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

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INTRODUCTION

In a previous paper (McDonald & Scothorne, 1986) we described histological changes in the 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; Kosuda & Bigazzi, 1979) and in other species including man (Shulman, Zappi, Ahmed & Davis, 1972; Samuel, Kolk, Rumke & Van Lis, 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 (Kosuda & Bigazzi, 1979).

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, Harrison & MacMillan, 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 of Anatomy were used.

Left unilateral vasectomies were performed, with sterile precautions. Anaesthesia was produced by intraperitoneal injection of pentobarbitone sodium, supplemented by inhalation of ether. 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 cremaster muscle and skin were closed with catgut and silk sutures respectively and the wound protected by a plastic dressing. 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 and epididymis were demonstrated by injection of India ink beneath the serosa through a 30 G needle. In some cases, ink was injected directly into the granuloma or into the lumen of the ductus, using gentle pressure through a 27 G 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

Unoperated rats

The general layout of the rat male reproductive tract is shown in Figure 1. In each of the 26 unoperated control animals ink was injected at one or more of the sites marked with an asterisk in Figure 2, which summarises 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.

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

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.

The ductus deferens. A total of 21 ink injections was made beneath the serosa of the scrotal portion of the ductus deferens. The exact position of each is shown in Figure 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

Figs. 1–5. The testis, epididymis and ductus deferens have been reflected to expose their lateral aspects. In Figures 2, 4 and 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).

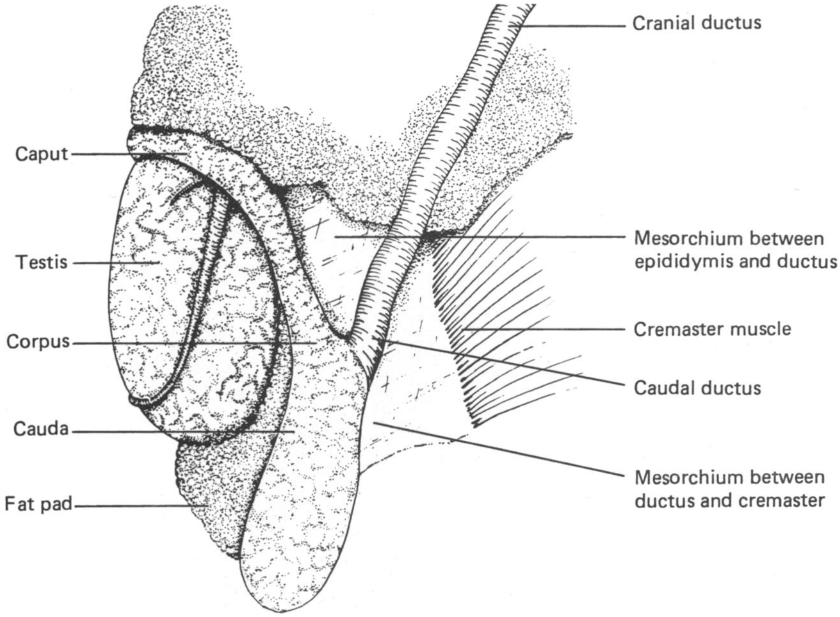

Fig. 1. General layout of the unoperated rat, left reproductive tract.

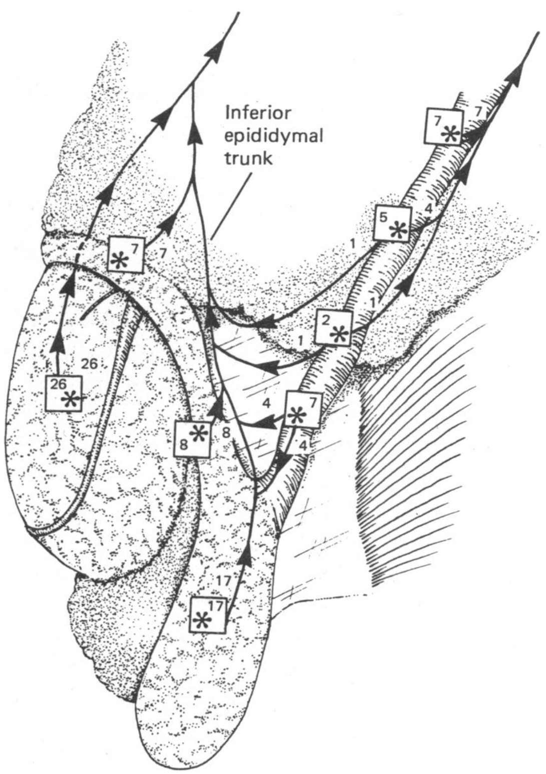

Fig. 2. Summary of lymphatic drainage of left testis, epididymis and ductus deferens in unoperated control animals.

