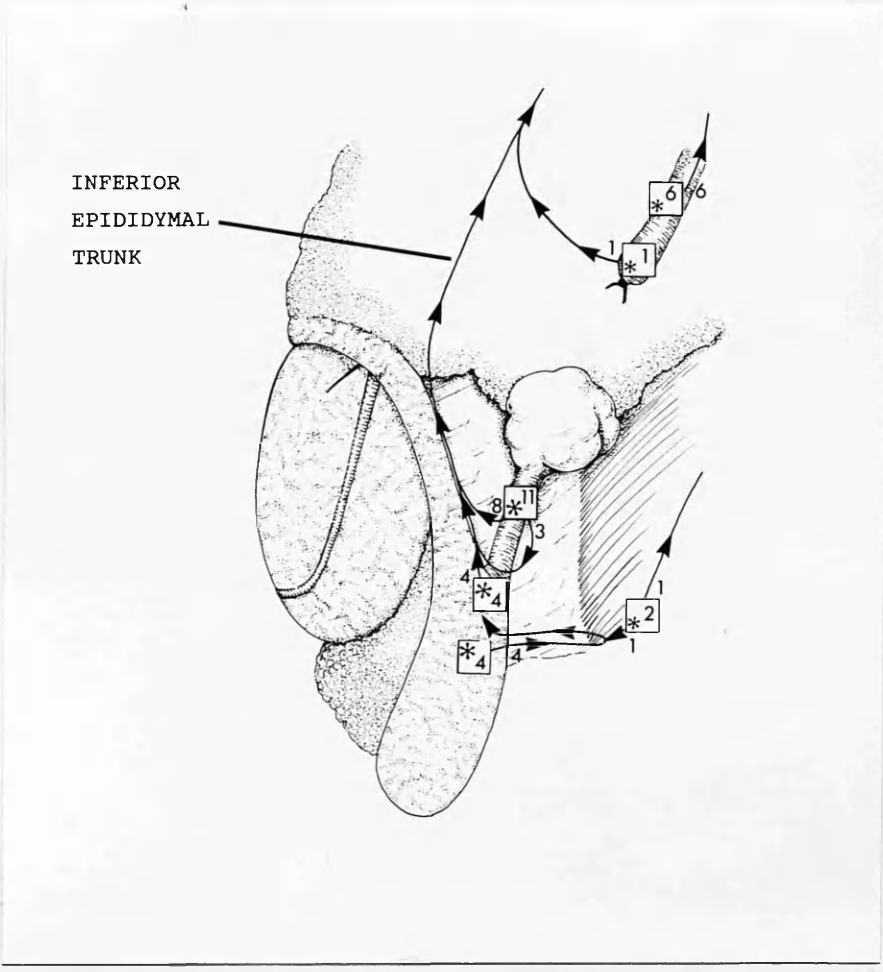

Fig. 2.5.

CHAPTER 3

The response of the regional lymph node to epididymal sperm granulomas after vasectomy.

SUMMARY

This study on unilaterally vasectomised and sham-operated Albino Swiss rats is a further investigation of whether variations in the lymphatic drainage of vasal sperm granulomas were responsible for the previously reported variability in the response of the testicular lymph nodes to vasectomy. In the vasectomised animals, the ductus deferens was transected at its junction with the epididymis. As a result, sperm granulomas developed in the epididymis, from which lymph invariably drains to the testicular node. In spite of the presence of epididymal granulomas in all rats 12 weeks after vasectomy, not all testicular nodes responded. It is concluded that variations in the lymphatic drainage of vasal granulomas were not wholly responsible for the variable lymph node responses found previously, but that additional unknown factors had a role.

INTRODUCTION

Chapter 2 showed that variations in the lymphatic drainage pattern of vasal granulomas may have explained, at least in part, the variable lymph node response to vasectomy reported previously (McDonald & Scothorne, 1986 & Chapter 1). The present chapter investigates whether such variations were the sole reason for the variable nodal response. The problem was approached by carrying out

the vasectomy at the junction of the ductus deferens with the epididymal duct in the expectation that an epididymal granuloma would invariably result. If variations in lymphatic drainage of vasal granulomas were solely responsible for the absence of a response in the testicular nodes of certain individuals in the previous studies (McDonald & Scothorne, 1986 & Chapter 1) then, in the present study, if all the testicular nodes were to receive lymph, and thereby spermatozoal antigens, from epididymal granulomas, they would all be expected to show a response.

MATERIALS AND METHODS

Young adult Albino Swiss rats, from an inbred colony maintained in the Department, underwent left unilateral vasectomy or sham operation with sterile precautions. Their ages ranged from 10 to 19 weeks and their weights from 226 g to 354 g. Anaesthesia was by intraperitoneal injection of pentobarbitone sodium supplemented by inhalation of ether.

In the experimental animals, using a binocular dissecting microscope, about 2 mm of the ductus deferens was excised between two silk ligatures, as close as possible to the junction with the epididymis. The vessels of the ductus were included in the ligatures. The cremaster muscle and scrotal skin were closed with catgut and silk sutures respectively and the wound protected with a plastic dressing.

For sham-operated control animals, the procedure was identical except that the two ligatures were tied only loosely round the ductus deferens, which was not transected.

All rats were inspected after operation for good healing and the absence of cryptorchidism.

All animals were sacrificed 12 weeks after operation by intraperitoneal injection of pentobarbitone sodium. Immediately after death the pattern of extrinsic lymphatic drainage of the left testis was determined, for each animal, as described in Chapter 1. The lymphatic injection study (Chapter 2) showed that lymphatics draining epididymal granulomas invariably united with those of the testis to reach a common node. In the present study granulomas were not injected directly because they are very friable and therefore difficult to inject with sufficient dye to be confident of accurately recording their lymphatic drainage pattern. In order to standardize the experimental procedure, material was used only from those rats whose left testis was found to drain to the left renal node, without interruption by another node.

The left renal nodes were excised, trimmed free from surrounding connective tissue and weighed on a "Microforce" electronic balance (Robal, Salisbury.). The nodes were then fixed in Bouin's fluid for 24 hours and processed for wax histology. Both left and right testes and epididymides were inspected and the positions of all sperm granulomas noted.

Serial 5 μm sections of the lymph nodes were cut, mounted and stained with toluidine blue and eosin.

Lymph node sections were assessed for signs of an immune response, in particular the number, size and cellular content of cortical nodules and the width and cellular content of medullary cords. In addition, the total number of cross-sectional profiles of cortical nodules appearing in every tenth section of each node was determined. This method of quantitation was adopted because it reflected both the number and size of the cortical nodules. A cortical nodule was defined as a circular or oval area in the diffuse cortical tissue containing medium and large lymphocytes (lymphoblasts) and tingible-body macrophages.

RESULTS

All rats 12 weeks after left unilateral vasectomy at the junction of the ductus deferens and epididymis had sperm granulomas at the cauda of the epididymis (Fig. 3.1). Some also had granulomas in the caput and/or corpus. One vasectomised rat (V2) had an atrophic left testis; the testes of the other experimental and control rats appeared normal. The abnormal left testis was associated with a granuloma in the caput as well as at the cauda of the epididymis.

In 8 out of 9 vasectomised rats, the testis drained directly to the left renal lymph nodes while 10 out of 12 controls showed this pattern. One of these sham-

operated rats was eliminated from the study as it had two left renal nodes and was therefore not comparable to the others. The weights of the left renal lymph nodes and their means and standard deviations are shown in Table 3.1.

Two-tailed Student's t-test showed a significant difference between the mean nodal weights of the vasectomised and control groups ($p < 0.05$). Four of the eight nodes from vasectomised rats had weights more than 3 standard deviations from the control mean; these are underlined in Table 3.1.

The general histology of the lymph nodes from vasectomised and sham-operated animals was examined.

The control nodes were similar in every way to those described by Kazeem et al. (1982); they were of the haemolymph variety with poorly developed cortical nodules, paracortex and medullary cords (Fig. 3.2).

The histological response of the nodes from vasectomised animals was variable. In 7 out of the 8 nodes, the cortical nodules were clearly larger and more numerous than in controls and contained medium-sized lymphocytes, lymphoblasts and tingible-body macrophages; mitotic figures were numerous. Many of the cortical nodules were sufficiently developed to have light and dark poles. In only four of these specimens (V2, V3, V4 & V5), however, were medullary cords distinctly thicker than in controls (Fig. 3.3). Thickened cords had a greater content of plasma cells than controls, fewer giant cells and less

haemosiderin. On histological examination one experimental node (V6), with an unbranched afferent lymphatic, was indistinguishable from controls in every way (Fig. 3.4). In all nodes the paracortex was relatively poorly developed.

The results of the enumeration of the cortical nodule profiles is shown in Table 3.2. Two-tailed Student's t-test showed a significant difference in the mean number of cortical nodule profiles in the experimental and control groups ($p < 0.02$). In 7 of the 8 nodes from the vasectomised group the number of profiles exceeded the mean of the controls by more than 3 standard deviations and are underlined in Table 3.2. Two of these, however, were only just over this limit.

DISCUSSION

This study was undertaken to investigate the cause(s) of the absence of a response to vasectomy in the testicular lymph nodes of certain rats reported in previous work (McDonald & Scothorne, 1986 & Chapter 1).

One possible explanation is that the sperm granuloma, the presumed site of entry of spermatozoal autoantigen into lymphatics, was draining to another node and that, consequently, the testicular node was not being stimulated. By performing the vasectomy at the site where the ductus deferens leaves the epididymis, sperm granulomas were induced to form at the cauda, which Chapter 2 has shown always drains to the testicular node.

In spite of this, however, the testicular nodes still showed a range of weights and cortical nodule content. One experimental node (V6) in the present study was, in every respect, indistinguishable from controls even though it had an epididymal granuloma. That this node was unresponsive despite the fact that it received all the lymph from the granuloma shows that variations in the lymph drainage pattern of granulomas do not fully explain the variability of nodal histology after vasectomy. This is consistent with the results of Bigazzi et al. (1977) who found that not all rats of an inbred strain had detectable antisperm antibodies in the blood after vasectomy. The other factors responsible for the variability are as yet unknown, but may include the rate of sperm antigen presentation.

The responsive nodes in this study were rather less reactive in appearance than those in the previous study (McDonald & Scothorne, 1986); the reason for this is unknown.

It is concluded that variations in the lymphatic drainage of sperm granulomas may have contributed to the variability of the lymph node response to vasectomy reported previously (McDonald & Scothorne, 1986 & Chapter 1) but do not fully explain it; other factors, still to be defined, must contribute.

TABLE 3.1.

WEIGHTS OF TESTICULAR LYMPH NODES 12 WEEKS AFTER VASECTOMY AND SHAM OPERATION

VASECTOMY		SHAM OPERATION	
Rat No.	Wt. of Testicular Lymph Node (mg)	Rat No.	Wt. of Testicular Lymph Node (mg)
V1	6.74	S1	8.38
V2	<u>15.11</u>	S2	6.22
V3	<u>15.76</u>	S3	7.61
V4	<u>13.65</u>	S4	6.95
V5	<u>11.09</u>	S5	4.92
V6	7.71	S6	6.21
V7	4.72	S7	7.39
V8	8.19	S8	4.66
		S9	6.34
	Mean = 10.37		Mean = 6.52
	S.D. = 4.14		S.D. = 1.22

Numbers underlined are those which exceed the mean for sham-operated controls by more than 3 x S.D.

TABLE 3.2.

TOTAL NUMBER OF CORTICAL NODULE PROFILES

IN EVERY TENTH SECTION OF THE TESTICULAR LYMPH NODE

VASECTOMY		SHAM OPERATION	
Rat No.	No. Profiles	Rat No.	No. Profiles
V1	20	S1	5
V2	199	S2	12
V3	337	S3	10
V4	150	S4	3
V5	83	S5	4
V6	5	S6	0
V7	45	S7	1
V8	27	S8	1
		S9	0
	Mean = 108		Mean = 4
	S.D. = 115		S.D. = 4

Numbers underlined are those which exceed the mean for sham-operated controls by more than 3 x S.D.

Fig. 3.1. The left male reproductive tract after vasectomy at the junction of the epididymis and ductus deferens. The testis (T), epididymis (E) and ductus deferens (D) are shown, as are the ligatures at the transected ends of the ductus deferens (L). A sperm granuloma (G) has formed on the epididymis at the vasectomy site.

Fig. 3.2. Histological appearance of the testicular lymph node 12 weeks after sham operation. The cortex (C) is quiescent and the medullary cords (M) are poorly developed.
Toluidine blue & eosin. X 100.

Fig. 3.3. Histological appearance of a testicular node showing a response 12 weeks after vasectomy. The cortex contains cortical nodules (N) and the medullary cords (M) are thickened and rich in plasma cells.
Toluidine blue & eosin. X 100.

Fig. 3.4. Histological appearance of the testicular node (V6) showing no response 12 weeks after vasectomy. The cortex (C) is quiescent and the medullary cords (M) poorly developed.
Toluidine blue & eosin. X 100.

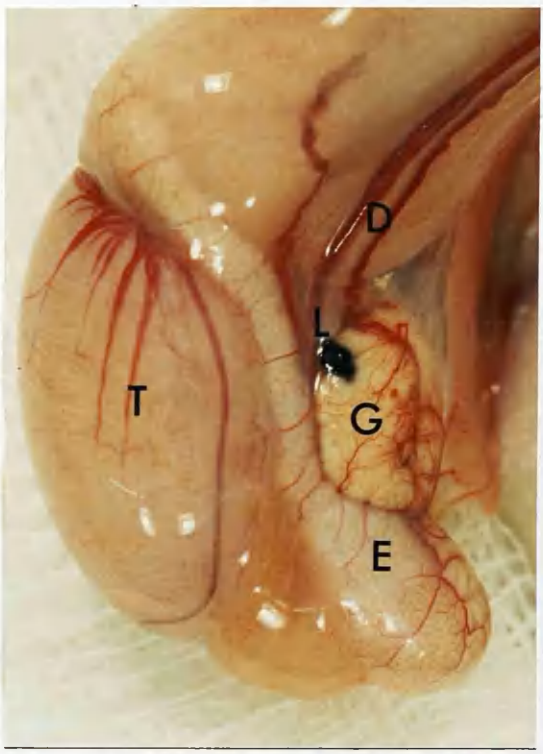

Fig. 3.1.

