

The structure, innervation and location of arteriovenous anastomoses in the equine foot

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Abbreviations

AVA = arteriovenous anastomosis; **CA** = catecholamine
CGRP = calcitonin gene-related peptide; **NA** = noradrenaline
NPY = neuropeptide Y; **SP** = substance P
VIP = vasoactive intestinal peptide

Summary

In the foot of the horse, arteriovenous anastomoses (AVAs) of epithelioid type occurred in the dermis of the coronary band, in the coronary and terminal papillae, in neurovascular bundles and at the entrance to and along the length of the dermal laminae. A particular feature of the epithelioid segment of AVAs in the horse, compared with that of other species, was the height and surface complexity of many of the endothelial cells. They extended into the lumen, forming undercut and tunnel-like areas which correlated with the characteristic surface marking of AVAs observed in vascular casts. The number of cell organelles, including the concentration of vesicles in the luminal cytoplasm, suggested cells with a high metabolic activity. The luminal surface possessed numerous microvilli and long cytoplasmic cell processes which appeared to surround material in the lumen.

The innervation of AVAs was more dense than that of the arteries and consisted of adrenergic and peptidergic nerves. Noradrenaline- and neuropeptide Y-containing nerves were identified as the vasoconstrictor components of the nerve supply and occurred along arteries and formed dense plexuses around AVAs. Calcitonin gene-related peptide, substance P and vasoactive intestinal polypeptide are vasodilators and were present in single nerve fibres which accompanied arteries and AVAs along the length of the dermal laminae. In this study the distribution, density and innervation of AVAs in the equine foot are correlated with their proposed role in the development of acute laminitis. The release of vasoactive peptides from diseased organs remote from the foot may induce inappropriate prolonged dilatation of AVAs and thus contribute to the laminar ischaemia of acute laminitis.

Introduction

Arteriovenous anastomoses (AVAs) are normal precapillary structures which connect the arterial and venous sides of the circulation. They occur in the peripheral circulation of terrestrial and marine mammals and birds and are important in

thermoregulation (Molyneux and Bryden 1981). Cutaneous AVAs are characterised by a trisegmental structure comprising arterial, intermediate and venous segments (Grosser 1902) in which the distinctive intermediate segment lacks an internal elastic lamina, possesses a slit- or star-shaped lumen and in some species the media contains modified smooth muscle cells known as vascular myoepithelioid cells (Schumacher 1916). Epithelioid-type AVAs are more densely innervated than contiguous vessels and, depending upon species and regional specificity, sensory, adrenergic, dopaminergic, cholinergic and peptidergic nerves have been described (see review, Hales and Molyneux 1988).

Epithelioid-type anastomoses have been reported in the equine foot by Talukdar *et al.* (1972), Schummer *et al.* (1981), Rooney (1984), Molyneux and Pollitt (1987) and Pollitt and Molyneux (1987, 1990). AVAs have been implicated in the pathophysiology of laminitis (Hood *et al.* 1978), a condition of the equine foot causing lameness, in which the peripheral tissues of the foot undergo ischaemia leading to necrosis and sometimes loss of the hoof.

Adrenergic and peptidergic nerves associated with AVAs in the equine foot have been reported (Molyneux and Pollitt 1987) and in the present study the distribution, structure and innervation of AVAs were examined by histological, ultrastructural, histochemical and immunofluorescent techniques.

Materials and methods

Five clinically normal Australian ponies (2 male, 3 female), aged 1–15 years (mean age 3.5 years) were killed with an intravenous injection of pentobarbitone sodium and the forelimbs were immediately amputated at the carpal joint. A 12-gauge plastic cannula was inserted into the medial palmar artery of each limb, firmly ligated and perfused with 1 litre of warm normal saline containing 5000 units heparin. During the perfusion the limb was flexed, extended and made to bear weight until the perfusate was clear of red blood cells. The heparinised saline was held 200 cm above the cut end of the common digital artery and the perfusate entered the circulation of the limb at maximal flow rate.

For histological, electron microscopic and immunofluorescent studies the feet were fixed by perfusion with appropriate glutaraldehyde/paraformaldehyde mixtures (see below). After fixation the feet were stored at 4°C overnight then sectioned with a band saw into 8 parts extending from the coronary band to the toe; these were designated as level L1 for the coronary band region, L4–L5 for the middle region and L8 for the toe. Tissue blocks were stored at 4°C in the buffer of the fixative and later trimmed to remove excess hoof and to reduce the specimens to a suitable size for processing.