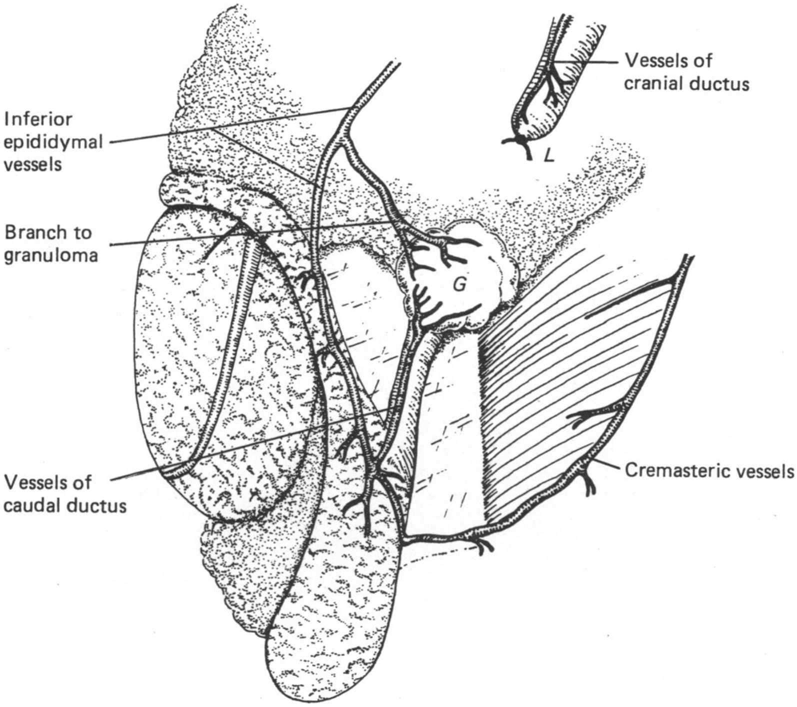

Fig. 3. The left male reproductive tract after vasectomy. A sperm granuloma (*G*) has formed at the vasectomy site. The ligature (*L*) at the cranial cut end of the ductus is visible. The blood vessels of the region are shown (see text).

scrotal ductus was injected, in three of these rats, the two sites drained independently, with no communicating lymphatics.

Vasectomised rats

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.

Vasal granulomas. Lymphatics from vasal granulomas accompanied, in a general way, the supplying blood vessels, which came from up to four sources (Fig. 3): always, from vessels of the ductus deferens caudal to the vasectomy site; usually, from branches of the inferior epididymal vessels; 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 (summarised in Fig. 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 two such cases, lymphatics from the cranial sector of the granuloma were found to accompany the vessels of the ductus cranially. Lymphatics from three 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

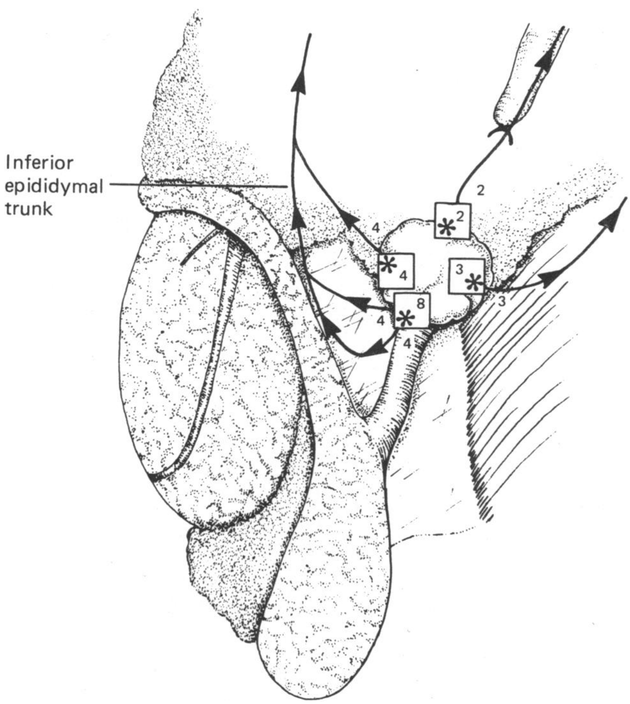

Fig. 4. Summary of lymphatic drainage of vasal sperm granulomas.