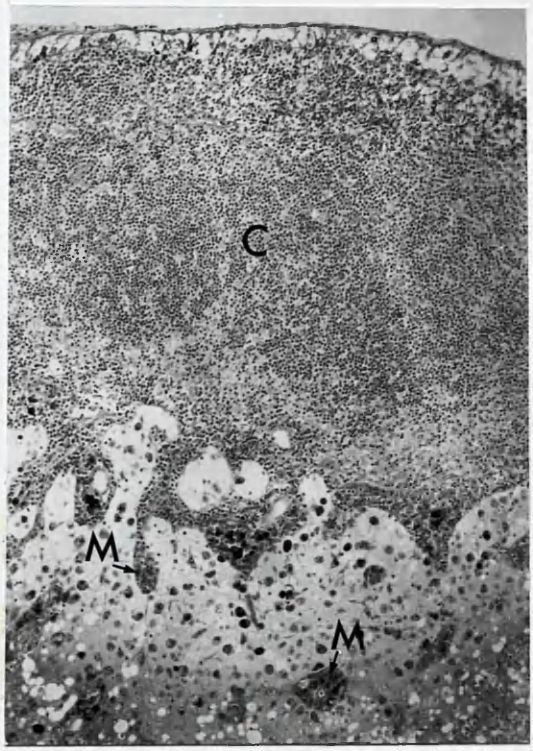

Fig. 3.2.

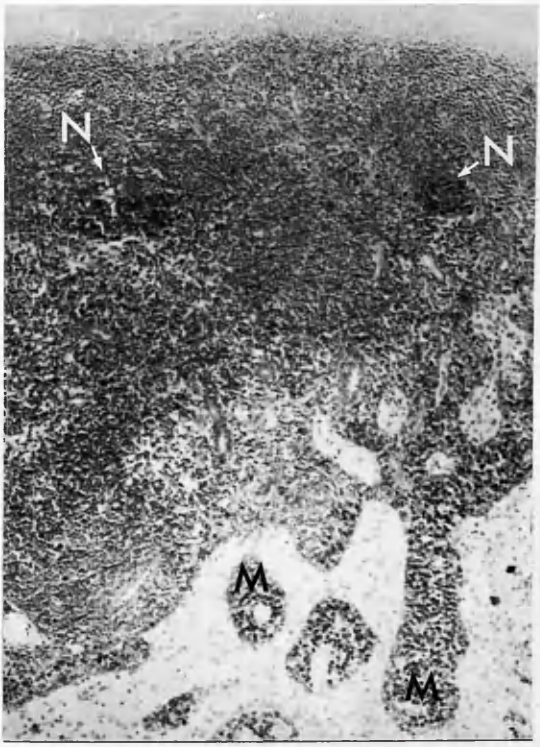

Fig. 3.3.

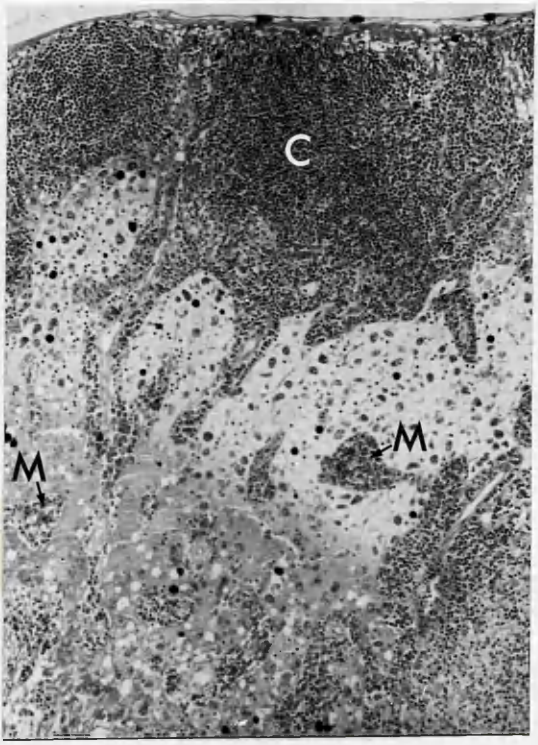

Fig. 3.4.

CHAPTER 4

On the mode of sperm autoantigen presentation to the regional lymph node of the testis after vasectomy.*

SUMMARY

The regional testicular lymph nodes of vasectomised rats and of sham-operated controls have been examined, in stained smears of cell suspensions, for the presence of spermatozoa, at intervals of 1 - 10 weeks after operation. Very few, if any, spermatozoa were found in either group. These findings differ from those reported in rams and boars by Ball and Setchell (1983), and indicate species differences in the mode of presentation of sperm autoantigens to the regional nodes after vasectomy.

INTRODUCTION

It is generally assumed that following vasectomy in rats, the sperm granuloma is the site of access of spermatozoal antigens to the immune system, since the remainder of the reproductive tract is usually normal histologically. These antigens might travel in lymphatics from the granuloma to the regional testicular lymph node carried either by lymphocytes or by macrophages, or as soluble antigens released after partial degradation by macrophages in the granuloma. A third possibility has been reported by Ball and Setchell (1983) who studied

* This study has been published in Journal of Anatomy 153 217 - 221 (1987).

vasectomised rams and boars and found large numbers of free spermatozoa in the testicular lymphatics intermittently between 1 week and 2 - 3 months after operation. In the regional lymph nodes they found phagocytosis of spermatozoa by sinus macrophages and, in some cases, the development of a granulomatous reaction.

In a previous study (McDonald & Scothorne, 1986) free or phagocytosed spermatozoa could not be found either in the epididymal lymphatics or in the lymph sinuses of the testicular nodes of vasectomised rats. It was impossible, however, to exclude with certainty the presence of spermatozoa in the diffuse cortical lymphoid tissue, where it was difficult to distinguish putative sperm heads from elongated nuclear profiles of blood capillary endothelium and reticular cells.

Because of this uncertainty, and because the results in rats appeared to differ so sharply from those of Ball and Setchell (1983) in rams and boars, it was decided to repeat the experiment, examining the testicular lymph nodes in stained smears of cellular suspensions instead of in sections. Although normal tissue architecture is obviously disrupted in such smears, the disaggregated cells are much more readily identified. In a pilot study, spermatozoa which had been deliberately added to the lymph node smear were identified without difficulty.

MATERIALS AND METHODS

Young adult Albino Swiss rats from an inbred colony maintained in the Department, were used. At operation, their ages ranged from 9 to 17 weeks and their weights from 245 g to 380 g. Experimental and control animals had been segregated from females since weaning and were housed one to three in each cage under standard conditions.

Thirty rats underwent left unilateral vasectomy and thirty underwent sham operation; all surgical procedures were performed as described in Chapter 3. Anaesthesia was by intraperitoneal injection of pentobarbitone sodium supplemented by inhalation of ether.

Five vasectomised and five sham-operated animals were sacrificed, by intraperitoneal injection of pentobarbitone sodium, at each of 1, 2, 4, 6, 8 and 10 weeks after operation.

The abdominal and left scrotal cavities were opened and the left testis, epididymis and ductus deferens exposed and inspected. The position of any sperm granulomas was noted.

In order to reveal the regional lymphatics, the left testis was injected beneath the tunica albuginea with 0.1 ml 3% pontamine sky blue. Great care was taken to avoid damage to the seminiferous tubules during this procedure, and at all stages of the dissection handling of the testis, epididymis and granuloma was kept to a minimum

to avoid the risk of damage and accidental extravasation of spermatozoa.

Once located, the first regional lymph nodes of the left testis were excised and trimmed free from surrounding connective tissue. Cytological smears were made from each node by the technique described by Trowell (1955). Six smears were produced from each node. They were fixed for 10 minutes in Heidenhain's "Susa" fixative, washed in running water, and stained in Mayer's haematoxylin.

The left and right testes and epididymides were examined and any abnormalities noted. The testes were then excised and weighed.

As a positive control, a smear was made from the testicular node from a normal unoperated rat. To this smear a suspension of epididymal sperm was deliberately added.

The best of the six smears of each node was examined for the presence of spermatozoa. 2 cm^2 of the smear was examined systematically at a magnification of X250. About 800 medium-power fields were examined from each node. During examination the labels on the slides were covered so that the observer did not know whether they were from vasectomised or sham-operated animals.

RESULTS

Three of the five rats 1 week after vasectomy showed signs of early granuloma formation in the epididymis and by 2 weeks all rats had developed granulomas within the

epididymis and/or on its surface, adjacent to the vasectomy site.

The node or nodes to which lymph from the left testis drained directly in each rat were the left renal, para-aortic, iliac or caudal nodes or any combination of these. These lymph nodes have been defined by Tilney (1971). Smears were prepared from the first regional node or nodes of each animal. In total 37 nodes from vasectomised animals and 42 from the sham-operated group were examined.

In the positive control smear, to which sperm had been deliberately added, sperm heads were easily recognised and sperm tails were also visible. By contrast, sperm heads were very sparse in smears of nodes from both vasectomised animals and from sham-operated controls. The few present were easily recognised but all had lost their tails.

In the smears from vasectomised animals spermatozoa were seen in 1 of 5 nodes one week after operation, 1 of 7 at two weeks, 2 of 5 at four weeks, 0 of 6 at six weeks, 3 of 7 at eight weeks and 2 of 7 at ten weeks. No more than 3 spermatozoa in total were seen in any of these smears.

In the smears from sham-operated controls spermatozoa were seen in 0 of 5 nodes one week after operation, 1 of 5 at two weeks, 0 of 8 at four weeks, 1 of 8 at six weeks, 1 of 7 at eight weeks and 1 of 9 at ten

weeks. No more than one spermatozoon was seen in any of these smears.

Macrophages were frequently seen in smears from both experimental and control groups but no evidence of phagocytosis of spermatozoa was seen.

All the left testes from vasectomised rats appeared healthy and their weights ranged from 1.28 g to 1.62 g (mean = 1.43 g; S.D. = 0.08 g).

In the sham-operated group one left testis 4 weeks postoperatively was markedly atrophic. The others all appeared normal and ranged in weight from 1.28 g to 1.58 g (mean = 1.44 g; S.D. = 0.08 g).

DISCUSSION

Antisperm antibodies are found in rat blood by 3 months after vasectomy (Bigazzi et al., 1977). There is also evidence that this antibody response is mediated, at least in part, by the regional lymph node of the testis (McDonald & Scothorne, 1986). In that paper, it was assumed that the site at which the immune system is exposed to sperm antigen is the sperm granuloma, where extravasated spermatozoa are in contact with macrophages, lymphocytes and plasma cells. From the granuloma, sperm antigens, either free or carried by lymphocytes or macrophages, may travel in lymphatics to the regional node. An alternative mechanism was suggested by Ball and Setchell (1983), who found spermatozoa within the regional lymphatics and lymph nodes of vasectomised sheep and pigs.

In the present study all the rats killed 2 weeks and more after vasectomy had well developed sperm granulomas, either within, or on the surface of, the epididymis. In addition 3 of 5 rats one week after vasectomy showed sperm extravasation and early granuloma formation. Thus in all but 2 of the vasectomised rats, spermatozoa were extravasated from the reproductive tract and could have entered the regional lymphatics.

The lymphatics of the testis were traced to the regional lymph node by means of injection of dye into the organ. The sperm granuloma, the assumed site of antigen release, was not itself injected because it is soft and friable and it was crucial to the study that sperm were not artificially released from the granuloma simply by the technical procedure of defining the regional lymphatics. The vasectomy was performed at the point where the ductus deferens arises from the epididymis so that the granuloma developed either within the epididymis or on its surface, adjacent to the cut end of the ductus. Chapter 2 showed that epididymal lymph drained to the same nodes as that from the testis. Thus injection of the testis was an indirect method of determining the regional node of the granuloma, and greatly reduced the risk of the accidental encouragement of entry of spermatozoa into the lymphatics.

Thorough search of the lymph node smears revealed very few sperm heads: a total of 17 from all the vasectomy material and 4 from all the controls. This in in marked contrast to the results of Ball and Setchell (1983), who

found spermatozoa to be present in large numbers in the testicular nodes of vasectomised rams and boars. The reason for the presence of the spermatozoa in smears from the control animals is unclear; it may be attributable to minor damage during dye injection into the testis.

All of the very small number of spermatozoa observed in this study were free. Although macrophages were numerous in the smears, none contained engulfed spermatozoa. It is possible that spermatozoa had been phagocytosed, and digested beyond recognition. It must be mentioned, however, that other workers have found spermatozoa to be markedly resistant to degradation (Bedford, 1976; Ball & Mitchinson, 1977).

It might have been anticipated that the rupture of the reproductive tract which precedes granuloma formation would have released appreciable numbers of spermatozoa into the lymphatics. However, only one sperm head was found in the nodes from all five animals one week after operation, of which three showed early granuloma formation. It might also have been anticipated that the continuing production of sperm by the testis, and their release at the granuloma, might have produced a progressive accumulation of spermatozoa in the node; sperm heads were, however, just as sparse in the later postoperative groups as in the earlier ones.

The contrast between the abundance of spermatozoa in the testicular lymph nodes of vasectomised sheep and pigs

(Ball & Setchell, 1983) and the paucity of spermatozoa in the rat nodes of the present study makes it clear that there are real and possibly important species differences in the mode of presentation of sperm autoantigens after vasectomy.

CHAPTER 5

A quantitative study of the effect of vasectomy on spermatogenesis.*

SUMMARY

A large sample of cross-sectional profiles of seminiferous tubules from the left testes of five Albino Swiss rats 6 months after left unilateral vasectomy was compared with those of sham-operated controls. Using the classification of Leblond and Clermont (1952), based primarily on the morphology of the spermatids, the frequency of each stage of the seminiferous cycle was recorded. Profiles were also analysed for distension, reduction in epithelial area and changes in spermatocyte numbers.

The absence of significant alterations in either the seminiferous cycle, the numbers of pachytene spermatocyte nuclei or epithelial area in the tubular profiles indicated that there was no alteration in spermatogenic rate after vasectomy.

The lack of tubular distension, reduction in spermatocytes per unit length of perimeter or of the presence of mature spermatozoa at inappropriate stages of the cycle indicated the absence of sperm retention. The study makes clear that, at least in Albino Swiss rats

* This study has been published in Journal of Anatomy 159 219 - 225 (1988).

6 months after vasectomy, the apparently healthy tubules were indeed normal.