To obtain fresh tissue for histochemical study, feet from one

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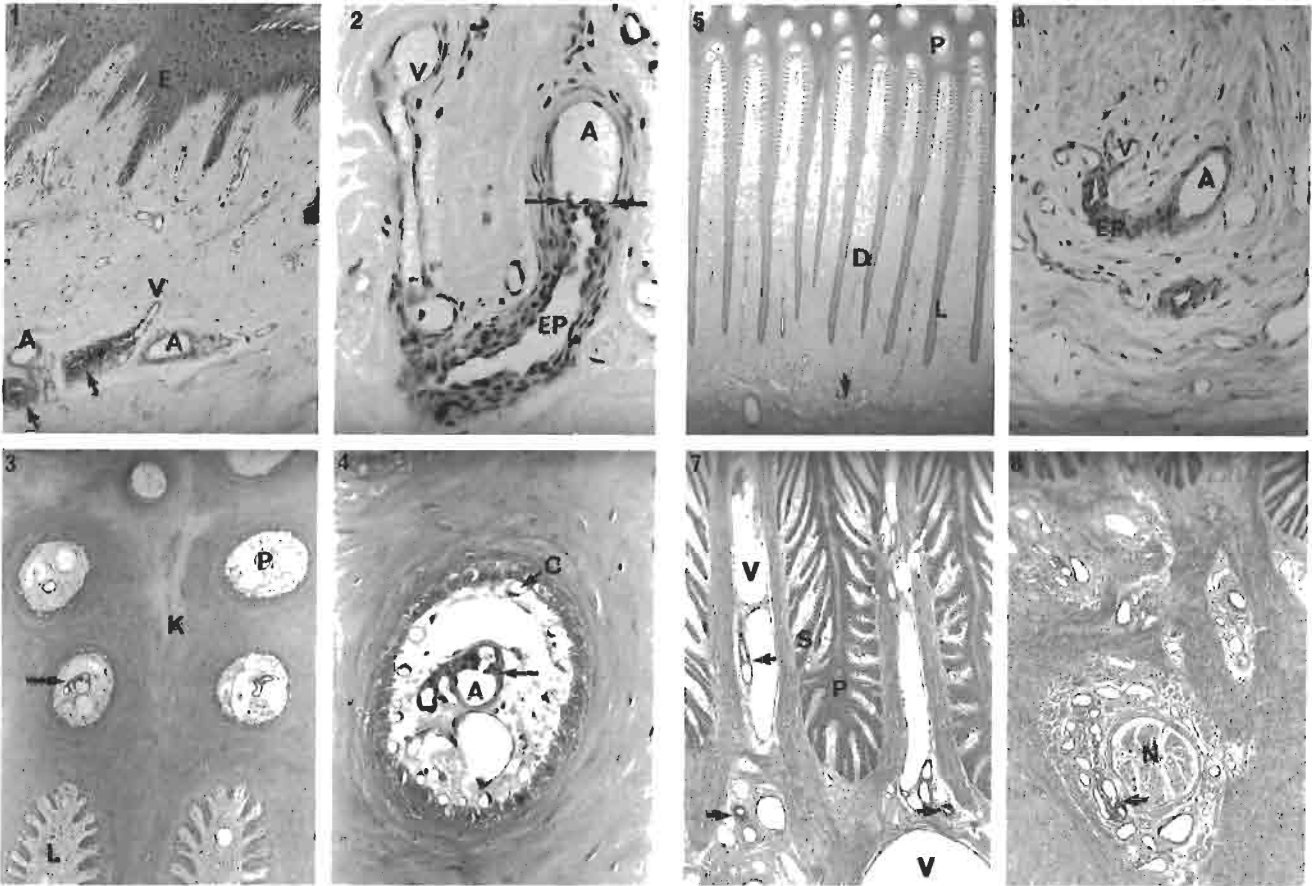


Fig 1: Light micrograph of coronary band region (level 1) of equine foot showing the position of two AVAs. Profiles of epithelioid segments of AVAs are indicated by arrows. Arteries of origin of AVAs (A); venous segment of AVAs (V); coronary band epithelium (E). Aldehyde fuchsin stain, x75. Fig 2: Light micrograph of AVA in coronary band region. Artery (A); sphincter at origin of AVA (arrows); intermediate (epithelioid) segment, EP, composed of 3–4 layers of modified smooth muscle cells with polygonal nuclei; thin-walled venous segment (V). Aldehyde fuchsin stain, x280. Fig 3: Light micrograph section of foot showing profiles of the distal ends of dermal laminae (L) and coronary papillae (P). AVA in coronary papilla indicated by arrow. Tissue of the hoof wall undergoing keratinisation (K). Aldehyde fuchsin stain, x60. Fig 4: Higher magnification of profile of coronary papilla shown in Figure 3. The papillary connective tissue contains a small AVA with prominent arterial sphincter (arrow). Artery, A. Profiles of capillaries (C) are adjacent to papillary epidermis. Thin-walled vessels either veins or lymphatics are present. Aldehyde fuchsin stain, x187. Fig 5: Light micrograph of section through hoof wall at level 2 showing coronary papillae (P), primary epidermal laminae (L), and dermal laminae (D). Note vessels extending into the dermal laminae towards the periphery and the position of AVA at the base of the laminae (arrow). Aldehyde fuchsin stain, x15. Fig 6: Higher magnification of AVA in Figure 5 at base of the laminae. Artery A, intermediate segment (EP), venous segment (V). Aldehyde fuchsin stain, x150. Fig 7: Light micrograph of section of horse foot showing primary (P) and secondary (S) epidermal laminae (level 6) and dermal laminae containing distended (perfused) blood vessels (V). AVAs (indicated by arrows) are present at the base of and within the laminae. Haematoxylin and eosin, x60. Fig 8: Light micrograph. A neurovascular bundle in the dermis at the base of the laminae contains an AVA (arrow) sectioned so that the arterial sphincter is demonstrated. Nerve bundle, N. Haematoxylin and eosin, x60.

animal were sectioned by band saw immediately after death, frozen and stored in liquid nitrogen and processed to demonstrate catecholamine (CA) fluorescence.

