

The pattern of lymphatic drainage of the rat testis

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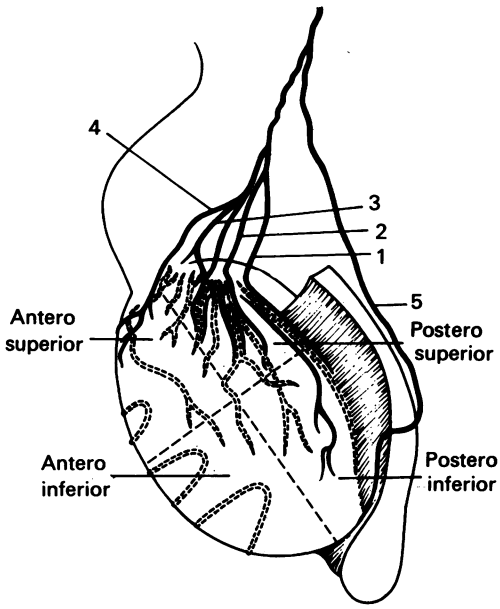
INTRODUCTION

The lymphatic drainage of the testis has long been neglected in considerations of testicular function. This is surprising when one reflects that the equilibrium which exists between the various cell types located within the membrana propria of the seminiferous tubule, and which determines the reproductive capacity of the mature male, must be dependent at least in part on a free flow of lymph from the testis: the activity of the cells of the interstitium is also likely to be influenced by changes in the quality and quantity of the testicular tissue fluids. Also it must be remembered that during the course of surgical operations in the inguinal region, which are commonplace, it is difficult to avoid injury to at least some of the lymphatic vessels draining the testis, and indeed the main testicular lymphatic trunk must often be divided. Although a number of observations have been made on the lymphatic drainage of mammalian testes (Cooper, 1830; Ludwig & Tomsa, 1861; Mihalkovics, 1873; Gerster, 1877; Testut, 1894; Regaud, 1897; Most, 1899; Poirier, Cuneo & Delamere, 1903; Jamieson & Dobson, 1910; Higgins, 1925; Rouvière, 1932; Fischer, 1933; Barringer & Earl, 1941; Paul, 1950; Macmillan, 1953; Ottaviani, 1954; Engeset, 1959; Azzalli & Didio, 1965*a, b, c, d*; Fawcett, Heidger & Leak, 1969; Setchell, Vogelmayr & Waites, 1969), these have been mainly concerned with man; it was therefore decided to undertake a detailed examination of the lymphatic drainage of the rat testis, since this animal is in common laboratory use.