INTRODUCTION

The effect of vasectomy on spermatogenesis shows well recognized species variations, ranging from marked impairment in guinea pigs, to temporary depression in dogs. In the rat, most workers have found the testes to be histologically normal after vasectomy, but some report atrophy of the seminiferous epithelium (reviewed by Fawcett, 1979). Some of the abnormal findings may have been due to non-specific side-effects of the operation such as disturbed blood supply, cryptorchidism or infection (Heller & Rothchild, 1974). Testicular degeneration may also occur when a sperm granuloma develops in the caput of the epididymis (Bedford, 1976), presumably by obstructing the outflow of spermatozoa and fluid from the seminiferous tubules. Certain strains of rat may be more susceptible to testicular changes after vasectomy (Neaves, 1978).

Although many papers report that after vasectomy rat seminiferous tubules appear "normal" by both light and electron microscopy, few have looked for more subtle alterations of function which might only be detected by collating the results of detailed analyses of individual tubular profiles. One such report (Lamano-Carvalho et al., 1984), using Wistar rats, described an increased frequency of sectional profiles at Stages VII to VIII of the

seminiferous cycle, which inferred retention of sperm in the tubules. The present study is concerned with whether similar changes follow vasectomy in the Albino Swiss rat, the strain used throughout this thesis. As well as recording alterations in the seminiferous cycle this study aims to investigate two corollaries:

1) that an alteration in the cycle would imply changes in the timing of the steps of spermatogenesis.

2) that identification of all the stages of the cycle would determine whether spermatozoa were being retained in the testis, more profiles with mature spermatozoa then being expected.

An alteration in the rate of spermatogenesis might be reflected not only in an upset cycle but in the numbers of spermatogenic cells in the seminiferous epithelium and perhaps in a change in the thickness of the epithelium. Retention of sperm in the testicular tubules might produce an increase in cross-sectional areas of the tubules and/or dilatation of their lumina, with thinning of the epithelium.

This chapter presents the results of an investigation into all these possibilities. The study was performed at 6 months after operation to determine, in addition, if the changes reported by Lamano-Carvalho et al. (1984), at 30 days after operation, might represent only transient sequelae of vasectomy.

MATERIALS AND METHODS

The testes used in this study were those retained from the rats, 6 months after vasectomy and sham operation, whose lymph nodes were described in Chapter 1. At sacrifice, the left and right testes were excised, trimmed of fat and immersed in Bouin's fixative for 24 hours. They were then blotted and weighed, halved longitudinally and replaced in fresh Bouin's fluid for a further 24 hours before being processed for wax histology.

The quantitation was performed on the testis from the operated (left) side. Only testes which appeared grossly normal were included in the study, giving five left testes from both the vasectomised and sham-operated groups. Blocks were cut until a complete longitudinal sectional profile was exposed and a single random 5 μm section taken, stained with periodic acid-Schiff's reagent and counterstained with Ehrlich's haematoxylin.

For each testis the percentage of a sample of tubules at each stage of the seminiferous cycle was determined. This was followed by measurement of tubular cross-sectional areas and counts of spermatocyte nuclei in all Stage VII tubules.

1) Investigation of the cycle of the seminiferous epithelium.

In each testis section a sample was taken of those tubules which had been cut transversely or near transversely, and which therefore presented circular or

oval profiles. This was done by examining the testis at a magnification of X160 and using the Vernier scale on the microscope stage. The testis sections had been mounted on the microscope slides in such a way that the longitudinal axis of the testis lay approximately parallel to the horizontal scale. The section of the testis was scanned in a horizontal direction at 1 mm intervals on the vertical axis. On each scan the reading on the horizontal scale was noted of all those circular or oval profiles which lay totally within the microscope field. Once the scans were complete each tubule noted could be positioned in the centre of the field, and the reading on the vertical scale recorded. Thus a representative random sample of cross-sections of a large number of seminiferous tubules was defined.

The stage of the seminiferous cycle of each of these tubule profiles was then determined using the classification of Leblond and Clermont (1952). The examination was performed under oil immersion at magnifications of X1000 and X1600 as appropriate. In the rat the whole of a particular transverse tubular profile is usually at the same stage of the cycle. In the occasional tubule where more than one stage occurred the predominant one was recorded.

For each testis the percentage of the sample of tubules at each stage of the cycle was calculated. The means and standard errors of the means for each stage in the vasectomised and control groups were obtained. At

those stages where the two standard errors did not overlap the means were compared by two-tailed Student's t-test.

2) Measurement of tubular cross-sectional areas and spermatocyte counts.

For each testis section all the Stage VII tubules were identified. Stage VII was chosen for this part of the study since it was the most frequent stage and thus gave a large sample for analysis; it was also readily identifiable without the use of oil immersion. For each profile, the form-factor and four parameters were quantitated by means of a MOP-AMO2 electronic planimeter (Kontron, Munich.). The form-factor is a measure of the degree of roundness of a structure; a perfect circle would give a form-factor of 1, any other shape a form-factor between 0 and 1; the less circular the structure, the lower the score. Only those profiles with a form-factor greater than or equal to 0.85 were included in this part of the study. The four parameters measured in each profile were:

i) total perimeter of profile;

ii) total area of profile;

iii) area of lumen;

iv) absolute number of pachytene spermatocyte nuclei.

From this information two further parameters were calculated:

v) area occupied by seminiferous epithelium in each tubule;

vi) number of pachytene spermatocyte nuclei per mm perimeter.

For each of parameters (ii) - (vi) the mean tubular value was calculated for each testis and the means of the vasectomised material compared with those of sham-operated controls by two-tailed Student's t-test.

RESULTS

Nine rats underwent left unilateral vasectomy. When these animals were killed it was found that three of them had left and right testes which were obviously smaller, softer and darker than normal. The abnormal left testes weighed 0.7 g, 0.8 g and 1.0 g. They were, therefore, eliminated from the study as was another testis which, although grossly normal, was cryptorchid. The remaining five left testes which were studied were normal in appearance and ranged in weight from 1.2 g to 1.6 g. All vasectomised animals had developed sperm granulomas at the vasectomy site and/or in the epididymis. The five left testes from sham-operated rats all appeared normal and ranged in weight from 1.4 g to 1.6 g. Student's t-test showed no significant difference between the weights of the experimental testes studied and the controls.

Histological examination of the sections to be staged showed two left testes from vasectomised rats to have occasional atrophic tubules which contained only Sertoli cells. The atrophic tubule profiles were grouped together suggesting that they might be sections through a single tubule. It must be emphasized, however, that the great

majority of tubular profiles in these two testes appeared to be active in spermatogenesis, as did all the tubules in the other experimental material, and in all the controls.

Careful comparison of the testes of vasectomised and sham-operated rats showed no obvious differences between the active seminiferous tubules of the two groups. The few atrophic tubules seen in two of the five experimental testes were markedly different from the healthy ones. There were clearly two populations of tubules in these two testes - functional and atrophic - rather than a spectrum of degeneration. The present study is concerned with detecting subtle changes in apparently normal tubules, so these atrophic ones were excluded from both analyses.

The results of the two quantitative studies were as follows:

1) Investigation of the cycle of the seminiferous epithelium.

In all the tubular profiles examined, the histological appearance of the spermatogonia, spermatocytes and spermatids, at each stage, was as described by Leblond and Clermont (1952).

The total number of sectional profiles of tubules staged was 1,566 from vasectomised, and 1,289 from sham-operated rats. The percentage of profiles at each stage of the seminiferous cycle was calculated for each rat. The means and standard errors of the means of these percentages for both vasectomised and control animals are

shown in Fig. 5.1. This shows that, for both vasectomised and sham-operated groups, sectional profiles at Stages I, VI and VII were most frequent, and those at Stages III, IV, V, IX, X and XI least frequent. The frequency of random sectional profiles of any stage of the cycle is directly related to the length of the particular segments of the tubules at that stage and, in a general way, directly related to the duration of that particular stage at any given tubular cross-section. Although the relation between the duration of a particular stage and segment length is only a general one and subject to considerable variation (Leblond & Clermont, 1952), Fig. 5.1 does demonstrate a close correspondence between relative frequencies in the vasectomised and control groups. Only at one stage (VI) does the difference between the two groups reach significance at $p = 0.05$ by two-tailed Student's t-test. An increase in rate of any particular stage would shorten the segment length and therefore decrease the frequency, while slowing would lengthen the segment and increase the frequency of sectional profiles.

Particular importance is attached to the absence of any effect of vasectomy on the frequency of profiles at Stage IX and to the absence of mature spermatozoa in the lumen. This corresponds to the finding in normal animals (Leblond & Clermont, 1952) and argues strongly against any damming back of mature sperm by retrograde pressure following vasectomy.

2) Measurement of tubular cross-sectional areas and spermatocyte counts.

The two-tailed Student's t-test showed no significant difference in any of the five parameters used to compare tubular cross-sectional areas and spermatocyte numbers after vasectomy and sham operation. The means and standard deviations are shown in Table 5.1.

In order to examine the reproducibility of the results, the analysis was repeated on one testis section. For each tubule profile the difference between the first and second values of the above five parameters was recorded. For each parameter, the values of the differences, irrespective of whether they were positive or negative, were summed and the total error thus obtained expressed as a percentage of the total of the first set of values. The errors of the total tubular profile area, luminal area, area occupied by the seminiferous epithelium, total number of pachytene spermatocyte nuclei per profile and their number per unit length of perimeter were 0.5, 4.2, 1.1, 5.3, and 5.5 percent respectively.

DISCUSSION

The aim of this study was to detect subtle changes in spermatogenesis in apparently healthy testicular tubules after vasectomy. Three atrophic left testes were, therefore, eliminated from the experiment and the very occasional degenerate tubules in a further two specimens were not evaluated. The testicular atrophy was bilateral,

suggesting an immunological aetiology but the cause of the occasional tubular degenerations in two rats is unclear.

In this paper, staging of the seminiferous tubules was carried out according to the classification of Leblond and Clermont (1952) which defines 14 stages of the seminiferous cycle, based on the development of the acrosome in the spermatids. That adopted by Lamano-Carvalho et al. (1984), although generally corresponding to Leblond and Clermont's classification and adequate for their purposes, does not allow distinction between Stages II and III, nor between Stage IV and Stage V.

The frequencies of the tubular stages in the control testes of the present study were generally similar to those found by Leblond and Clermont (1952). The most marked differences were at Stage VII, which was reduced in frequency compared with that of Leblond and Clermont (1952), and at Stages III, VI and VIII, which were all found to be more frequent in the present study. These discrepancies may be due to environmental factors, or to differences of observer or of strain. The strain used by Leblond and Clermont is not stated.

The present results show that, after vasectomy, only Stage VI had a frequency differing from that in the control group at the 0.05 level of significance by Student's t-test. However, given that there are 14 stages, there is a 0.7 probability that one of them would show a change at the 0.05 level as the result of chance, and this may be the explanation for the increase in Stage VI

suggested by the t-test. It is concluded that there is no important alteration in the seminiferous cycle after left unilateral vasectomy. This is supported by the observation that there was no change in the relationship of the spermatids, on which the staging was primarily based, with the spermatogonia and spermatocytes; the spermatogonia and spermatocytes were always at the stage of development described by Leblond and Clermont (1952) for the corresponding stage of spermatid development. If there were to be an alteration in the rate of some step in spermatogenesis it would have been expected to put the development of the spermatogonia, spermatocytes and spermatids out of phase with each other.

Lamano-Carvalho et al. (1984) attributed their finding of a higher frequency of Stages VII and VIII, the stages with the most mature spermatids, to sperm accumulation within the testes as a result of vasectomy, and speculated that this might have been due to partial denervation of the myoepithelial cells, with reduced peristaltic tubular contraction. The present results indicate that, at least in Albino Swiss rats 6 months after vasectomy, there was no retention of mature spermatozoa in the seminiferous tubules. Sections of tubules at Stage IX were consistently devoid of mature spermatozoa, after both vasectomy and sham operation, as they were, at that stage, in the original description of Leblond and Clermont (1952).

If the rate of spermatogenesis were to be reduced as a result of vasectomy it might have been expected to produce any or all of the following changes in each tubular profile:

1) a decrease in the total area;

2) a reduction in the area of the seminiferous epithelium;

3) a reduction in the absolute number of pachytene spermatocyte nuclei;

4) a reduction in the number of pachytene spermatocyte nuclei per unit length of perimeter.

On the other hand damming back of sperm into the testis as a result of blockage might have been expected to produce any of the following changes in the tubular profiles:

1) an increase in the total area as a result of tubular distension;

2) an increase in the area of the lumen;

3) a reduction in the number of pachytene spermatocyte nuclei per unit length of perimeter due to thinning of the epithelium.

That there was no significant difference in any of the parameters studied after vasectomy compared with sham operation suggests strongly that there was neither alteration in the rate of spermatogenesis nor damming back of sperm into the testicular tubules as a result of vasectomy.

The results of this detailed analysis of the cycle of the seminiferous epithelium and of other tubular parameters make clear that, at least in Albino Swiss rats

6 months after vasectomy, there was neither alteration in spermatogenesis nor retention of spermatozoa in the apparently healthy testicular tubules.

TABLE 5.1.

MEANS AND STANDARD DEVIATIONS OF THE FIVE PARAMETERS USED TO STUDY TUBULAR CROSS-SECTIONAL AREAS AND SPERMATOCYTE COUNTS AFTER VASECTOMY AND SHAM OPERATION

	VASECTOMY	SHAM OPERATION
Total tubular profile (mm^2)	7.37 +/- 0.30	8.22 +/- 0.88
Luminal area (mm^2)	1.73 +/- 0.32	2.05 +/- 0.41
Area occupied by seminiferous epithelium (mm^2)	5.64 +/- 0.21	6.17 +/- 0.54
Total number of pachytene spermatocyte nuclei per profile	45.0 +/- 7.90	48.1 +/- 5.70
Number of pachytene spermatocyte nuclei per mm perimeter	44.9 +/- 8.39	45.7 +/- 5.40

Fig. 5.1. Means and standard errors of means of percentage of tubular profiles at each stage of the cycle of the seminiferous epithelium 6 months after vasectomy and sham operation.

