Vascular Morphology of the Golden Hamster Spermatic Cord

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Summary. The morphology of the convoluted testicular artery and the pampiniform plexus of the golden hamster was studied by light microscopy and corrosion cast techniques combined with scanning electron microscopy. The artery was found to be totally enclosed by the pampiniform plexus, except for minor superficial areas where the artery was exposed. Although no direct connection between the artery and the vein was found in the area of apposition, the arterial and venous walls reduced their thickness by sharing a single tunica adventitia, which seemed well suited to the transfer of substances by diffusion. Many band-like structures of the venous walls were found in the deep part of the spermatic cord, suggesting that these may act as barriers to slow down the venous blood velocity. The venous wall here and there showed a stick-like endothelial bridge, suggesting that it may prevent the veins from over distension. In addition to the close relation between the artery and the vein, lymphatic vessels and mast cells were distributed widely within the connective tissue of the arterio-venous walls and venous walls. Mast cells were situated mainly in the area of apposition, especially at the base of the protruding venous wall. These morphological findings suggest that mast cells may be involved in the counter-current transfer mechanism in the spermatic cord of the golden hamster.

Materials and Methods

Forty adult golden hamsters (Mesocricetus auratus) weighing 100-120 g, were used in this study. Under ether and Nembutal anesthesia (5 mg/100 g body weight, I/P), the thoracic aorta was cannulated closely above the diaphragm. Ringer solution was perfused through the same cannula followed by a fixative, i.e., either 2.5% glutaraldehyde in 0.1 M phosphate buffer for electron microscopy, or Bouin's fluid or 10% formalin for light microscopy.

Light microscopy

Paraplast-embedded samples were sectioned serially at 6 μm and stained either with Masson's trichrome, alcian blue-safranin or toluidine blue. For distinction between venules and the lymphatics, a solution containing 0.5-1.0% silver nitrate and 0.2-0.3% India ink was introduced through the cannula (according to the method of Mori, 1969).

Perfused and unperfused samples were excised and cut into small blocks (1-2 mm thick), which were subsequently fixed for 2 h in 2.5% glutaraldehyde in 0.1 M phosphate buffer. They were then rinsed in the...
same phosphate buffer and post-fixed in 1% osmium tetroxide for 2 h. The blocks were then dehydrated in graded alcohol and embedded in Epon 812 or Araldites 6005. The 1.0-1.5 μm sections were stained with toluidine blue and were observed.

Scanning electron microscopy
For the preparation of corrosion casts, methacrylic methyl ester monomer (Acry ester M., Mitsubishi Rayon Co., Ltd. Japan) was semipolymerized according to the methods described by MURAKAMI (1970, 1975). The semipolymerized ester was mixed with 25-30% hydroxypropyl methacrylate, 0.1% sudan III and 1-1.5% benzoyl peroxide (catalyst). Further, the mixture was supplemented with 1-1.5% N-N-Dimethyl aniline (accelator) just before injection. The resin, then, was perfused through the same cannula as described above followed by perfusion with normal saline solution. The animals were immersed in warm water (60°C) for 4 h, and then were macerated in warm 2-5% sodium hydroxide for 12-20 h. The macerated specimens were rinsed in distilled water for 1-2 h, and then in 5% trichloroacetic acid to eliminate remnant tissue elements (PANNARALE et al., 1986). Finally, the casts were air dried, mounted on stubs, and coated with gold to be observed by a scanning electron microscope (Hitachi, S-430) at 15 kV.

Some tissue samples were fixed for 2 h in 2.5% glutaraldehyde and post-fixed in 1% osmium tetroxide for another 2 h. The osmicated tissues (1-2 mm thick) were then dehydrated in graded ethanol, critical point-dried and coated with gold by sputtering in a vacuum evaporator. The samples were observed as described above.