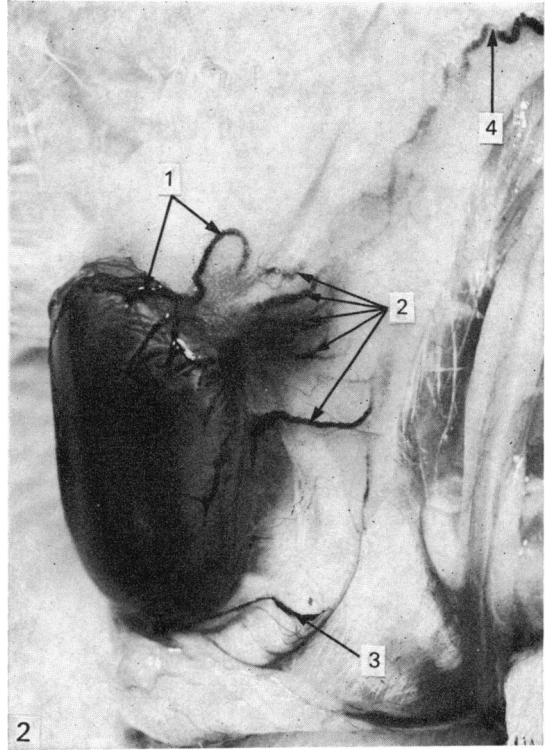
MATERIALS AND METHODS

The testes of 48 mature albino rats chosen at random were withdrawn through a midline suprapubic incision using intraperitoneal Nembutal anaesthesia (50 mg/kg) and injected immediately beneath the tunica albuginea with various contrast media into the anteroinferior quadrant, a site devoid of superficial lymphatic vessels. The contrast media used included 0.5 ml 20% India ink in physiological suspension injected by means of a micropipette drawn out from a microhaematocrit tube in a Bunsen microflame; this technique prevented escape of intratesticular fluid. Radioopaque contrast media such as 35% Micropaque (Damancy & Co.) suspension in normal saline, and white and yellow Chromopaque were also used. Thereafter the testes were observed *in situ* and then removed for further examination using microdissection techniques, radiography (80 kV for 0.40 second at 60 mA using Kodirek film) and microradiography (15 kV at 12 mA for 25 minutes using Kodak high resolution plates S.P.O. 652). In six animals, following the introduction of India ink, the testes were subjected to histological examination; 6 μ m sections were cut

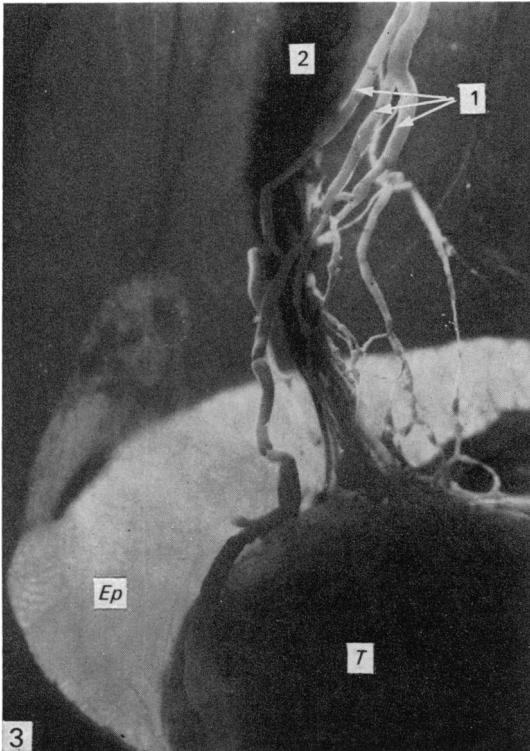
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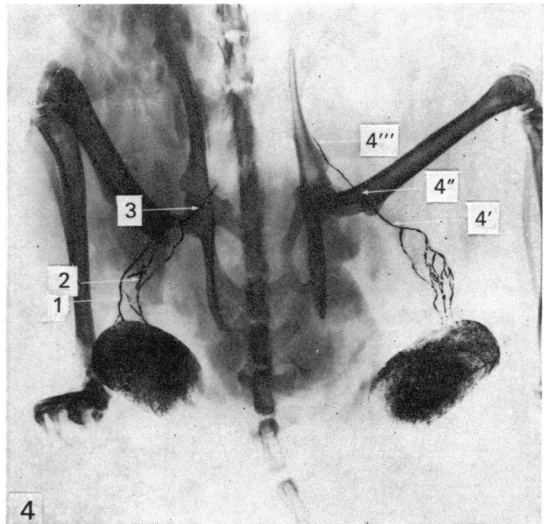
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after fixation in Bouin for 24 hours and stained with Weigert's iron haematoxylin and eosin.

RESULTS

Following the injection of the contrast media lymphographic studies showed that the lymph flow follows an orthograde route in a caudocranial direction from the lower to the upper pole and diagonally from the anteroinferior to the posterosuperior quadrant of the testis (Figs. 1, 2). The collecting vessels contain numerous bicuspid valves with short intervalvular segments in the earlier part of their course (Figs. 3, 4). Histologically, the fine lymph vessels of the membrana propria of the seminiferous tubules (Figs. 5, 6, 7) were clearly seen, and were not to be confused with branches of the testicular artery and veins.

Eleven to fifteen collecting vessels emerge from the surface of the testis in three groups. The superior group is located at the level of the upper pole, the middle group emerges from the mediastinum, the inferior group from the lower pole of the organ.

The superior testicular lymphatics, after their exit from the tunica albuginea, surround the ductuli efferentes, one, two or three vessels lying in front (Fig. 8) and several behind. The latter join the middle group almost immediately, after a short diagonal course, medial to the caput epididymidis. The former quickly reach the fatty body and join to form a single superior testicular trunk which itself follows a curved course medial to the superior epididymal artery and then crosses the medial surface of the testicular artery to join the main testicular lymphatic trunk.