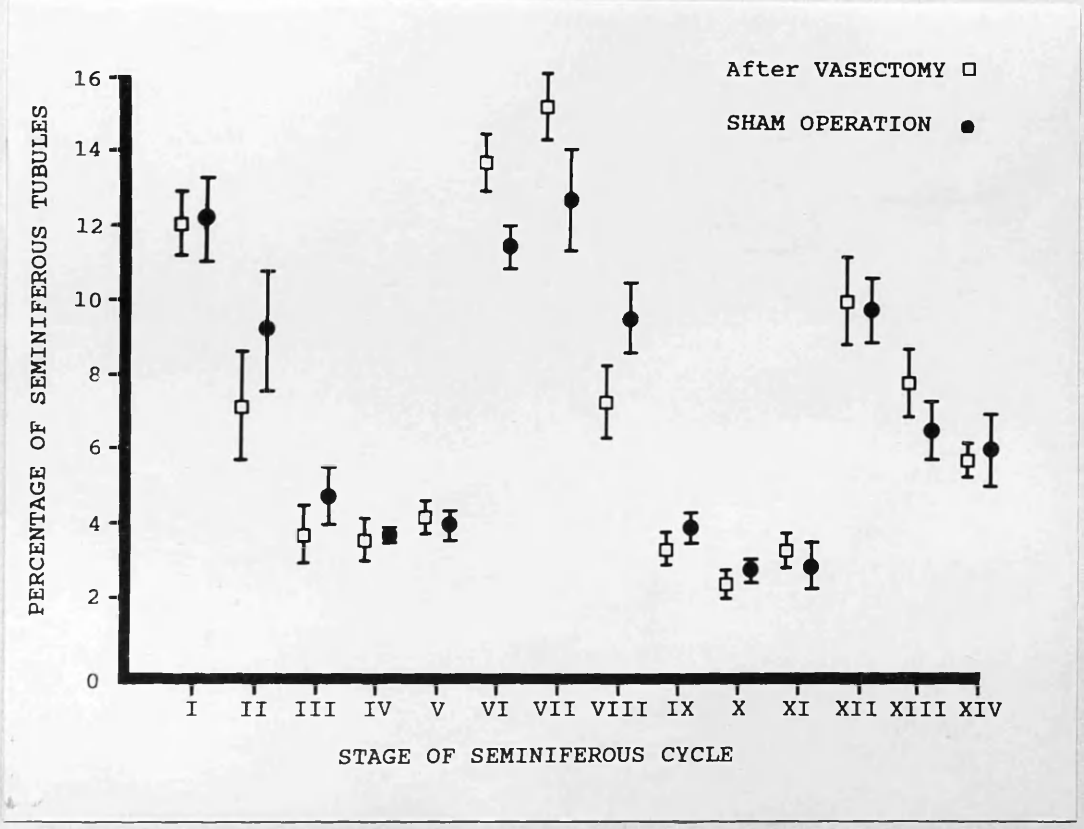
After VASECTOMY □
SHAM OPERATION ●
PERCENTAGE OF SEMINIFEROUS TUBULES
16
14
12
10
8
6
4
2
0
I II III IV V VI VII VIII IX X XI XII XIII XIV
STAGE OF SEMINIFEROUS CYCLE

Fig. 5.1.

CHAPTER 6

The effect of testicular biopsy on the regional lymph node of the testis.*

SUMMARY

The immunological effects of testicular biopsy are largely unknown. This study on young adult Albino Swiss rats shows no changes in the regional testicular lymph nodes as compared to those from sham-operated controls at either 1 or 3 months after operation. The findings contrast markedly with those of vasectomy after which there are obvious histological changes in the nodes consistent with antisperm antibody formation. The difference is likely to be related to the absence of sperm granulomas or other inflammatory changes in the testis after testicular biopsy.

INTRODUCTION

Vasectomy is not the only surgical procedure to damage the epithelial barrier of the reproductive tract. Testicular biopsy, by severing seminiferous tubules, also risks an immune response against spermatozoa. Testicular biopsy is used in the investigation of male infertility when reduced sperm counts are associated with normal hormone levels and an obstructive aetiology is therefore suspected (Lewis-Jones & Lynch, 1987). Because of the clear evidence for an immune response after vasectomy and

* This study has been published in Clinical Anatomy 1 179 - 185 (1988).

the well documented role of antisperm antibodies in male infertility, the widely assumed safety of testicular biopsy may be questioned. This study investigates the effect of testicular biopsy on the regional lymph nodes in rats.

MATERIALS AND METHODS

Young adult male Albino Swiss rats, from an inbred colony maintained in the Department, were used. Their ages at operation ranged from 18 to 24 weeks and their weights from 320 g to 390 g. Rats underwent left-sided testicular biopsy or a control sham operation with sterile precautions. Anaesthesia was by intraperitoneal injection of pentobarbitone sodium supplemented by inhalation of ether.

For testicular biopsy, the ventral aspect of the upper lateral quadrant of the left testis was exposed through a short scrotal incision. A small hole was made in the tunica albuginea with a 25G hypodermic needle. Some seminiferous tubules were drawn out by needle-point forceps and transected. The excised tubules were weighed on a "Microforce" electronic balance (Robal, Salisbury.). The weight of the tubules was about 1 percent of testis weight, comparable to that removed in human testicular biopsy (Cosentino et al., 1986). The cut ends of the tubules were returned to the testis, the cremaster and skin were closed with catgut and silk sutures respectively and a plastic dressing applied. A pilot study showed that

the tunica albuginea healed well without suturing. The whole operation took about fifteen minutes, the testis being exposed for about ten. For a sham operation, the same area of the testis was exposed for ten minutes, being kept moist with sterile water, and the skin and muscle incisions then closed as above. All animals were observed frequently for good healing and for the absence of cryptorchidism.

After 1 or 3 months, biopsied and control rats were sacrificed by intraperitoneal injection of pentobarbitone sodium. The scrotal cavities were opened and the testes and epididymides inspected and any abnormalities noted. The regional lymphatics and lymph nodes were identified by injecting 0.05 ml of 3% pontamine sky blue, beneath the tunica albuginea. In the biopsied rats, the injection was made well away from the biopsy site. The lymphatic drainage pattern thus revealed was recorded. To standardize the lymph node measurements, only the left renal node as defined by Tilney (1971) was used. The left testis drains to this node in more than 50 percent of rats and material was collected until five animals showed this drainage pattern in each group.

Once located, the node was carefully excised, trimmed free from surrounding connective tissue and weighed. It was then placed in Bouin's fixative for 24 hours. The testes were excised, trimmed and weighed and placed in Bouin's fluid. After 24 hours they were halved transversely and replaced in Bouin's fluid for a further

24 hours. After fixation, all tissue was processed for wax histology.

The lymph nodes were sectioned serially at 5 μm and stained with toluidine blue and eosin. The testes were also sectioned at 5 μm and the biopsy site located. Sections were stained with periodic acid-Schiff's reagent and Ehrlich's haematoxylin.

On the lymph node sections, a quantitative analysis was made of the number and size of cortical nodules by totalling the number of cortical nodule profiles on every tenth section of the series. The results of this analysis and the weights of the lymph nodes and testes were compared by two-tailed Student's t-test.

RESULTS

The findings in all aspects of this study were similar at 1 and 3 months after biopsy.

On inspection the biopsy site in all animals had healed very well and the testis appeared essentially normal (Fig. 6.1). A small scar filled the pin-hole in the tunica albuginea and the immediately surrounding tunica appeared thickened. There was an occasional cream-coloured, necrotic tubule near the lesion (Fig. 6.2). The right testes of the experimental rats were normal, as were the testes of both sides in the controls.

One month after operation the biopsied testes ranged in weight from 1.41 g to 1.60 g (mean = 1.48 g; S. D. = 0.05 g) and the controls ranged from 1.37 g to

1.64 g (mean = 1.52 g; S. D. = 0.11 g). Three months after biopsy and sham operation the ranges were 1.43 g to 1.62 g (mean = 1.57 g; S. D. = 0.08 g) and 1.46 g to 1.65 g (mean = 1.58 g; S. D. = 0.08 g) respectively. Two-tailed Student's t-test showed no significant difference between the two groups at either interval.

Histological section through the biopsy site (Fig. 6.3) showed that the defect in the tunica albuginea had healed by scar tissue which also extended for a short distance into the underlying tissue. At the biopsy site there were some atrophic tubules and occasional macrophages but there was no inflammatory response, neutrophils, lymphocytes and plasma cells being absent. The great majority of tubule profiles elsewhere in the testis were normal but occasional atrophic tubules were seen, some of which contained PAS-positive debris.

One month after operation the experimental left renal lymph nodes ranged in weight from 4.85 mg to 7.11 mg (mean = 6.18 mg; S. D. = 0.87 mg) and the controls ranged from 3.40 mg to 8.22 mg (mean = 6.26 mg; S. D. = 1.78 mg). Three months after testicular biopsy and sham operation, the ranges were 4.81 mg to 7.40 mg (mean = 6.28 mg; S. D. = 0.96 mg) and 4.86 mg to 6.57 mg (mean = 5.79 mg; S. D. = 0.72 mg) respectively (Table 6.1). Two-tailed Student's t-test showed no significant difference between the two groups at either interval.

The histology of the rat left renal lymph node, which is of the haemolymph variety, has been described by

Kazeem et al. (1982). As in this earlier description, the nodes in the present study were very unreactive; at 1 and 3 months after testicular biopsy they resembled those of controls in every way (Fig. 6.4 & 6.5). In both groups, the cortex was poorly developed with very few cortical nodules, the paracortex was unreactive and the medullary cords were atrophic, containing some haemosiderin-laden macrophages and giant cells but very few plasma cells.

The counts of the numbers of cortical nodule profiles in every tenth section revealed minimal numbers - no more than 4 profiles in any experimental or control node (Table 6.2). All profiles seen were small with none of the features of high activity such as light and dark poles or a small lymphocyte corona.

DISCUSSION

Gordon et al. (1965) and Rowley et al. (1969) found reduced sperm counts after testicular biopsy in humans while Cosentino et al. (1986) found no significant alteration of rat testicular function if 1 percent or less of the testis was removed but impairment if 10 percent or more was excised. Ansbacher and Gangiai (1975) report that circulating antisperm antibodies are absent after testicular biopsy in oligo- or azoospermic men while Hjort et al. (1974) found low titres of antisperm antibodies following biopsies to assess testicular health after orchidopexy.

Vasectomy in the rat is followed within 3 months by lymph node changes which clearly indicate a humoral immune response (McDonald & Scothorne, 1986); the present study shows that testicular biopsy is not.

The difference in response may be due to one - or both - of two factors. The first, and more likely one, is that after vasectomy there is extensive leakage of spermatozoa, with the formation of one or more large granulomas. Antigenic dosage is therefore probably high. By contrast, at the biopsy site there were no obvious signs, gross or microscopic, of leakage of spermatozoa or of a consequential inflammatory reaction.

The second factor which must be considered is that the high local concentrations of steroids which are known to occur within the testis may have exerted a local immunosuppressive effect. This is at present no more than speculative, but a number of steroid hormones have been shown to interfere with immune reactions (Lahita, 1984) and a similar mechanism has been invoked by Head and Billingham (1985) to explain the privileged status of the testis as a site for tissue allografts.

Whatever the reasons may be, the present results show no evidence for an immune response after a single testicular biopsy in rats, and offer some reassurance about the safety of the procedure in man.

TABLE 6.1.

WEIGHTS OF TESTICULAR LYMPH NODES 1 AND 3 MONTHS AFTER TESTICULAR BIOPSY AND SHAM OPERATION

TESTICULAR BIOPSY		SHAM OPERATION	
Rat No.	Wt. of Testicular Lymph Node (mg)	Rat No.	Wt. of Testicular Lymph Node (mg)
1 month			
TB 1	6.67	STB 2	8.22
TB 2	5.86	STB 3	6.99
TB 5	7.11	STB 4	6.56
TB 6	6.41	STB 5	3.40
TB 11	4.85	STB 6	6.14
	Mean = 6.18		Mean = 6.26
	S.D. = 0.87		S.D. = 1.78
3 months			
TB 12	6.77	STB 7	5.81
TB 13	6.16	STB 8	4.86
TB 15	4.81	STB 9	6.40
TB 16	6.25	STB 10	6.57
TB 17	7.40	STB 11	5.31
	Mean = 6.28		Mean = 5.79
	S.D. = 0.96		S.D. = 0.72

TABLE 6.2.

TOTAL NUMBER OF CORTICAL NODULE PROFILES

IN EVERY TENTH SECTION OF THE TESTICULAR LYMPH NODE

TESTICULAR BIOPSY		SHAM OPERATION	
Rat No.	No. Profiles	Rat No.	No. Profiles
1 month			
TB 1	4	STB 2	1
TB 2	0	STB 3	0
TB 5	0	STB 4	0
TB 6	1	STB 5	4
TB 11	0	STB 6	0
3 months			
TB 12	1	STB 7	0
TB 13	1	STB 8	0
TB 15	0	STB 9	0
TB 16	0	STB 10	0
TB 17	1	STB 11	4

Fig. 6.1. Left testis one month after testicular biopsy. The indistinct biopsy site is marked by arrow A. A necrotic tubule is shown by arrow B.

Fig. 6.2. Close-up of the biopsy site seen in Fig. 6.1. Arrow A shows the biopsy site. The tunica albuginea is more opaque around the site. Arrow B shows a nearby necrotic tubule.

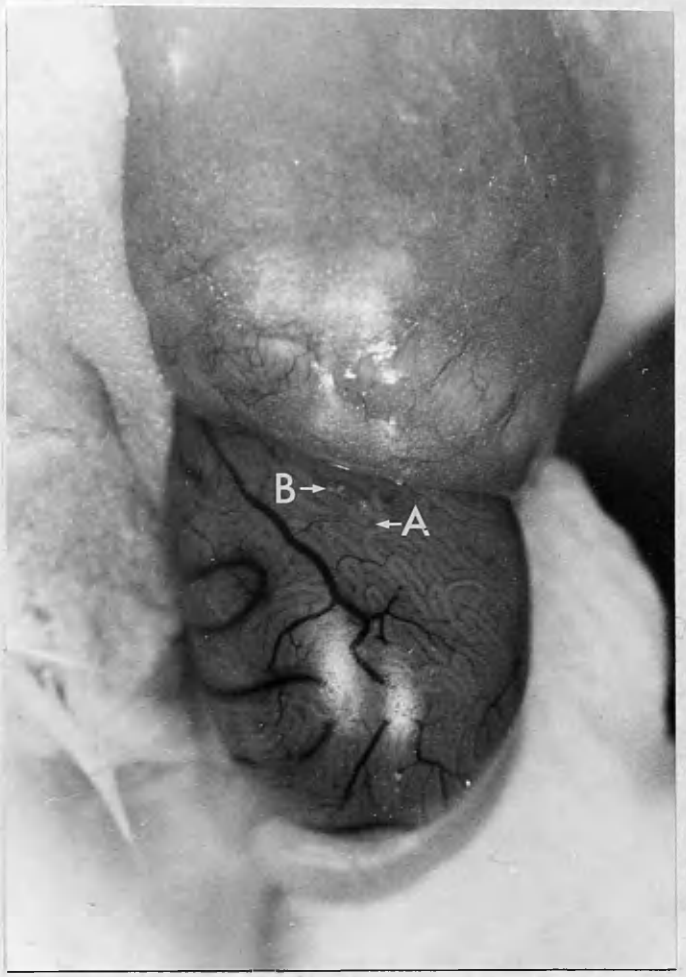

Fig. 6.1.