RESULTS
A single, long testicular artery arose from the abdominal aorta slightly below the origin of the renal artery. The artery was divided into three portions. The straight abdominal portion, which was the initial part of the testicular artery, originated from the aorta and extended to its first arterial branch, the cranial epididymal artery. The spermatic cordal portion was defined as that part between the origin of the cranial epididymal artery and the cranial pole of the testis. Finally, the testicular portion of the artery terminated as a serpentine loop on the testicular surface. Of these three portions, only the spermatic cordal portion was closely related to the pampiniform plexus.

After giving off the cranial epididymal artery, the testicular artery began to convolute along the transverse plane. This convolution gradually increased as it descended to the testis, and reached its maximum at the middle of the cord. The convolutions then slowly decreased and disappeared close to the cranial pole of the testis (Fig. 1). The mean number of spiral loops was 20.28, with a range from 17 to 22 in five hamsters. In addition to the convolutions, a retrograde loop was also found, especially in the middle of the cord (Fig. 2).

In addition, several testicular veins ran parallel and anastomosed with each other to form a delicate network, the pampiniform plexus (Figs. 3, 4). The number of the veins gradually decreased towards the proximal portion of the artery. Both the deep and superficial arterial surfaces were entirely covered.
by this complicated venous plexus, except in some superficial areas where the artery was free (Fig. 4). In the convolutions and retrograde loop, one or two layers of veins surrounded the arterial loop in its deep part. On the other hand, there was only one layer of veins covering the artery in the superficial aspect (Fig. 4). A capillary network formed from the epididymal artery was also found between testicular artery and pampiniform plexus.

The tunica adventitia of the arterial and venous compartments joined together to form a single connective tissue septum (Figs. 5, 6, 8). Although the artery and the veins formed such a closely related structure, direct connections (shunts) between them were not found throughout the observations.

Many band-like structures of the venous walls, varying in size and length, were found in the deep part of the spermatic cord (Figs. 4, 5). A stick-like structure was also found to project from the venous wall to reach the opposite wall. This structure, occurred either between the apposing area and the anastomosing venous wall (Fig. 5) or between two area of apposition (Fig. 7), and was regarded as an endothelial bridge (GREENBERG et al., 1985). In observation of the cross section, the bridge was round in shape and composed of an inner endothelial cell layer, and a smooth muscle cell layer in the middle and the central connective tissue. The components of the bridge thus corresponded to those of the venous wall.

In light microscopy, the testicular artery was a small muscular type of artery consisting of a prominent internal elastic membrane and 3-4 layers of smooth muscle cells in the tunica media. The vein was composed of a single smooth muscle layer in the media with an incomplete internal elastic membrane (Fig. 8). The muscle layers varied in number from 0 to 3 in the venous wall, being thicker at the site where the venous wall emerged from the area of apposition but rather thinner at this area itself.

Lymphatics and mast cells were widely distributed in the connective tissue of the arterio-venous wall as well as in the endothelial bridge. Mast cells occurred predominantly at the base of the protruding venous wall rather than in the apposing area (Fig. 8). They were located closer to the venous or lymphatic side than to the arterial side (Fig. 9). Large lymphatic capillaries were found in the apposing area where the venous wall protruded into the venous lumen. On the other hand, smaller lymphatic capillaries were located within the area of apposition and in the bridge.

Fig. 2. Scanning electron micrograph of the resin cast of the testicular artery showing the retrograde loop (arrowhead) at the middle part of the spermatic cordal portion. ×45

Fig. 3. Scanning electron micrograph of the superficial aspect of a vascular cast. Several of the testicular veins anastomose to each other forming, the pampiniform plexus (PP). A testicular artery. ×100
DISCUSSION

Although no direct connections were found between the hamster testicular artery and veins, there was a capillary network between the epididymal artery and the pampiniform plexus. Such a network has also been found in the bull (HEES et al., 1984) and rat (OHTSUKA, 1984), but the endogenous testosterone levels in the blood of the epididymal artery did not differ from those in the systemic artery in the rat (FREE and JAFFE, 1978). Therefore, it seems that this kind of shunt may not be indispensable to the transferring mechanism in the spermatic cord.