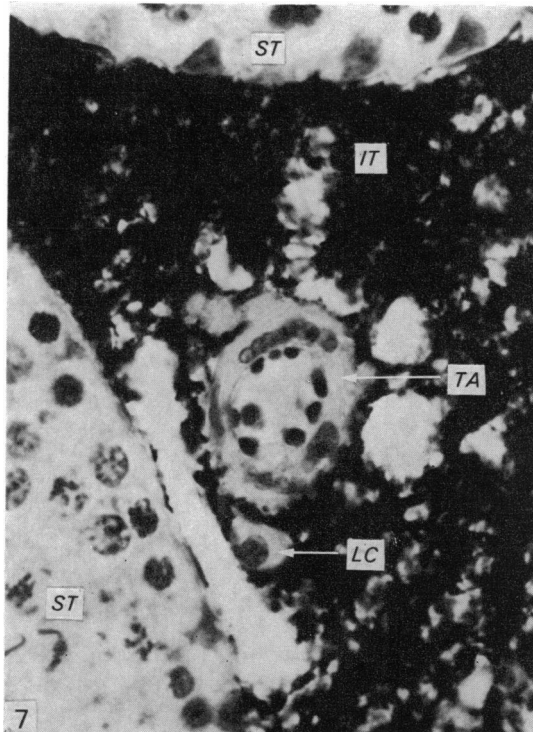
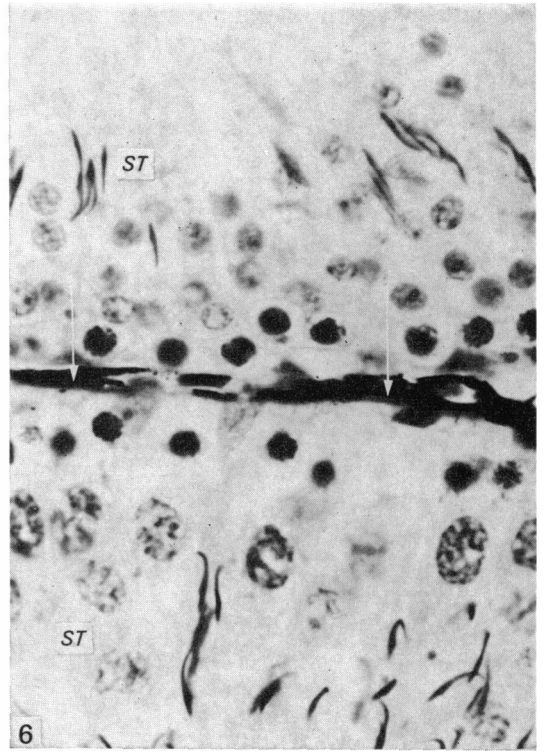
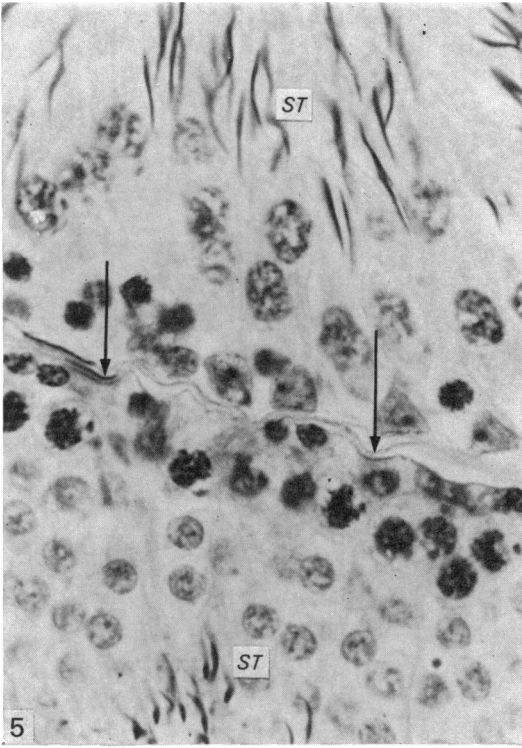
The middle testicular lymphatic vessels constitute a major group of collecting vessels emerging from the surface of the mediastinum where the testicular artery reaches the testis. Proximal, distal, medial and lateral collecting vessels can be recognized in this group, located around the testicular artery. The proximal collecting vessel is hidden between the testicular artery and the ductuli efferentes. The distal collecting vessel, located on the opposite side of the artery, is easily identified in the mesorchium as it leaves the tunica albuginea either as one vessel or as a group