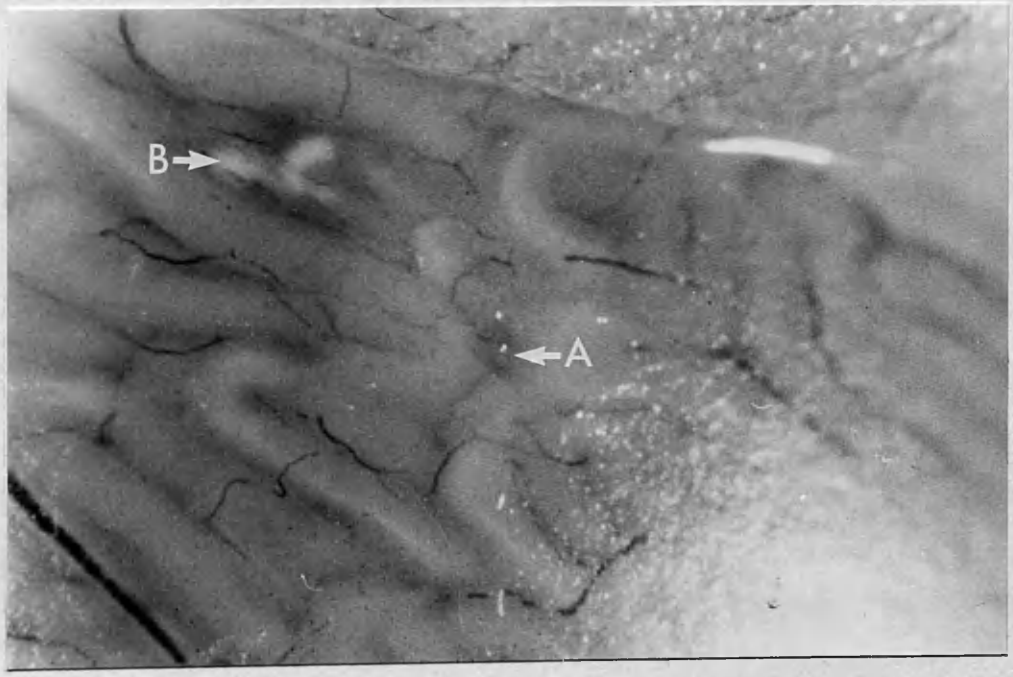

Fig. 6.2.

Fig. 6.3. Section through the biopsy site one month after operation. The break (arrow) in the tunica albuginea (T) produced by the biopsy is clearly visible. Scar tissue (S) covers the biopsy site and extends into the testis for a short distance. Three atrophic tubules (A) are seen.
Periodic acid-Schiff & haematoxylin. X 25.

Fig. 6.4. Testicular lymph node three months after sham operation. It shows unreactive cortex (C), no cortical nodules and poorly developed medullary cords (arrows).
Toluidine blue & eosin. X 100.

Fig. 6.5. Testicular lymph node three months after testicular biopsy. It shows unreactive cortex (C), no cortical nodules and poorly developed medullary cords (arrows). It is similar to testicular nodes from control animals (Fig. 6.4).
Toluidine blue & eosin. X 100.

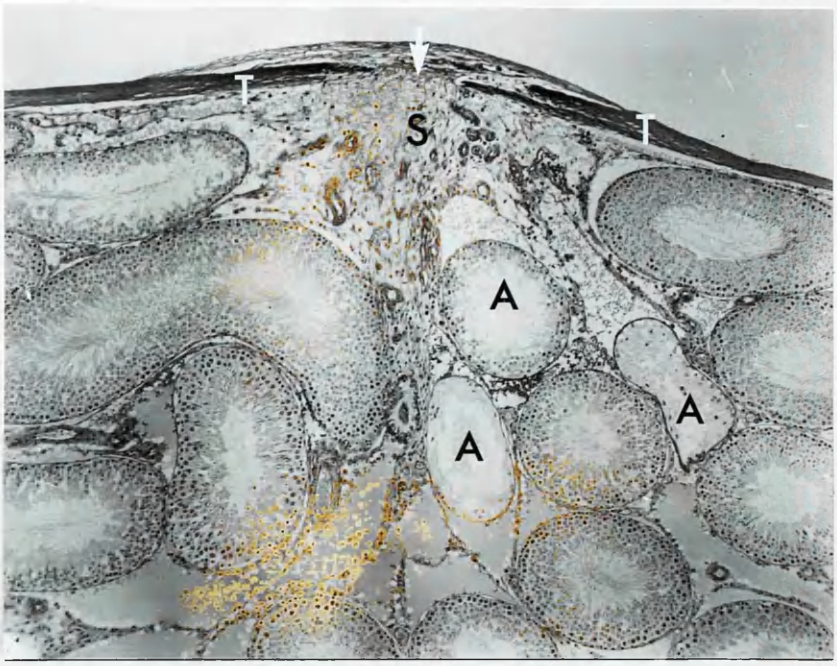

Fig. 6.3.

Fig. 6.4.

Fig. 6.5.

DISCUSSION

In the introduction to this thesis the main clinical anxieties associated with vasectomy were described. The six studies presented contribute to the body of information forming the scientific background to these problems. The effects of vasectomy show marked variations between species and to date it has been difficult to determine a clear and detailed sequence of events following the operation in any single species. In order to gain a sound understanding of the biological processes involved, the Department's vasectomy project aims to make a detailed study of the effects of the procedure in its Albino Swiss rats. This inbred colony has been maintained in the Department since 1974 and it was fortuitous that the Albino Swiss strain showed changes in the testicular lymph nodes following vasectomy; it is likely that the nodes of certain other strains of rat would have been unresponsive since several breeds never develop antisperm antibodies in their blood after vasectomy (Bigazzi et al., 1977). In addition, quite by chance, the lymphatic drainage pattern of the testis in the Albino Swiss rat is relatively simple with few lymphatic trunks and nodes; experiments to detect lymph node changes following vasectomy failed in the Lewis and Dark Agouti strains because of their complex and variable pattern of lymph vessels and nodes draining the testis. The six experiments described in this thesis form part of the

ongoing project to investigate the effects of vasectomy with special reference to autoimmunity to spermatozoa.

By piecing together information from previous work in the Department (McDonald, 1980; McDonald & Scothorne, 1986; Al-Saffar, 1987), from the results presented in this thesis and from work currently in progress, the likely sequence of events following vasectomy in the Albino Swiss rat can now be described.

The initial effects of vasectomy.

The testis of the Albino Swiss rat, at least initially, is unaffected by vasectomy and continues to produce spermatozoa. The ductuli efferentes, caput and corpus of the epididymis also continue to function normally (McDonald, 1980). In the hamster it has been shown that, in the days following vasectomy, intraluminal pressure rises throughout the epididymis but most markedly in the cauda and ductus deferens proximal to the vasectomy site (Howards & Johnson, 1979). The high frequency of sperm granuloma formation in the cauda and ductus deferens suggests that these are also the regions where intraluminal pressure is highest in the Albino Swiss rat. The Albino Swiss rat, like rats and hamsters in general, shows no marked distension of the ductus deferens or epididymal duct following vasectomy.

First appearance of sperm granulomas.

In the Albino Swiss rat, sperm granulomas invariably form within 2 weeks of vasectomy (Chapter 4). They mostly

form at the vasectomy site but may form instead in the cauda epididymidis. It is unusual for the initial granuloma to form at any part of the reproductive tract other than the vasectomy site or cauda epididymidis.

Increase in epididymal intraluminal pressure.

Following vasectomy but before sperm granuloma formation, the continued production of spermatozoa and fluid by the testis leads to an increase in intraluminal pressure in the epididymis and proximal ductus deferens. Direct measurements of intraluminal pressure have been performed in the hamster and guinea pig. In both species, it has been shown that the intraluminal pressure is not equal throughout the vasectomised reproductive tract but becomes progressively higher from proximal to distal regions of the epididymis. In the guinea pig, the intraluminal pressure in the distal cauda is particularly high relative to more proximal regions. Pressure in the seminiferous tubules, however, is similar before and after operation (Howards & Johnson, 1979).

Although continued sperm production by the testis is primarily responsible for the rise in intraluminal pressure in the epididymis, clearly other factors influence the distribution of the pressure. The responsible factor is likely to be the activity of the smooth muscle cells surrounding the duct. By an unknown mechanism, which may be related to the increasing thickness of the smooth muscle layer as the epididymal duct passes distally, the intraluminal pressures are

higher in progressively more distal regions of the epididymal duct. Granuloma formation relieves the intraluminal pressure.

Intraluminal pressure has not been measured in the rat but its presence in the cauda epididymidis and proximal ductus is suggested by the preferential formation of granulomas in these regions and by the areas of epididymal epithelial thinning close to epididymal granulomas.

Although not studied in the Albino Swiss rat, it is widely recognized that vasectomy severs the sympathetic nerves supplying the ductus deferens and the cauda epididymidis (Hodson, 1970). The role of this denervation of smooth muscle in the build-up of pressure in the cauda and proximal ductus is unclear but it is of interest that the administration of the sympathetic blocking drug guanethidine to unoperated rats results in sperm granuloma formation in the cauda (Evans et al., 1972).

Mechanism of initial sperm granuloma formation.

Whatever the causal force, the continued passage of spermatozoa into the cauda epididymidis and proximal ductus is likely to result in a rise in their intraluminal pressures. In the Albino Swiss rat, sperm granulomas form at the vasectomy site almost certainly because of weakening of the wall of the ductus resulting from necrosis of the tissue compressed within the ligature; granulomas never form at other sites on the ductus

deferens. In the cauda, although there is no obvious tubular distension, indirect evidence for increased intraluminal pressure is seen histologically in the regions of epithelial thinning, frequently multiple, observed in the epididymal duct near epididymal granulomas. A caudal granuloma forms by extravasation of spermatozoa through an epithelial discontinuity at one of these areas of thinning.

The observation that, in Albino Swiss rats, first or second granulomas frequently develop in the cauda need not necessarily imply that the duct wall at this site is intrinsically weaker than in the caput or corpus; it may be that leakage occurs because, as in hamsters, local intraluminal pressures are higher. Paralysis of smooth muscle resulting from the severance of sympathetic nerves may be a factor in allowing thinning and perforation of the wall to occur (Evans et al., 1972). Whatever the mechanism, if the granuloma does not form at the vasectomy site it usually forms at the cauda. In the hamster, it has been shown that granuloma formation relieves the intraluminal pressure (Howards & Johnson, 1979).

Mechanism of subsequent granuloma formation.

Once a sperm granuloma has formed, it may drain spermatozoa indefinitely. Al-Saffar (1987), studying Albino Swiss rats at intervals up to 18 months after vasectomy, noted that when granulomas were found only at the vasectomy site they showed a progressive increase in size with time. However, for reasons which are unclear,

once a granuloma has formed at the vasectomy site or in the cauda epididymidis, one or more further granulomas may form in more proximal regions of the epididymis. This may happen at any time after initial granuloma formation but is more commonly seen six months and more after operation. Al-Saffar (1987) also noticed that once a more proximal granuloma formed, the distal one decreased in size presumably because spermatozoa were no longer draining into it and those already present were phagocytosed by macrophages in the granuloma wall.

Work is in progress to determine whether a second granuloma forms because spermatozoa are no longer draining freely into the initial one. This might occur if the route of sperm escape from the epididymal lumen into the granuloma became blocked, if the enlarging granuloma compressed nearby more proximal regions of the duct thereby inhibiting the free drainage of the sperm or if tension in the connective tissue surrounding an enlarging granuloma prevented its further expansion. To date there is insufficient evidence to state which possible mechanism is the cause. Nevertheless, for whichever reason, it seems that in some rats the initial granuloma fails to relieve the intraluminal pressure in the epididymal duct and the process of epithelial thinning, spermatozoal leakage and granuloma formation repeats at a site closer to the testis.

Effects of vasectomy on the testis.

Following unilateral vasectomy, the testes remain normal in many Albino Swiss rats. Occasionally, however, the ipsilateral testis is found to be atrophic. In many of the rats showing ipsilateral testicular degeneration, inspection reveals a sperm granuloma in the caput epididymidis. These changes have been found as early as 9 weeks after vasectomy. The mechanism by which a caput granuloma produces degeneration of the associated testis is unclear and is currently under investigation. It seems that the caput granuloma prevents free drainage of sperm and fluid from the ductuli efferentes and the initial segment of the epididymal duct and therefore fails to relieve the intraluminal pressure; the mechanism is likely to be similar to that by which an initial granuloma at the vasectomy site or the cauda epididymidis is sometimes followed by the formation of a second granuloma elsewhere. The thin layer of smooth muscle surrounding the tubules in the ductuli efferentes and proximal caput epididymidis and the cilia of the ductuli efferentes seem unable to prevent the intraluminal pressure being transmitted to the testicular tubules. The seminiferous epithelium succumbs to the pressure it has itself generated. A series of experiments, in which granulomas were induced to form in the caput epididymidis by ligation of the epididymis about 0.5 cm distal to the initial segment, suggested that when the intraluminal pressure rises the testis may become

markedly distended and show dilatation of the seminiferous tubules and ductuli efferentes. Within a few days of the distension, however, the testis becomes markedly atrophic, being only about half of its original weight. The seminiferous epithelium degenerates leaving only Sertoli cells and occasional sperm precursors in the tubules. It is probable that the testicular changes are irreversible.

In some Albino Swiss rats, bilateral testicular atrophy follows left unilateral vasectomy. This is mainly found 6 months and more after operation. The bilateral nature of this atrophy at first suggested an immunological mechanism but the recent finding of some partially degenerated testes showing no inflammatory cells indicates that the degeneration is likely to be non-immunological in aetiology. A possible explanation of the atrophy may be that a sperm granuloma in the region of the narrow inguinal canal, through which the abdominal and scrotal cavities of rats are continuous, might press on the testicular vessels of both sides and interfere with the blood flow. The same mechanism may account for some instances of unilateral atrophy where no caput granuloma was found. To date no evidence of autoimmune orchitis has been found in vasectomised Albino Swiss rats.

