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The Arteriovenous Connection in the Spermatic Cord in the Variable Squirrel (*Callosciurus finlaysoni*)

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ABSTRACT

In the variable squirrel (*Callosciurus finlaysoni*), four to five ramifying arterioles arose directly from the testicular artery and then gave off numerous small capillaries. The capillaries made a series of anastomoses with neighbouring counterpart capillaries to become a complicated network. Some of the capillaries drained into a small venule, which were connected directly with the testicular vein (pampiniform plexus), to form an arteriovenous connection (A-V shunt) between the testicular artery and the pampiniform plexus. This A-V shunt appeared to make the transfer of substances from the pampiniform plexus to the testicular artery more efficient. The shunt might control the volume of the blood draining into the testis. In addition, the capillaries were covered by vesiculated cells which were located adjacent to the pericytes of the capillaries. The vesiculated cells contained abundant mitochondria, rough endoplasmic reticulum, Golgi complex and cytoplasmic vesicles. The vesiculated cells could provide support for the capillaries and prevented their collapse when the shunts were closed.

Key words : *Callosciurus finlaysoni*, arteriovenous connection, spermatic cord.

INTRODUCTION

A high concentration of testosterone is essential for spermatogenesis in the testis (Steinberger *et al.*, 1973) and for prolonging the life span of the epididymal sperm (Reeves, 1987). It has been demonstrated that in order to maintain high concentrations of testosterone intratesticulary, testosterone has to be transferred from the pampiniform plexus to the testicular artery. This has been shown in the rat (Free and Jaffe, 1978), ram (Ginther *et al.*, 1974), rhesus monkey (Dierschke *et al.*, 1975), cow (Amann and Ganjam, 1976) and man (Bayard *et al.*, 1975). The structure of the vasculature in this area has been modified to some extent for this purpose. Although there is no doubt that a countercurrent system is present in this area, there is disagreement as to the way testosterone is transferred into the arterial blood. Some suggestion has been made that testosterone is transferred by diffusion (Free *et al.*, 1973; Free, 1977). Alternatively, some reports have suggested that it is achieved through an arteriovenous connection (A-V shunt) in the cord (Noordhuizen-Stassen *et al.*, 1985). However, there was no morphological evidence of the A-V shunts in this area. Recently, the first evidence of the A-V shunts had been reported in the common tree shrew (Rerkamnuaychoke *et al.*, 1991b).

In this study, the arteriovenous connection between the testicular artery and the pampiniform plexus in the variable squirrel spermatic cord is described. The results of this study encourage the previous data and strongly indicate that there are A-V shunts in this area.

MATERIALS AND METHODS

Ten adults variable squirrel (*Callosciurus finlaysoni*), weighing 180-250 g, were used in this study. The animals were fed for two weeks and selected the healthiness for the experiments. Under ether and pentobarbital anaesthesia (5 mg/100 g. body weight, i.p.) the thoracic aorta was cannulated just above the diaphragm. Ringer solution was perfused through the cannula followed by fixative, 5 % glutaraldehyde in 0.1 M cacodylate buffer for electron microscopy, or Bouin's fluid or 10% formalin for light microscopy. The whole spermatic cord was collected and processed as below.

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Light microscopy

Two paraplast embedded spermatic cords were handled through routine histological preparations, sectioned at 6-7 μm and stained with Masson's trichrome or hamatoxylin and eosin.

Scanning electron microscopy (SEM)

For the preparation of corrosion casts, methacrylic methyl ester monomer (Acry ester M., Mitsubishi Rayon Co. Ltd, Japan) was semipolymerised according to the method described by Murakami (1975). The semipolymerised ester was mixed with 25-30% hydroxypropyl methacrylate, 0.1% Sudan III and 1-1.5% N-N-dimethyl aniline (accelerator) just before injection. The resin-injected samples were immersed in warm water (60°C) for 4 hr, macerated in warm 2-5% sodium hydroxide for 12-20 hr, and then washed in running water followed by distilled water for 1-2 hr. Finally, the casts were air-dried, mounted on stubs, and coated with platinum-palladium before examined with a Hitachi S-4000 scanning electron microscope at 5-10 kV.