Fig. 1. A drawing of the testis *in situ* demonstrating the course of the lymphatic collecting vessels (in black) following the intratesticular injection of contrast medium (e.g. India ink). The testicular artery and veins are shown in interrupted outline. The medial aspect of the right testis is depicted divided superficially into four quadrants. The superficial testicular lymphatic vessels are shown draining the anterosuperior, posterosuperior and posteroinferior quadrants. The numbers in the diagram express the order in which each vessel is filled by the medium when injection is made into the lower pole of the testis.

Fig. 2. A photograph of the lymphatic vessels *in situ* in a fresh specimen immediately after the intratesticular injection of India ink. 1, superior lymphatic group; 2, middle lymphatic group; 3, inferior lymphatic group; 4, main testicular lymphatic trunk.

Fig. 3. Photograph of the lymphatic collecting vessels (1) emerging from the medial surface of the testis (*T*). Note their relationship to the testicular vascular bundle (2). The caput and corpus epididymidis (*Ep*) are visible on the lateral side of the testicular vessels. White Chromopaque (intratesticular injection) was used as the contrast medium and the specimen was cleared using the Spalteholz technique. $\times 16$.

Fig. 4. A radiograph showing the orthograde flow of contrast medium injected deep to the tunica albuginea of the testes. The right testis shows a lymphatic vessel from the upper pole (1) and vessels from the mediastinum (2) which join as a single vessel at (3). On the left side three lymphatic collecting vessels emerge at the level of the mediastinum testis and form a network from which a single vessel arises and traverses the inguinal region (4', 4'') to reach the posterior abdominal wall (4'''). Microopaque injection.



of very short vessels forming anastomoses with the medial and lateral members of the group. A study of the various specimens suggested that this vessel is the forerunner of the main testicular lymphatic trunk. The medial collecting vessel, occasionally double, is found in front of the artery. The lateral collecting vessel, on the other hand, lies behind the testicular artery between it and the corpus epididymidis; it is sometimes double. The vessels of the middle group ascend, lying superficial to the artery and the pampiniform plexus (Fig. 9), dividing and rejoining to form a thin polygonal network (Fig. 10); a terminal vessel joins the main testicular lymphatic trunk.

Lymphatics of the inferior testicular lymphatic group arise from the lower pole of the testis and are easily recognized. One or two long vessels emerging from the tunica albuginea reach the mesorchium; the inferior testicular trunk, as representative of the group, crosses the medial face of the corpus epididymidis and in most cases joins with the inferior epididymal trunk at right angles or high up on the corpus epididymidis.

After 3½ minutes the contrast media outlined the main testicular lymphatic trunk which accepts the lymph drainage of the three testicular lymphatic groups (Fig. 11) and accompanies the testicular blood vessels. It runs on the dorsal body wall, lies retroperitoneally and reaches the para-aortic lymph nodes.