An investigation was carried out on the apparently normal testes of vasectomised Albino Swiss rats to determine whether, despite the healthy appearance of the tubules, there may have been some subtle disturbance of the cycle of the seminiferous epithelium, tubular

dimensions or the timing of spermatozoal release (Chapter 5). Since a delay in spermatozoal release at 30 days after operation in Wistar rats had been suggested by Lamano-Carvalho et al. (1983), it was decided to study the testes at 6 months after vasectomy in case this previous report was the result of temporary disturbance of testicular function following operation. The results indicated that, at 6 months after vasectomy, those tubules which appeared healthy were indeed normal. The significance of the very occasional atrophic tubules seen among the normal ones was unclear and merits further investigation. The effect of vasectomy on the interstitial cell population of the Albino Swiss testis has not been studied.

Testicular changes in vasectomised patients are documented but their aetiology and significance are unclear. They may play a role in the low fertility after vasovasostomy. Whether caput granuloma formation and associated testis degeneration occurs in man is totally unknown.

The fate of spermatozoa produced following vasectomy.

Apart from the presence of intraluminal neutrophils and macrophages at regions of epididymal epithelial thinning and leakage, Al-Saffar (1987) could find no evidence of any phagocytosis of spermatozoa by intraluminal macrophages in the epididymal duct of the vasectomised Albino Swiss rat. There was also no evidence

that epithelial cells were involved in the phagocytosis of spermatozoa although they are likely to have been active in fluid absorption. These finding are similar to those of Bedford (1976) in Sprague-Dawley rats.

Following leakage of spermatozoa from the reproductive tract a localised inflammatory response occurs. The extravasated pool of spermatozoa is infiltrated by neutrophils and a layer of macrophages and chronic inflammatory cells forms around it. No ultrastructural studies have yet been performed on the granulomas of Albino Swiss rats but it is assumed that the macrophages phagocytose spermatozoa, as they do in other strains (Bedford, 1976). Al-Saffar (1987) reported that single granulomas progressively increased in size up to 18 months after vasectomy but that once second granulomas formed the more distal ones became smaller as spermatozoa were phagocytosed.

Several questions about granulomas remain to be answered:

1) Do the number and type of chronic inflammatory cells surrounding the mass of sperm change with time?

2) Once macrophages have degraded the spermatozoa adjacent to them do they become less active or do they move so as to phagocytose more deeply placed spermatozoa?

3) Is there a continuous recruitment of monocytes to the sperm granuloma?

4) Do any immunological barriers develop in the wall of the granuloma?

5) Do granulomas have lymphatics in their walls?

6) Are granulomas immunologically privileged sites?

Sperm granulomas as sites of spermatozoal autoantigen release.

The huge mass of extravasated spermatozoa, the surrounding chronic inflammatory cells and the active appearance of the macrophages at light microscopy are convincing evidence that the sperm granuloma is a major site of release of spermatozoal autoantigen to the immune system in vasectomised Albino Swiss rats. Further evidence for the role of the granuloma in the genesis of the lymph node response following vasectomy comes from the testicular biopsy study (Chapter 6) in which, despite the disruption of seminiferous tubules, the absence of a granuloma was associated with the lack of nodal changes.

No attempt has been made to look for immune-complex deposition indicative of antigen leakage from other regions of the Albino Swiss reproductive tract in the manner that has been carried out for other species (Bigazzi, 1979; Tung & Alexander, 1980).

Al-Saffar (1987) found no evidence of altered numbers of epididymal intraepithelial lymphocytes following vasectomy; the intraepithelial helper T-cell population is a possible vehicle of antigen presentation to the regional lymph nodes. Immunocytochemistry needs to be performed to examine the effect of vasectomy on the relative and

absolute numbers of suppressor and helper T-cells in the epididymal epithelium.

Route and mode of spermatozoal autoantigen presentation.

Spermatozoal autoantigen, having escaped from the reproductive tract, travels to the regional lymph node of the sperm granuloma. The mode by which the transfer of autoantigen occurs is not known. Presumably the transfer of antigen occurs either by breakdown products passing to the nodes as small fragments or soluble antigens or by activating lymphocytes or macrophages. Transfer of whole spermatozoa is rare in Albino Swiss rats (Chapter 4) although common in sheep and pigs (Ball & Setchell, 1983). The basis of this species difference is unclear but may lie in differences in cellular connections between the macrophages surrounding the mass of spermatozoa in the granuloma, in the distribution of lymphatics to the granuloma or in the histological features of the endothelium of the lymphatic vessels at the granulomas.

It is also possible that sperm antigen in a similar form to that entering lymphatics gains access to the bloodstream and travels to the spleen. Al-Saffar (1987), however, was unable to detect any morphological changes in the spleens of vasectomised Albino Swiss rats to indicate their involvement in the generation of an autoimmune response to spermatozoa.

It has been shown that epididymal spermatozoa are more immunogenic than ejaculated spermatozoa. It is

believed that factors secreted into the seminal plasma by the seminal vesicles, prostate and bulbourethral glands inhibit the immunogenicity of the spermatozoa. This complex subject has been reviewed by James and Hargreave (1984). To date little is known of the identity of the immunosuppressive factors or their mechanism of action. It is believed that they may be very important in inhibiting immune responses to spermatozoa deposited in the female reproductive tract at coitus. They may also, by suppressing local immune responses, be of aetiological significance in vaginal infections and in cervical carcinomas. It must be emphasized that the site of leakage of spermatozoa from the epididymis and ductus deferens following vasectomy is at a considerable distance proximal to the sites where immunosuppressive factors are believed to be added; the spermatozoa extravasated following vasectomy are therefore potentially immunogenic.

Lymph node changes following vasectomy.

In whatever form the lymph-borne spermatozoal antigen is transmitted, its arrival in the lymph node stimulates obvious morphological changes indicative of a humoral response in many inbred Albino Swiss rats (McDonald & Scothorne, 1986 & Chapter 1). The parameters used to detect the response were the volumes or weights of the nodes, which might have increased in either a humoral or cell-mediated response, and the development of cortical nodules and medullary cords, both of which would indicate a humoral response.

Cortical nodules are clusters of medium-sized and large lymphocytes, rich in tingible-body macrophages with many mitotic figures in the lymphoid cells. The reticular cells of the cortical nodules are the specialized dendritic cells, so-called because of their shape. These dendritic cells are members of a wider group of antigen-presenting cells, which includes Langerhans cells, veiled cells and interdigitating reticulum cells. They are believed to trap antigen and to be involved in immunological programming of clones of plasma cell precursors and memory B-lymphocytes (reviewed by Raviola, 1986). No whole spermatozoa were seen in the cortical nodules of any vasectomised rat.

The activity of all the cells of a particular clone of memory cells or plasma cells is directed against the same antigenic determinant (reviewed by Fleming, 1985). Spermatozoa possess several autoantigens and the regional lymph nodes are likely to produce several clones of cells, each programmmed to react against the particular autoantigen which stimulated its formation. Following vasectomy, the medullary cords of the regional lymph nodes enlarge and are filled with plasma cells which secrete antibody into the efferent lymph. No doubt, as in other humoral responses, antibody-producing plasma cells are also dispersed throughout the body and especially to the medullary cords of other lymph nodes.

The morphological changes in the lymph nodes indicative of immune activity resulting from vasectomy are first seen at 6 weeks after operation in Albino Swiss rats (McDonald & Scothorne, 1986). The first visible changes are the increased number and size of cortical nodules. As the time after operation increases, the cortical nodules continue to develop and, by 9 weeks, form light and dark poles with a corona of small lymphocytes. The functional significance of the polarity and coronas of cortical nodules in lymph node responses is not understood. By 9 weeks after operation the medullary cords have also increased in thickness and in their content of plasma cells; their development is even greater at 12 weeks after operation. By 6 months, however, the response wanes (Chapter 1) and the cortical nodules, although active, are smaller and have lost their coronas although they retain their polarity. The medullary cords are also reduced in thickness. By 9 months after vasectomy the cortical nodules and medullary cords are again poorly developed, although the latter still contain more plasma cells and less haemosiderin than controls.

The significance of the waning of the lymph node response is unclear. Feedback mechanisms are postulated, by which adequate levels of circulating antibody inhibit further recruitment of plasma cells by binding to Fc receptors on helper T-cells (reviewed by Fleming, 1985). However, it has been found that in other strains of

rat there is a gradual decrease in circulating antisperm antibody titres beyond about 3 months after vasectomy (Bigazzi et al., 1977); the waning lymph node response may reflect a similar decline in antibody production.

At all time intervals after vasectomy it was impossible to determine whether there was increased T-cell activity in the paracortex of the regional lymph nodes. There was certainly no marked increase in the volume of the paracortex relative to the volume of the node. The scattered lymphoblasts and mitotic figures in the thymic-dependent areas may have been cells of B-lineage migrating from the outer cortex to the medulla.

In the initial study to 3 months following vasectomy (McDonald & Scothorne, 1986) and in its continuation to 6 and 9 months (Chapter 1), it was found that several testicular nodes were similar to controls even though they received lymph directly from the testis. Detailed study of the lymphatic drainage of the epididymis and ductus deferens revealed that in some cases the nodes may have failed to receive lymph from sperm granulomas (Chapter 2). Subsequent work (Chapter 3), in which the vasectomy was carried out in such a way that the resulting granuloma always drained to the testicular node, did not completely eliminate the variability of the response of the node even though all the rats were from the same inbred colony; a range in the degree of nodal response existed, with one animal in the study showing no response at all.

There may be many explanations for the variability in the response. Perhaps the range in the magnitudes of the responses of different rats reflects variations in the rate of spermatozoal breakdown at the granuloma; this in turn could reflect variations in the areas of the macrophage-spermatozoal interface of the granulomas. The variations in the nodal responses might also result from variations in levels of immunosuppressive steroid hormones in the blood of different individuals. In the non-responders it is also possible that the rate of antigen presentation to the regional lymph node was such as to render the animals tolerant to their own spermatozoa.

The immune response to spermatozoa in relation to other autoimmune phenomena.

Autoimmunity to body constituents may arise for three general reasons:

1) Non-antigenic body components are altered by an extraneous factor such as a virus, drug or toxic chemical. The alteration in chemical structure renders the component autoantigenic.

2) Immunological tolerance breaks down; this could occur because of:

a) elevated helper T-cell function,

b) reduced suppressor T-cell function,

c) inappropriate activity of B-lymphocytes and plasma cells.

The factors leading to the breakdown of tolerance are not understood.

3) Immunological barriers sequestering autoantigenic material from the immune system become defective.

Little is known of the aetiology of many human autoimmune diseases; the subject is reviewed by Robbins and Kumar (1987). Those conditions in which viruses may be implicated include insulin-dependent diabetes mellitus and chronic active hepatitis. Immunohaemolytic anaemia is a well-documented side-effect of certain drugs. Other autoimmune diseases seem most likely to be related to defects of the immune system. B-cell dysfunction is believed to underlie systemic lupus erythematosus and Sjogren's syndrome. Myasthenia gravis is often associated with thymic abnormalities. T-cell defects are implicated in Hashimoto's thyroiditis and Graves' disease. Various immunological defects have been noted in primary biliary cirrhosis. The frequent association of two or more autoimmune diseases in the same patient suggests a single, as yet unknown, precipitating factor.

In all the above examples it seems that there is either an alteration in the antigen or a defect in the immune system. They, therefore, do not resemble the response to spermatozoa following vasectomy which results solely from exposure of sequestered antigen to the immune system.

Two disease processes occur in the eye which may resemble the immune response following vasectomy in resulting from the release of sequestered antigen; they are sympathetic ophthalmia and phacoantigenic uveitis. Sympathetic ophthalmia has been reviewed by Wilson and Wilson (1987) and phacoantigenic uveitis by Schlaegel and O'Connor (1987).

1) Sympathetic ophthalmia is a serious condition in which inflammation of one eye, following surgery or a perforating injury, elicits a uveitis of the good eye which progresses to permanent loss of function. It can be averted if the injured eye is enucleated before the uveitis appears in the opposite eye. The condition closely resembles experimental allergic uveitis, a condition which has been induced in guinea pigs by administration of retinal antigen-S, a soluble substance derived from rod outer segments. It is currently postulated that sensitization to antigen-S is responsible for sympathetic ophthalmia but there is some speculation that altered cell-mediated immunity in these patients also contributes to the aetiology.

2) Phacoantigenic uveitis is a rare complication of cataract surgery in which an inflammatory response is elicited by particles of lens material retained in the anterior chamber. It is likely that the response occurs because the lens fibres, normally sequestered from the immune system by the thick lens capsule, are exposed to

the immune system. It is unclear why the condition is so rare and it has been suggested that in affected patients the release of lens antigens is associated with a loss of T-cell tolerance.

Achievements of the Department's vasectomy project.

The Department's vasectomy project is succeeding in its aim to determine the sequence of events following vasectomy in the Albino Swiss rat. However, much work remains to be done to fill the gaps in our knowledge indicated in this discussion. To date the achievements have been the mapping of the lymphatic drainage pattern of the epididymis and ductus deferens, the demonstration of the importance of the lymphatic route of antigen presentation and the participation of the regional lymph nodes in the humoral response which follows vasectomy. The effects of vasectomy on the epididymis and the mechanisms of granuloma formation are gradually being determined. Recently attention has been given to the effect of vasectomy on the testis; a detailed study of spermatogenesis and spermatozoal release in apparently healthy testicular tubules is presented in this thesis. The various mechanisms by which vasectomy may result in testicular changes and their relation to granuloma formation is currently under investigation. It is hoped that the results of these studies will alert clinicians about possible mechanisms of testicular change following vasectomy in man and encourage them to research whether

processes similar to those described in the Albino Swiss rat occur in their patients.

REFERENCES

Alexander, N. J. (1972) Vasectomy: long-term effects in the rhesus monkey. Journal of Reproduction and Fertility 31 399 - 406.

Alexander, N. J. (1973) Ultrastructural changes in rat epididymis after vasectomy. Zeitschrift fur Zellforschung und Mikroskopische Anatomie 136 177 - 182.

Alexander, N. J., Wilson, B. J. & Patterson, G. D. (1974) Vasectomy: immunologic effects in rhesus monkeys and men. Fertility and Sterility 25 149 - 156.