Transmission electron microscopy (TEM)

Four middle portion of the spermatic cords were excised and cut into smaller blocks (1-2 mm thick), which were subsequently fixed in 5 % glutaraldehyde in 0.1 M cacodylate buffer for 2 hr. They were rinsed in the same cacodylate buffer and postfixed in 1% osmium tetroxide for 2 hr. The specimens were then dehydrated in a graded series of ethanol and embedded in the mixtures of Epon 812 and Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and observed with a JEM-1200EX transmission electron microscope at 80 kV.

RESULTS

Testicular artery and pampiniform plexus

The testicular artery was a small muscular type of artery consisting of 3-4 layers of smooth muscle cells in the tunica media. The testicular vein (pampiniform plexus), on the other hand, was composed of a single smooth muscle layer in the tunica media and shared a common tunica adventitia with the apposed artery. About one-third of the initial portion, the artery ran straight and then, the artery began to become convoluted. The degree of convolution gradually increased as the artery ran caudally to the testis and was prominent in the middle and distal portions of the cord. In addition, the initial part of the vein ran straightly and at the middle of the cord, the vein also divided into several branches, anastomosing with each other to become as pampiniform plexus which almost covered the artery in the inner.

Morphological architecture of A-V shunt

A small ramifying arterioles arose directly from the testicular artery. In the initial portion, the tunica media of the arteriole consisted of two smooth muscle cell layers. The muscle layers became thinner as the vessel advanced peripheraly, thereafter, the arteriole gave rise to numerous capillaries. These capillaries were long, tortuous and twisted into U or S shapes in their courses (Figures 1 and 2). The capillary usually made a series of anastomoses with theirs counterparts derived from the same arteriole. However, they occasionally anastomosed with the capillaries derived from a different arteriole to make a connection between capillary networks of different arteriolar origins. The capillary network was arranged in a localised mass and was situated on the surface of the spermatic cord (Figures 1-3). Only a few portions of the network penetrated deep into the spermatic cord. Some capillary branches drained into a small venule which was directly connected with the testicular vein (Figures 2 and 3). Thus a direct connection between the testicular artery and the pampiniform plexus was formed (Figure 4). In the initial portion of the cord, the capillary network was small, simple and A-V shunts were rare, compared with the middle and distal portions. The capillary closed to the venule had a more dilated lumen as compared with the remaining capillaries.

Ultrastructure of the arteriovenous connection

The capillary network contained three kinds of cell, namely, endothelial cells, pericytes and vesiculated cells (Figure 5). The name 'vesiculated cell' was adopted because of the presence of numerous cytoplasmic vesicles (Rerkamnuaychoke *et al.*, 1991b). The vesiculated cells which were large, irregular in shape and difficult to located the boundaries of the cells. Mitochondria, rough endoplasmic reticulum, golgi complex and vesicles were found more frequently in the cell bodies region than in the cell process. The elongated cellular processes of the vesiculated cell made contact with the adjoining cells and surrounded the capillary. Abundant collagen fibres, some marcophages and occasional fibroblasts were found in the tunica adventitia of the arteriovenous connection.

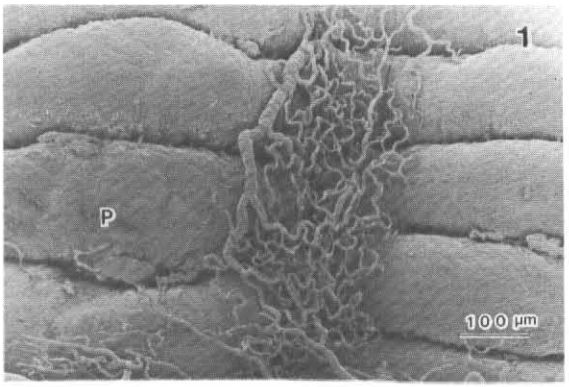

Figure 1 SEM of corrosion cast showing the capillary network on the pampiniform plexus (P). The capillaries are long, tortuous and twist in their course.