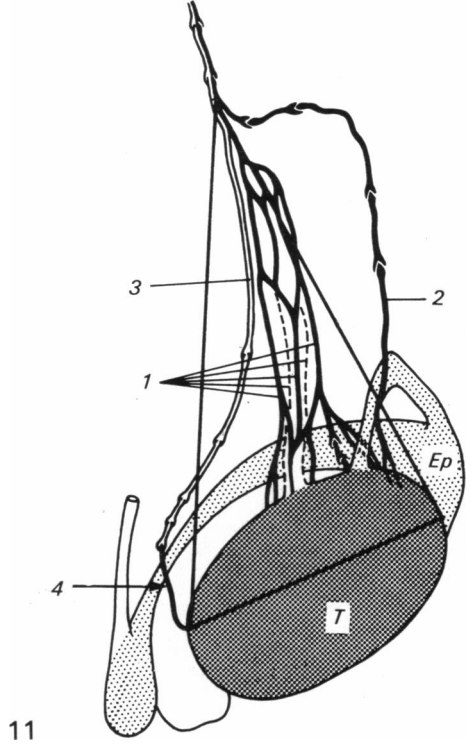
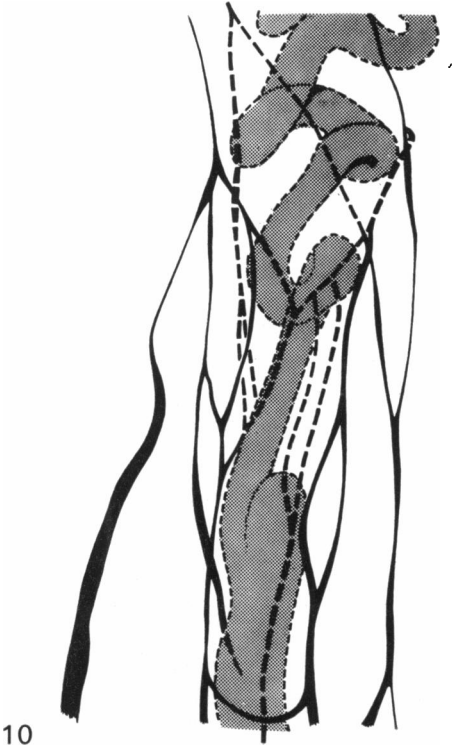
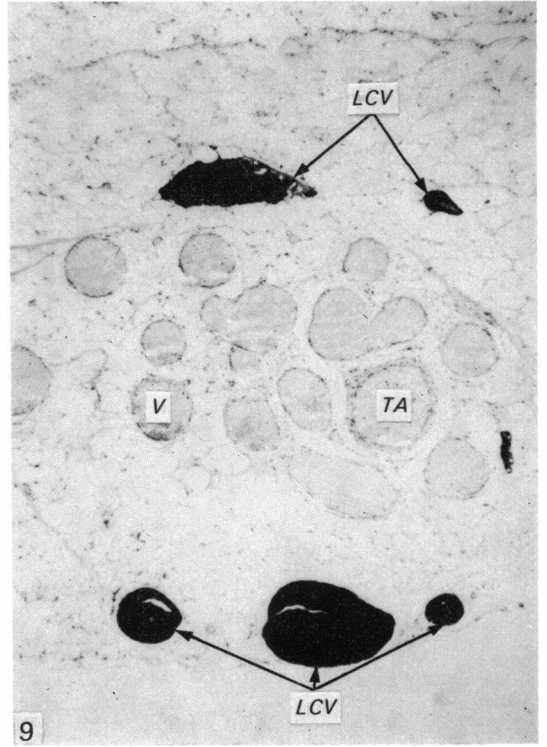
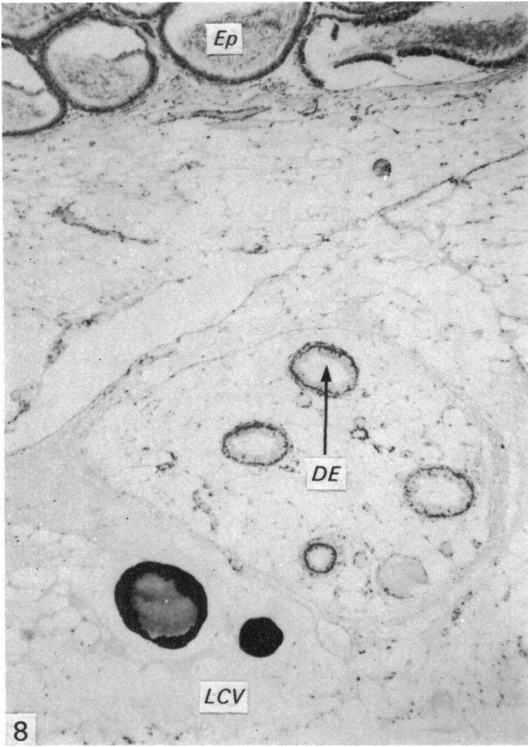
DISCUSSION