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.

Excurrent duct system of vasectomised rats (*Fig*. 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 four 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. 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 four rats, a lymphatic passed from the cauda towards the cremaster, but soon looped back to the cauda (Fig. 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

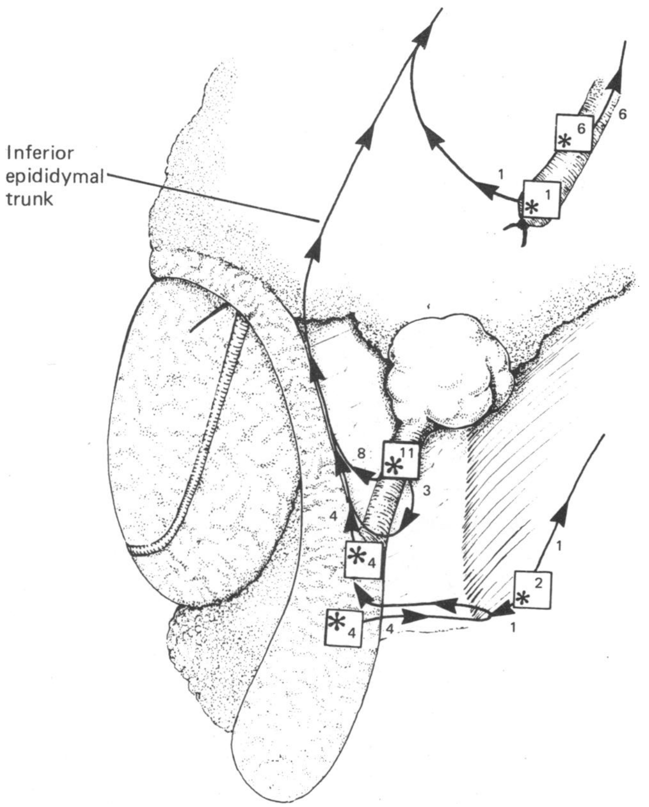

Fig. 5. Summary of lymphatic drainage of the epididymis and ductus deferens of vasectomised rats.

cauda, two 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 eleven 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. 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 six 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. 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 three 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, we believe, 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 authors, however, we found no case 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. We therefore looked specifically for such an arrangement but found that lymphatics always drained 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. Moreover, the injection of epididymal granulomas has shown that their lymph always reaches the testicular node–a finding in conformity with our previous observation that all rats with epididymal granulomas have a responsive testicular node (McDonald & Scothorne, 1986).

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 study (McDonald & Scothorne, 1986). 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 emphasised that lymphatics of the caudal sector of vasal granulomas would be unaffected. We can, therefore, answer the two questions posed in the Introduction:

(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 cranial 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.

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, drain 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.

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REFERENCES

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.

KAZEEM, A. 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.

KOSUDA, L. L. & BIGAZZI, P. E. (1979). Influence of genetic factors on the production of autoantibodies to spermatozoa in vasectomised rats and mice of different inbred strains. In *Vasectomy: Immunologic and Pathophysiologic Effects in Animals and Man* (ed. I. H. Lepow & R. Crozier), pp. 289–300. London: Academic Press.

McDONALD, S. W. & SCOTHORNE, R. J. (1986). On the response of the regional testicular lymph node after unilateral vasectomy in rats. *Journal of Anatomy* **144**, 49–59.

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.

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.

SAMUEL, T., KOLK, A. H. J., RUMKE, P. & VAN LIS, J. M. J. (1975). Auto-immunity to sperm antigens in vasectomised men. *Clinical and Experimental Immunology* **21**, 65–74.

SHULMAN, S., ZAPPI, E., AHMED, U. & DAVIS, J. E. (1972). Immunologic consequences of vasectomy. *Contraception* **5**, 269–278.

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