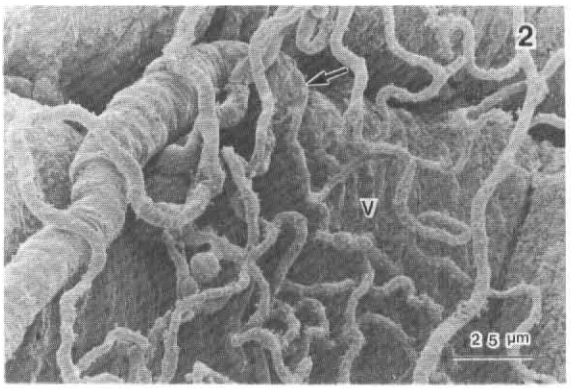

Figure 2 SEM of corrosion cast showing the venule (arrow) branching from the testicular vein (V).

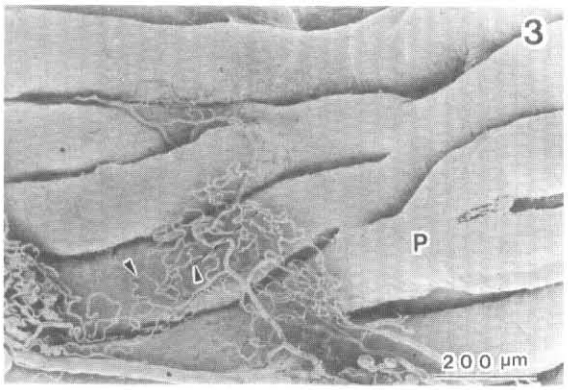

Figure 3 SEM of corrosion cast showing the arteriovenous connection (arrowheads) in the capillary network on the pampiniformplexus (P).

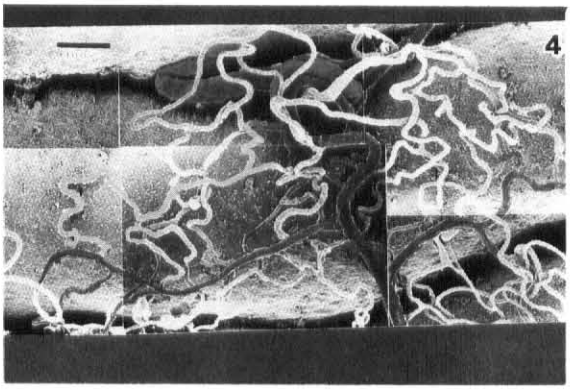

Figure 4 The high magnification and recoloring of the Figure 3 showing the A-V shunt.
blue = venule of the testicular vein.
red = arteriole of the testicular artery.
yellow = arteriovenous connection.

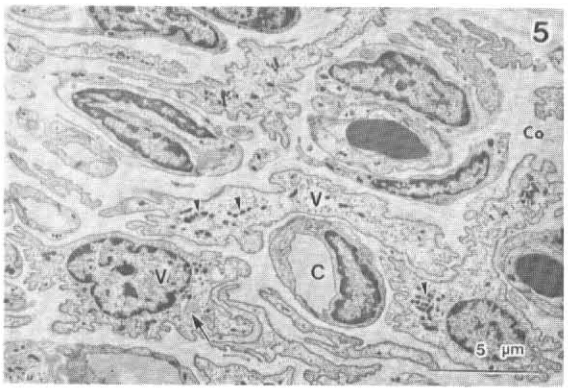

Figure 5 TEM of capillary network in spermatic cord. The network is dominated by the capillaries (C) and vesiculated cells (V).
arrowheads = mitochondria;
arrow = vesicle ; Co = collagen.