Alexander, N. J. (1977) Vasectomy and vasovasostomy in rhesus monkeys: the effect of circulating antisperm antibodies on fertility. Fertility and Sterility 28 562 - 569.

Alexander, N. J. & Schmidt, S. S. (1977) Incidence of antisperm antibody levels and granulomas in men. Fertility and Sterility 28 655 - 657.

Alexander, N. J. & Tung, K. S. K. (1977) Immunological and morphological effects in the rabbit. Anatomical Record 188 339 - 350.

Alexander, N. J. & Anderson, D. J. (1979) Vasectomy: consequences of autoimmunity to sperm antigens. Fertility and Sterility 32 253 - 260.

Alexander, N. J. & Tung, K. S. K. (1979a) Effects of vasectomy in rhesus monkeys. In Vasectomy: Immunologic and Pathophysiologic Effects in Animals and Man. I. H. Lepow & R. Crozier (ed.). London: Academic Press, pp. 423 - 458.

Alexander, N. J. & Tung, K. S. K. (1979b) Vasectomy in the rabbit: immunological and morphological effects. In Vasectomy: Immunologic and Pathophysiologic Effects in Animals and Man. I. H. Lepow & R. Crozier (ed.). London: Academic Press, pp. 355 - 377.

Al-Saffar, R. A. S. (1987) Morphological studies of the immune response to vasectomy. Ph.D. thesis, University of Glasgow.

Amann, R. P. (1962) Reproductive capacity of dairy bulls. III. The effect of ejaculation frequency, unilateral vasectomy, and age on spermatogenesis. American Journal of Anatomy 110 49 - 67.

Anderson, D. J. & Alexander, N. J. (1981) Antisperm antibody titres, immune complex deposition and immunocompetence in long-term vasectomized mice. Clinical and Experimental Immunology 43 99 - 108.

Ansbacher, R. (1974) Bilateral vas ligation: sperm antibodies. Contraception 9 227 - 237.

Ansbacher, R. & Gangiai, M. P. (1975) Testicular biopsy: sperm antibodies. Fertility and Sterility 26 1239 - 1242.

Ansbacher, R., Hodge, P., Williams, A. & Mumford, D. M. (1976) Vas ligation: humoral sperm antibodies. International Journal of Fertility 21 258 - 260.

Ball, R. Y. & Mitchinson, M. J. (1977) Degradation of phagocytosed spermatozoa by mouse peritoneal macrophages in vitro. Journal of Reproduction and Fertility 49 409 - 411.

Ball, R. Y., Naylor, C. P. E. & Mitchinson, M. J. (1982) Spermatozoa in an abdominal lymph node after vasectomy in a man. Journal of Reproduction and Fertility 66 715 - 716.

Ball, R. Y. & Setchell, B. P. (1983) The passage of spermatozoa to regional lymph nodes in testicular lymph following vasectomy in ram and boars. Journal of Reproduction and Fertility 68 148 - 153.

Bansal, N., Majumdar, S. & Chakravarti, R. N. (1986) Frequency and size of atherosclerotic plaques in vasectomized diabetic monkeys. International Journal of Fertility 31 298 - 304.

Bedford, J. M. (1976) Adaptations of the male reproductive tract and the fate of spermatozoa following vasectomy in the rabbit, rhesus monkey, hamster and rat. Biology of Reproduction 14 118 - 142.

Berg, J. W. (1954) An acid-fast lipid from spermatozoa. Archives of Pathology 57 115 -120.

Bigazzi, P. E., Kosuda, L. & Harnick, L. (1977) Sperm autoantibodies in vasectomized rats of different inbred strains. Science 197 1282 - 1283.

Bigazzi, P. E. (1979) Immunopathological findings in vasectomized rabbits. In Vasectomy: Immunologic and Pathophysiologic Effects in Animals and Man. I. H. Lepow & R. Crozier (ed.). London: Academic Press, pp. 339 - 353.

Bigazzi, P. E., Alexander, N. J., & Silber, S. J. (1979) Studies on testicular biopsies from vasectomized men. In Vasectomy: Immunologic and Pathophysiologic Effects in Animals and Man. I. H. Lepow & R. Crozier (ed.). London: Academic Press, pp. 459 - 469.

Boettcher, B. (1977) Isolation of spermatozoal antigens. In Immunological Influence on Human Fertility. B. Boettcher (ed.). London: Academic Press, pp. 105 - 110.

Brannen, G. E., Kwart, A. M. & Coffey, D. S. (1974) Immunologic implications of vasectomy. I. Cell-mediated immunity. Fertility and Sterility 25 508 - 514.

Bullock, J. Y., Gilmore, L. L. & Wilson, J. D. (1977) Autoantibodies following vasectomy. Journal of Urology 118 604 - 606.

Cambell, W. B., Slack, R. W. T., Clifford, P. C., Smith, P. J. B. & Baird, R. N. (1983) Vasectomy and atherosclerosis: an association in Man? British Journal of Urology 55 430 - 435.

Chapman, E. S., Heidger, P. M., Harrison, R. M., Roberts, J. A., Dominigue, G. J. & Schlegel, J. U. (1978) Vasectomy in rhesus monkeys. IV. Electron microscopic studies of the seminiferous epithelium. Anatomical Record 192 41 - 54.

Chapman, E. S. & Heidger, P. M. (1979) Spermatic granuloma of the vas deferens after vasectomy in rhesus monkeys and men: light and electron microscopic study. Urology 13 629 - 639.

Choi, Y. L. & Reiner, L. (1983) Autoimmune response following vasectomy. New York State Journal of Medicine 83 819 - 822.

Clarkson, T. B. & Alexander, N. J. (1980) Long-term vasectomy: effects on the occurrence and extent of atherosclerosis in the rhesus monkey. Journal of Clinical Investigation 65 15 - 25.

Conway, E. A. (1937) Cyclic changes in lymphatic nodules. Anatomical Record 69 487 - 513.

Cooper, T. G. & Hamilton, D. W. (1977) Phagocytosis of spermatozoa in the terminal region and gland of the vas deferens of the rat. American Journal of Anatomy 150 247 - 268.

Cosentino, M. J., Sheinfeld, J., Erturk, E. & Cockett, A. T. K. (1986) The effect of graded unilateral testicular biopsy on the reproductive capacity of male rats. Journal of Urology 135 155 - 158.

Croft, B. T. & Bartke, A. (1976) Quantitative study of spermatogenesis in vasectomized mice. International Journal of Fertility 21 61 - 64.

Edwards, I. S. & Errey, B. (1982) Pain on ejaculation after vasectomy. British Medical Journal 284 1710.

Esho, J. O., Cass, A. S. & Ireland, G. W. (1973) Morbidity associated with vasectomy. Journal of Urology 110 413 - 415.

Esk, P. C. & Pabst, R. (1981) Improved results of vasovasostomy after sparing of nerves during vasectomy. Fertility and Sterility 35 363 - 364.

Evans, B., Gannon, B. J., Heath, J. W. & Burnstock, G. (1972) Long-lasting damage to the internal male genital organs and their adrenergic innervation in rats following chronic treatment with the antihypertensive drug guanethidine. Fertility and Sterility 23 657 - 667.

Fahrenbach, H. B., Alexander, N. J., Senner, J. W., Fulgham, D. L. & Coon, C. J. (1980) Effects of vasectomy on the retinal vasculature of men. Journal of Andrology 1 299 - 303.

Fallon, F., Jacobo, L. & Bunge, R. E. (1978) Restoration of fertility by vasovasostomy. Journal of Urology 119 85 - 86.

Fawcett, D. W. (1979) Interpretation of the sequelae of vasectomy. In Vasectomy: Immunologic and Pathophysiologic Effects in Animals and Man. I. H. Lepow & R. Crozier (ed.). London: Academic Press, pp. 3 - 23.

Fleming, S. (1985) Immunophysiology: the immune response. In Muir's Textbook of Pathology, 12th Ed. J. R. Anderson (ed.). London: Edward Arnold, pp. 6.1 - 40.

Flickinger, C. J. (1972a) Alterations in the fine structure of the rat epididymis after vasectomy. Anatomical Record 173 277 - 300.

Flickinger, C. J. (1972b) Ultrastructure of the rat testis after vasectomy. Anatomical Record 174 477 - 494.

Friedman, N. B. & Garske, G. L. (1949) Inflammatory reactions involving sperm and the seminiferous tubules: extravasation, sperm granulomas and granulomatous orchitis. Journal of Urology 62 363 - 374.

Fuchs, E. F. & Alexander, N. J. (1983) Immunologic considerations before and after vasovasostomy. Fertility and Sterility 40 497 - 499.

Galle, J. & Friend, D. S. (1977) The fate of sperm after vasectomy in the guinea pig: fine structure, freeze-fracture and cytochemistry. Laboratory Investigation 37 79 - 95.

Goldacre, M. J., Holford, T. R. & Vessey, M. P. (1983) Cardiovascular disease and vasectomy. Findings from two epidemiologic studies. New England Journal of Medicine 308 805 - 808.

Gordon, D. L., Barr, A. B., Herrigel, J. E. & Paulsen, C. A. (1965) Testicular biopsy in man. I. The effect upon sperm concentration. Fertility and Sterility 16 522 - 530.

Grewal, R. S. & Sachan, M. S. (1968) Changes in testicle after vasectomy. An experimental study. International Surgery 49 460 - 462.

Gupta, A. S., Kothari, L. K., Dhruva, A. & Bapna, R. (1975) Surgical sterilization by vasectomy and its effect on structure and function of the testis in man. British Journal of Surgery 62 59 - 63.

Gupta, I., Dhawan, S., Goel, G. D. & Saha, K. (1975) Low fertility rate in vasovasostomized males and its possible immunologic mechanism. International Journal of Fertility 20 183 - 191.

Hadley, M. A. & Dym, M. (1983) Spermatogenesis in the vasectomized monkey: quantitative analysis. Anatomical Record 205 381 - 386.

Harris, J. D. & Lipshultz, L. I. (1981) The effect of vasectomy on Sertoli cell function in rats. Investigative Urology 18 305 - 307.

Head, J. R. & Billingham, R. E. (1985) Immune privilege in the testis. II. Evaluation of potential local factors. Transplantation 40 269 - 275.

Heller, G. V. & Rothchild, I. (1974) The influence of the surgical technique used for vasectomy on testis function in rats. Journal of Reproduction and Fertility 39 81 - 84.

Hernandez-Jauregui, P. & Olivera, J. (1975) A study of the morphology of the Leydig cells of the rat after unilateral vasectomy. Journal of Reproduction and Fertility 45 405 - 407.

Hjort, T., Husted, S. & Linnet-Jepsen, P. (1974) The effect of testis biopsy on autosensitization against spermatozoal antigens. Clinical and Experimental Immunology 18 201 - 212.

Hodson, N. (1970) The nerves of the testis, epididymis and scrotum. In The Testis. A. D. Johnson, W. R. Gomes & N. L. Vandemark (ed.). London: Academic Press, pp. 47 - 99.

Howards, S. S. & Johnson, A. L. (1979) Effects of vasectomy on intratubular hydrostatic pressure in the testis and epididymis. In Vasectomy: Immunologic and Pathophysiologic Effects in Animals and Man. I. H. Lepow & R. Crozier (ed.). London: Academic Press, pp. 55 - 67.

Isojima, S., Li, T. S. & Ashitaka, Y. (1968) Immunologic analysis of sperm-immobilizing factor found in sera of women with unexplained sterility. American Journal of Obstetrics and Gynaecology 101 677 - 683.

James, K. & Hargreave, T. B. (1984) Immunosuppression by seminal plasma and its possible clinical significance. Immunology Today 5 357 - 363.

Jarow, J. P., Budin, R. E., Dym, M., Zirken, B. R., Noren, S. & Marshall, F. F. (1985) Quantitative pathologic changes in the human testis after vasectomy. A controlled study. New England Journal of Medicine 313 1252 - 1256.

Jenkins, J. L., Muir, V. Y., Blacklock, N. J., Turk, J. L. & Hanley, H. G. (1979) Consequences of vasectomy: an immunological and histological study related to subsequent fertility. British Journal of Urology 51 406 - 410.

Johnson, A. L. & Howards, S. S. (1975) Intratubular hydrostatic pressure in the testis and epididymis before and after vasectomy. American Journal of Physiology 228 556 - 564.

Jones, R. (1973) Epididymal function in vasectomized rabbits. Journal of Reproduction and Fertility 36 199 - 202.

Kazeem, A. A. (1979) Regional lymphatics and lymph nodes of the testis with reference to the testis as an immunologically privileged site. Ph. D. thesis, University of Glasgow.

Kazeem, A. A., Reid, O. & Scothorne, R. J. (1982) Studies on hemolymph nodes. I. Histology of the renal hemolymph node of the rat. Journal of Anatomy 134 677 - 683.

Kennedy, S. W. & Simon, B. W. (1974) Spermatic granuloma in vasectomized rats. Texas Reports on Biology and Medicine 32 857.

Kennedy, S. W. & Heidger, P. M. (1980) Fine structure of the spermatic granuloma of the rat vas deferens following vasectomy. Anatomical Record 198 461 - 474.

Kibrick, S., Belding, D. L. & Merrill, B. (1952) Methods for the detection of antibodies against mammalian spermatozoa. II. A gelatin agglutination test. Fertility and Sterility 3 430 - 438.

Kisker, C. T., Wu, K. K., Culp, D. A., Hackett, J. G., Alexander, N. J., Hess, E. V. & Houk, J. L. (1979) Blood coagulation studies in vasectomy. In Vasectomy: Immunologic and Pathophysiologic Effects in Animals and Man. I. H. Lepow & R. Crozier (ed.). London: Academic Press, pp. 105 - 120.

Kolk, A. (1979) Isolation of sperm-specific autoantigens. In Vasectomy: Immunologic and Pathophysiologic Effects in Animals and Man. I. H. Lepow & R. Crozier (ed.). London: Academic Press, pp. 223 - 239.

Kuwahara, M. & Frick, J. (1975) The ligation of the male reproductive organs and the role of the spermatic cyst. Andrologia 7 1 - 14.

Kwart, A. M. & Coffey, D. S. (1973) Sperm granulomas: an adverse effect of vasectomy. Journal of Urology 110 416 - 422.

Lahita, R. G. (1984) Sex hormones and immunity. In Basic and Clinical Immunology. D. P. Stites, A. Stobo, H. H. Fudenberg & J. V. Wells (ed.). Los Altos, California: Lange Medical Publications.