The microanatomical findings in the rat testis described in this work confirmed early reports establishing the circulation of lymph between and around the seminiferous tubules, in different species of animals and in Man, but failed to establish the presence of fibrous septa or septula containing lymphatics (Ludwig & Tomsa, 1862). The rat testis, therefore, consists of a glandular parenchyma formed by seminiferous tubules closely related to the blood and lymph circulations. The lymphatics are easily displayed by injecting contrast media deep to the tunica albuginea, and outlining the contour of the seminiferous tubules. The vessels lie in the interstitium so that the Leydig cells are in intimate contact with the lymphatic capillaries. Hundeiker (1969) studied the parenchyma of the bull testis, and Fawcett *et al.* (1969) examined the interstitial tissue of the testes of the guinea-pig and chinchilla. Their results confirm once again the location of the lymphatics between the seminiferous tubules. The lymphatic parenchymal network in the rat communicates with a sub-albugineal plexus which itself acts as a pathway for the drainage of intratesticular lymph; at the same time the collecting vessels leaving the testis become organized as three groups, namely mediastinal (cf. Ottaviani, 1931), superior (from the upper pole) and inferior (from the lower pole).

The interstitial fluid of the rat testis requires an uninterrupted and efficient pathway of drainage, and in the absence of septula, which are poorly developed in the rat (Regaud, 1897), numerous lymphatic collecting vessels emerge from the

Figs. 5 and 6. Photomicrographs of rat testis to show a lymphatic capillary between two adjoining tubular sections. Stained with Weigert's iron H/E. $\times 400$. (5) No injection (black arrows). (6) Injected with India ink (white arrows).

Fig. 7. Histological section of testis stained with Weigert's iron haematoxylin and eosin following injection of India ink suspension. *IT*, interstitial tissue loaded with India ink; *TA*, branch of the testicular artery; *LC*, Leydig cell; *ST*, seminiferous tubule. $\times 400$.



tunica albuginea at various levels. The majority of authors have concentrated their attention on the number of the collecting vessels which emerge from the tunica in different species (Most, 1899; Poirier *et al.* 1903; Jamieson & Dobson, 1910; Azzalli & Didio, 1965*a,b,c,d*). Workers who have made precise studies in the rat, among them Ottaviani (1931) and Macmillan (1953), have briefly described three lymphatics which join to form one single collecting trunk, described in this work as the main testicular lymphatic trunk. The recognition of the three main routes of lymphatic drainage from the surface of the rat testis establishes a constancy in the morphological pattern.

SUMMARY

The basic pattern of the lymphatic drainage of the testis is described in the mature albino rat following studies *in vivo* and the examination of dissected specimens *in vitro*. Microlymphography was undertaken using various contrast media. Three groups of lymphatics leave the surface of the testis – superior, medial and inferior, from the upper pole, from the mediastinum and from the lower pole respectively.

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Fig. 8. A photomicrograph demonstrating two lymphatic collecting vessels (LCV) belonging to the superior testicular lymphatic group on the medial surface of the ductuli efferentes (DE). The arrow points to one of four ductular cross sections. Ep, epididymis. Stained with Weigert's iron H/E. $\times 350$.

Fig. 9. A photomicrograph demonstrating the lymphatic collecting vessels (LCV) which contain India ink as a contrast medium following intratesticular injection. The LCV form a circle around the veins of the pampiniform plexus. One of the veins is shown at V; the veins surround the testicular artery (TA). Stained with Weigert's iron H/E. $\times 350$.

Fig. 10. A diagram based on photographs and stereoscopic observations to illustrate the polygonal lymphatic network around the convoluted part of the testicular artery 1.5 cm from the mediastinum testis. Note the anastomoses between the lymphatics of the middle group which drain the medial surface of the testis (continuous black lines) and those from the lateral surface of the testis lying behind the testicular artery (interrupted black lines). As the lymphatics ascend they show variation in calibre, constrictions and attenuations alternating with dilatations which contrast with the uniformity of the calibre of the artery. The lymph vessels converge at acute angles; connecting loops are easily seen.

Fig. 11. A drawing to show the basic pattern of the lymphatic drainage of the testis (T). The middle testicular lymphatic group (1) bisects the testicular lymphatic triangle bounded by the testis itself, the superior testicular trunk (2) and the inferior epididymal trunk (3) which receives the inferior testicular trunk (4).

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