Another factor involved in the transfer mechanism is blood flow velocity. WEERASOORIYA and YAMAMOTO (1985) suggested that blood flow velocity in the artery might be reduced according to the increase in luminal

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**Fig. 4.** A cross sectional view of the spermatic cord. In the superficial surface, the coiled testicular artery (A) is almost enclosed by one layer of veins (V), whereas in the deep part, there are two layers of veins between the artery. E endothelial bridge, Ep epididymal fat pad. ×140

**Fig. 5.** High magnification scanning electron micrograph showing the relation of the artery (A) and vein. The venous walls (V) varying in size and length project from the area of apposition into the lumen and reach the opposite wall. Some of them are stick like in appearance and deserve to be called an endothelial bridges (E). ×220

**Fig. 6.** The tunica adventitia of the artery and vein join together to form a single stratum connective tissue, an area of apposition (t). A arterial lumen. V venous lumen. ×440

**Fig. 7.** Scanning electron micrograph showing endothelial bridges (E) extending between two adjoining convoluted arteries (A). V venous wall. ×550
Figs. 5-7. Legends on the opposite page.
Fig. 8. Light micrograph of a semithin section showing the relation between the artery (A), vein (V), lymphatic capillaries (L) and mast cells (M). Most of the mast cells are found at the base of the venous walls, which they protrude from the area of apposition. Toluidine blue stain. ×240

Fig. 9. High magnification of a paraffin section showing the location of mast cells (M) within the arterio-venous wall. They are closer to the vein (V) and lymphatic capillary (L) than to the artery (A). Alcian blue-safranin stain. ×360
diameter from the initial part to the distal end in the rat testicular artery. In contrast, HEES et al. (1984) reported that, while the luminal diameter of the testicular artery was constant, the arterial wall thickness (intima and media) decreased regularly in an orthogonal direction.

In our observations, there were no changes in either diameter or thickness of the arterial wall from the initial portion to the distal end. Therefore, the decrease in blood flow velocity seems to be mainly due to convolutions of the artery as suggested by WAITES and MOULE (1960) and also the presence of the retrograde loop. On the other hand, the anastomosing venous wall running across the venous lumen can serve as a barrier to slow down the venous blood velocity. The decrease in blood flow velocity in both the arterial and venous compartments in the pampiniform plexus should facilitate the transfer of substances from the vein to the artery. In addition, the endothelial bridges presumably prevent the vein from excessive distension.

AMANN and GANJAM (1976) reported that the amount of steroid transferred across the pampiniform plexus was independent of the venous steroid concentration. Their results suggest the existence of a functional unit involved in the transfer mechanism. In the present study, many mast cells were found throughout the tunica adventitia of the arterio-venous walls and endothelial bridges, especially at sites close to lymphatic capillaries. Mast cells contain histamine in their granules, which increases vascular permeability (SCHWARTZ and AUSTEN, 1984). The present finding suggests that mast cells may play a role in the countercurrent transfer in the spermatic cord of the hamster.

In the female hamster, mast cells degranulate in response to the luteinizing hormone (LH) surge on proestrus (KRISHNA and TERRANOVA, 1985). The production of testosterone in the Leydig cell is apparently under the influence of LH (DYM, 1983). In contrast, testosterone concentration in the blood circulation regulates the amount of LH release as a feedback mechanism. A possibility thus is that degranulation of mast cells in the male hamster may also be induced by LH release or other physiological effects, and subsequently may increase the transfer of testosterone from the plexus or lymphatics to the artery.

The morphological architectures of the testicular artery and pampiniform plexus in the golden hamster, i.e., the presence of the retrograde loop, convolutions, endothelial bridges and the vascular wall attenuated by sharing a single tunica adventitia, suggest that the arterio-venous compartments in the plexus have a structure that is compatible with the requirements of a counter-current mechanism. It is also suggested that the permeability of the vascular wall may be increased by mast cells in the adventitial layer.

REFERENCES


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