Lamano-Carvalho, T. L., Favaretto, A. L. V., Ferreira, A. L. & Antunes-Rodrigues, J. (1984) Histophysiological study of vasectomized rats. Brazilian Journal of Medical and Biological Research 17 83 - 91.

Lauersen, N. H., Muchmore, E., Shulman, S., Marinescu, A. E., Graves, Z. R., Leeds, L. & Geller, S. A. (1983) Vasectomy and atherosclerosis in Macaca fascicularis: new findings in a controversial issue. Journal of Reproductive Medicine 28 750 - 758.

Law, H. Y., Bodmer, W. F., Mathews, J. D. & Skegg, D. C. G. (1979) The immune response to vasectomy and its relation to the HLA system. Tissue Antigens 14 115 - 139.

Leblond, C. P. & Clermont, Y. (1952) Spermiogenesis of rat, mouse, hamster and guinea pig as revealed by the "periodic acid-fuchsin sulphurous acid" technique. American Journal of Anatomy 90 167 - 215.

Lee, H. Y. (1986) A 20-year experience with vasovasostomy. Journal of Urology 136 413 - 415.

Lee, L. & McLoughlin, M. G. (1980) Vasovasostomy - comparison of macroscopic and microscopic techniques at one institution. Fertility and Sterility 33 54 - 55.

Lenzi, A., Valesini, G. & Dondero, F. (1985) Vasectomy: study of circulating immune-complexes and its correlation with antisperm immunity in man, with a twelve month follow-up study. Andrologia 17 158 - 165.

Lewis-Jones, D. I. & Lynch, R. (1987) Male infertility. British Journal of Hospital Medicine 37 488 - 502.

Linnet, L. & Hjort, T. (1977) Sperm agglutinins in seminal plasma and serum after vasectomy: correlation between immunological and clinical findings. Clinical and Experimental Immunology 30 413 - 420.

Linnet, L., Hjort, T. & Fogh-Andersen, P. (1981) Association between failure to impregnate after vasovasostomy and spermagglutinins in semen. Lancet 1 117 - 119.

Linnet, L., Moller, N. P. H., Bernth-Petersen, P., Ehlers, N., Brandslund, I. & Svehag, S. E. (1982) No increase in arteriosclerotic retinopathy or activity in tests for circulating immune complexes 5 years after vasectomy. Fertility and Sterility 37 798 - 806.

Livingstone, E. S. (1971) Vasectomy: a review of 3200 operations. Canadian Medical Association Journal 105 1065.

Lucas, P. L. & Rose, N. R. (1978) Immunological consequences of vasectomy: I. Prospective study through one-year postvasectomy. Archives of Andrology 1 249 - 255.

McDonald, S. W. (1980) Responses to vasectomy in the first regional lymph node of the testis in rats. B.Sc. thesis, University of Glasgow.

McDonald, S. W. & Scothorne, R. J. (1986) On the response of the regional testicular lymph nodes after unilateral vasectomy in rats. Journal of Anatomy 144 49 - 59.

MacDougall, M. K., McCowin, K., Derrick, F. C., Glover, W. L. & Jacobson, C. B. (1975) The effects of vasectomy on spermatogenesis in the dog, Canis familiaris: a meiotic analysis. Fertility and Sterility 26 786 - 790.

McGlynn, J. M. & Erpino, M. J. (1974) Effects of vasectomy on the reproductive system and sexual behaviour of rats. Journal of Reproduction and Fertility 40 241 - 247.

Mancini, R. E. (1976) Immunologic Aspects of Testicular Function. New York: Springer-Verlag.

Martin, D. C. (1981) Microsurgical reversal of vasectomy. American Journal of Surgery 142 48 - 50.

Mathews, J. D., Skegg, D. C. G., Vessey, M. P., Konice, M., Holborow, E. J. & Guillebaud, J. (1976) Weak autoantibody reactions to antigens other than sperm after vasectomy. British Medical Journal 2 1359 - 1360.

Mehrotra, R., Nath, P., Singh, K. M., Tandon, P., Kumar, H., Pandey, R. K. & Mehrotra, R. M. L. (1985) Changes in seminiferous tubules after vasectomy. Indian Journal of Pathology and Microbiology 28 371 - 378.

Miller, R. J., Killian, G. J. & Vasilenko, P. (1984) Effects of long- and short-term vasectomy on structural and functional parameters of the rat. Journal of Andrology 5 381 -388.

Millette, C. F. & Bellve, A. R. (1977) Temporal expression of membrane antigens during mouse spermatogenesis. Journal of Cell Biology 74 86 - 97.

Muir, V. Y., Turk, J. L. & Hanley, H. G. (1976) Comparison of allergic aspermatogenesis with that induced by vasectomy. I In vivo studies in the guinea pig. Clinical and Experimental Immunology 24 72 - 80.

Muir, V. Y., Turk, J. L. & Hanley, H. G. (1977) Comparison of allergic aspermatogenesis with that produced by vasectomy. II. In vitro studies of cell-mediated immunity to sperm after vasectomy in man and guinea pig. Clinical and Experimental Immunology 28 461 - 466.

Nagarkatti, P. S. & Rao, S. S. (1976) Cell-mediated immunity to homologous spermatozoa following vasectomy in the human male. Clinical and Experimental Immunology 26 239 - 242.

Neaves, W. B. (1974) The rat testis after vasectomy. Journal of Reproduction and Fertility 40 39 - 44.

Neaves, W. B. (1978) The effect of vasectomy on the testis of inbred Lewis rats. Journal of Reproduction and Fertility 54 405 - 411.

O'Brien, D. A. & Millette, C. F. (1984) Identification and immunochemical characterization of spermatogenic cell surface antigens that appear during early meiotic prophase. Developmental Biology 101 307 - 317.

O'Rand, M. G. & Romrell, L. J. (1977) Appearance of cell surface auto- and isoantigens during spermatogenesis in the rabbit. Developmental Biology 55 347 - 358.

Oslund, R. (1924) A study of vasectomy on rats and guinea pigs. American Journal of Physiology 67 422 - 443.

Owen, E. & Kapila, H. (1984) Vasectomy reversal. Review of 475 microsurgical vasovasostomies. Medical Journal of Australia 140 398 - 400.

Parslow, J. M., Royle, M. G., Kingscott, M. M. B., Wallace, D. M. A. & Hendry, W. E. (1983) The effect of sperm antibodies on fertility after vasectomy reversal. American Journal of Reproductive Immunology 3 28 - 31.

Perez-Clavier, R., Harrison, R. G. & Macmillan, E. W. (1982) The pattern of the lymphatic drainage of the rat epididymis. Journal of Anatomy 134 667 - 675.

Perrin, E. B., Woods, J. S., Namekata, T., Yagi, J., Bruce, R. A. & Hoffer, V. (1984) Long-term effect of vasectomy on coronary heart disease. American Journal of Public Health 74 128 - 132.

Petitti, D. B., Klein, R., Kipp, H. & Friedman, G. D. (1983) Vasectomy and the incidence of hospitalized illness. Journal of Urology 129 760 - 762.

Phadke, A. M. (1964) Fate of spermatozoa in cases of obstructive azoospermia after ligation of vas deferens in man. Journal of Reproduction and Fertility 7 1 - 12.

Plaut, S. M. (1973) Testicular morphology in rats vasectomized as adults. Science 181 554 - 555.

Poynter, H. (1939) Testis hormone secretion in the rat under conditions of vasectomy or isolation. Anatomical Record 74 355 - 379.

Raviola, E. (1986) The immune system. In Bloom and Fawcett: A Textbook of Histology. D. W. Fawcett (ed.). London: W. B. Saunders Co., pp. 406 - 435.

Requeda, E., Charron, J., Roberts, K. D., Chapdelaine, A. & Bleau, G. (1983) Fertilizing capacity and sperm antibodies in vasovasostomized men. Fertility and Sterility 39 197 - 203.

Richards, I. S., Davis, J. E. & Lubell, I. (1981) Current status of endocrinologic effects of vasectomy. Urology 18 1 - 6.

Ritchie, A. W. S., Hargreave, T. B., James, K. & Chisholm, G. D. (1984) Intra-epithelial lymphocytes in the normal epididymis. A mechanism for tolerance to sperm auto-antigens? British Journal of Urology 56 79 - 83.

Robbins, S. L. & Kumar, V. (1987) Disorders of immunity. In Basic Pathology. London: W. B. Saunders Co. pp. 129 - 181.

Rosenberg, C., Schwingl, P. J., Kaufman, D. W., Helmrich, S. P., Palmer, P. R. & Shapiro, S. (1986) The risk of myocardial infarction 10 or more years after vasectomy in men under 55 years of age. American Journal of Epidemiology 123 1049 - 1056.

Rowley, M. J., O'Keefe, K. B. & Heller, C. G. (1969) Decreases in sperm concentration due to testicular biopsy procedure in man. Journal of Urology 101 347 - 349.

Rumke, P. & Titus, M. (1970) Spermagglutinin formation in male rats by subcutaneously injected syngeneic epididymal spermatozoa and by vasoligation or vasectomy. Journal of Reproduction and Fertility 21 69 - 79.

Rumke, P. & Hellema, H. W. J. (1979) Immunologic studies in long-term follow-up of vasectomized men. In Vasectomy: Immunologic and Pathophysiologic Effects in Animals and Man. I. H. Lepow & R. Crozier (ed.). London: Academic Press, pp. 521 - 532.

Russell, L. D. (1977) Movement of spermatocytes from the basal to the adluminal compartment of the rat testis. American Journal of Anatomy 148 313 - 328.

Russell, L. D. (1978) The blood-testis barrier and its formation relative to spermatocyte maturation in the adult rat: a lanthanum tracer study. Anatomical Record 190 99 - 112.

Sackler, A. M., Weltman, A. S., Pandhi, V. & Schwarz, R. (1973) Gonadal effects of vasectomy and vasoligation. Science 179 293 - 295.

Samuel, T., Kolk, A. H. J., Rumke, P. & Van Lis, J. M. J. (1975) Auto-immunity to sperm antigens in vasectomized men. Clinical and Experimental Immunology 21 65 - 74.

Samuel, T. & Kolk, A. (1979) Auto-antigenicity of human protamines. In Vasectomy: Immunologic and Pathophysiologic Effects in Animals and Man. I. H. Lepow & R. Crozier (ed.). London: Academic Press, pp. 203 - 222.

Schlaegel, T. F. & O'Connor, G. R. (1987) Hypersensitivity uveitis. In Clinical Ophthalmology, Volume 4. T. D. Duane & E. A. Jaeger (ed.). London: Harper & Row, pp. 54.1 - 6.

Schmidt, S. S. (1979) Sperm granuloma: an often painful lesion. Fertility and Sterility 31 178 - 181.

Selikowitz, S. M. & Schned, A. R. (1985) A late post-vasectomy syndrome. Journal of Urology 134 494 - 497.

Shapiro, E. I. & Silber, S. J. (1979) Open-ended vasectomy, sperm granuloma and postvasectomy orchialgia. Fertility and Sterility 32 546 - 550.

Shulman, S., Zappi, E., Ahmed, U. & Davis, J. E. (1972) Immunologic consequences of vasectomy. Contraception 5 269 - 278.

Silber, S. J. (1978) Vasectomy and vasectomy reversal. Fertility and Sterility 29 125 - 140.

Smith, G. (1962) The effects of ligature of the vasa efferentia and vasectomy on testicular function in the adult rat. Journal of Endocrinology 23 385 - 399.

Steinberg, J. & Straus, R. (1947) Sperm invasion of the epididymis. Journal of Urology 57 498 - 503.

Sutherland, P. D., Matson, P. L., Masters, J. R. W. & Pryor, J. P., (1984) Association between infertility following reversal of vasectomy and the presence of sperm agglutinating activity in semen. International Journal of Andrology 7 503 - 508.

Thakur, A. N., Sheth, A. R. & Rao, S. S. (1972) Biochemical studies on rat testes and sex accessory organs after vasoligation operation. Fertility and Sterility 23 834 - 837.

Thomas, A. J., Pontes, J. E., Rose, N. R., Segal, S. & Pierce, J. M. (1981) Microsurgical vasovasostomy: immunologic consequences and subsequent fertility. Fertility and Sterility 35 447 - 450.

Tilney, N. L. (1971) Patterns of lymphatic drainage in the adult laboratory rat. Journal of Anatomy 109 369 - 383.

Trowell, O. A. (1955) The culture of lymph nodes in synthetic media. Experimental Cell Research 9 258 - 276.

Tumboh-Oeri, A. G. & Roberts, T. K. (1978) The induction of cell-mediated immunity to spermatozoa following vasectomy in inbred mice. International Journal of Andrology 1 81 - 85.

Tumboh-Oeri, A. G. & Roberts, T. K. (1979) Immunological and morphological consequences of vasectomy in the rabbit. Experientia 35 675 - 676.

Tung, K. S. K. (1979) Pathogenesis of testicular pathology in vasectomized guinea pigs. In Vasectomy: Immunologic and Pathophysiologic Effects in Animals and Man. I. H. Lepow & R. Crozier (ed.). London: Academic Press, pp. 407 - 421.

Tung, K. S. K. & Alexander, N. J. (1980) Monocytic orchitis and aspermatogenesis in normal and vasectomized rhesus macaques (Macaca mulatta). American Journal of Pathology 101 17 - 30.

Tung, P. S. & Fritz, I. B. (1978) Specific surface antigens on rat pachytene spermatocytes and successive classes of germinal cells. Developmental Biology 64 297 - 315.

Walker, A. M., Jick, H., Hunter, J. R. & McEvoy, J. (1983) Vasectomy and nonfatal myocardial infarction: continued observation indicates no elevated risk. Journal of Urology 130 936 - 937.

Wang, Y. F. & Holstein, A. F. (1983) Intraepithelial lymphocytes and macrophages in the human epididymis. Cell and Tissue Research 233 517 - 521.

Weinerth, J. L. (1984) Long-term management of vasovasostomy patients. Fertility and Sterility 41 625 - 628.

Wilson, F. M. & Wilson, F. M. (1987) Postoperative uveitis. In Clinical Ophthalmology, Volume 4. T. D. Duane & E. A. Jaeger (ed.). London: Harper & Row, pp. 55.1 - 18.

Yule, T. D., Montoya, G. D., Russell, L. D., Williams, T. M. & Tung, K. S. K. (1988) Autoantigenic germ cells exist outside the blood-testis barrier. Journal of Immunology 141 1161 - 1167.