DISCUSSION

There was controversy concerning the presence of an arteriovenous connection (A-V shunt) between the testicular artery and the pampiniform plexus in the spermatic cord. After ligation of the distal testicular artery on the testicular surface, the coloured solution (such as Indian ink) which was infused through the proximal testicular artery was visible in the testicular vein. By using this type of experiment, the presence of A-V shunt has been claimed in the ram (Noordhuizen-Stassen *et al.*, 1985), bull, goat, boar and dog (Wensing and Dijkstra, 1981). However, morphological evidence for A-V shunt was not clearly demonstrated. Noordhuizen-Stassen *et al.* (1985) suggested that the shunt opened or closed depending on the physiological condition and this property made it difficult for it to be observed. Hees *et al.* (1984) described the existence of A-V shunt in the bull between the small branches of the testicular artery and the pampiniform plexus by light microscopy on serial sections, however they failed to establish the presence of shunts by corrosion casts of SEM samples. In contrast, Amselgruber and Sinowatz (1987) reported that there were no A-V shunt in the bull spermatic cord. Recently, Rerkamnuaychoke *et al.* (1991b) reported the first evidence of the A-V shunt in the spermatic cord of the common tree shrew by light microscopy of serial section and corrosion cast technique of SEM. In the present study, it demonstrated the existence of the similar morphological features of A-V shunt in the spermatic cord of variable squirrel by light microscopy, TEM and by the corrosion cast technique combined with SEM. The corrosion cast technique of SEM is the most effective and accurate for tracking down or study the pattern of the vascular branches. This study showed that there were arteriovenous connections in the spermatic cord. In this study, there were three A-V shunts in the cord. Therefore the shunts must be numerous. However in order to have full access to expose the A-V shunt, it must severed the vascular cast to determine the underlying vascular branches. Then some vascular casts were broken during track down the shunt. On the otherhand it was possible that the shunt may be close or open depending on the physiological condition of the vessel at the time (Noordhuizen-Stassen *et al.*, 1985). Therefore, in some condition it was difficult to demonstrate the existence of the shunt.

The similar capillary network between the testicular arteries and pampiniform plexus in the area of the spermatic cord had been reported in the rat (Ohtsuka, 1984). However the capillaries derived from epididymal arterial branches were regarded as vasa vasorum of the testicular artery. The capillary networks in the squirrel spermatic cord were large and most of them were located on the surface of the cord. From the transmission electron microscopic observations, in addition, it was clear that the vesiculated cell was present in the capillary network of the squirrel spermatic cord, but was not present in the rat. There was, therefore, no doubt that the capillary network of variable squirrel are similar to that of the tree shrew. However, no modified smooth muscle cells were evidence as described in A-V shunt in other organs (Molyneux and Bryden, 1981).

A countercurrent system occured between the testicular artery and the pampiniform plexus. The testicular artery and pampiniform plexus modified their structures in some degree to suit the countercurrent system in this area and varied slightly between species. Some of these modified structures, such as the convoluted arteries and reduced vascular walls achieved by sharing a common tunica adventitia, were generally found in mammals with testes located in a scrotum. The other structural modifications, such as the presence of most cells in the common adventitial layer in the golden hamster (Rerkamnuaychoke, *et al.*, 1989) and the presence of a venous portal system in the bull and boar (Hees *et al.*, 1984; Amselgruber and Sinowatz, 1987; Rerkamnuaychoke, *et al.*, 1991a), were considered to be species-dependent modifications. These modifications would make the countercurrent transfer in this area more effective. In the spermatic cord of the variable squirrel, the modified structures were generally similar to those of the golden hamster except for the rare existence of mast cells. The presence of an A-V shunt was thought to be a species-specific modification of the variable squirrel as in the tree shrew. However, this did not prove that the transfer of substances occurs only by this shunt. In addition, the shunt may control the volume of blood draining into the testis. The present study indicated that there were arteriovenous connections between testicular artery and the pampiniform plexus in the squirrel.

The vesiculated cells contains rough endoplasmic reticulum, numerous mitochondria and golgi complex. It was therefore suggested that the vesiculated cells were active in synthesizing the substance which affected the capillaries. In addition, the adjoining vesiculated cell processes will reinforce the rigidity of the vascular wall and prevent the capillary from collapsing when the shunt was closed. The origin

and exact function of the vesiculated cell were still unknown and the further study was needed to define the exact functions of the cell.

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