Histology

Paraffin wax sections, 7 μ m thick, were prepared and stained with haematoxylin and eosin or with an aldehyde fuchsin, haematoxylin, orange G, fast green sequence (Molyneux 1965) which distinguishes the myoepithelioid segment of the anastomosis and demonstrates the absence of an internal elastic lamina.

To determine the range in size of anastomoses, the internal diameters of transverse sections of myoepithelioid segments of a number of AVAs from different regions of the foot were measured by light microscopy using a microscope with a stage micrometer and a graduated eyepiece graticule.

Histochemistry

Tissue CAs were demonstrated by the SPG method, a modified glyoxylic acid technique (de la Torre and Surgeon 1972). Unfixed tissue samples were partly thawed, cut into 5–10 mm thick pieces and frozen on to a precooled cryostat chuck at a temperature of -30°C ; sections of 30 μ m were cut and thawed onto glass slides pretreated with albumin which were then treated with SPG solution at room temperature. The sections were air-dried and then placed in an oven at 80°C for 5 min. They were mounted using fluorescence-free paraffin oil and then viewed in a Leitz fluorescence microscope with a Ploempak incident light attachment, using a 100 W ultra-high-pressure mercury lamp as the u.v. light source with a Leitz Broad Pass 350–360 nm excitatory filter and a Long Pass 515 nm suppression filter.

Electron microscopy

Tissue was fixed by perfusion with a mixture of 4% paraformaldehyde, 2.5% glutaraldehyde in 0.067 M-sodium cacodylate buffer, pH 7.4 (after Karnovsky 1965) for 4 h and stored in buffer at 4°C. The tissue was post fixed in 1% osmium tetroxide in cacodylate buffer for 1 h at room temperature, then stained *en bloc* in 5% aqueous uranyl acetate as this procedure tends to extract glycogen. The tissue was dehydrated through a series of alcohols, cleared in propylene oxide and embedded in an Epon-Araldite mixture. Blocks were cut with a diamond knife (Diatome) on a Reichert-Jung Ultramicrotome, stained with lead citrate (Reynolds 1963) and viewed in a Zeiss EM10 electron microscope.

Immunofluorescence

Tissue was perfused with a fixative composed of 4% paraformaldehyde in 0.1 M-phosphate buffer, pH 7.4, washed in several changes of phosphate-buffered saline (PBS; pH 7.4) containing 20% (w/v) sucrose, and then frozen onto pre-cooled brass studs. Sections 20 µm thick were cut on a cryostat and placed on gelatine-coated slides. For single labelling some slides were incubated with one of the following antibodies: calcitonin gene-related peptide (CGRP), substance P (SP), vasoactive intestinal peptide (VIP) and neuropeptide Y (NPY). Slides were then washed and incubated for a further 2 h in a species specific anti-rabbit or anti-rat IgG antibody conjugated to rhodamine or fluorescein respectively. For double labelling some slides were incubated with antisera raised against CGRP and NPY or CGRP and VIP. In these cases the anti-CGRP was visualised using species specific anti-rabbit IgG conjugated to rhodamine diluted with PBS to 1:20 and VIP and NPY were visualised using species specific anti-rat IgG conjugated to fluorescein diluted to 1:40.

Antibodies

Sections were incubated for 24 h at 4°C in polyclonal primary antiserum raised in rabbits against CGRP diluted with PBS to 1:500, SP diluted to 1:100 (Amersham, Bucks, UK), and in rats against VIP diluted to 1:200 and NPY diluted to 1:200 after the method of Morris *et al.* (1985). Antibody binding for each was visualised by secondary antibodies labelled with rhodamine (anti-rabbit IgG) or fluorescein isothiocyanate (anti-rabbit IgG) respectively. Immunohistochemical controls included (i) omission of the primary antiserum from the incubation and (ii) incubation with primary antiserum which was preabsorbed with its respective peptides at 10⁻⁶ M.

Results

Light microscopy

Anastomoses were located in the dermis of the coronary band region (Figs 1 and 2), in the coronary papillae (Figs 3 and 4) and at the base and along the length of the dermal laminae and in neurovascular bundles (Figs 5, 6, 7 and 8). The largest AVAs occurred in the coronary band and at the base of the dermal laminae beside the tips of the primary epidermal laminae. A prominent arterial sphincter (Figs 2 and 4) was present at the origin of the intermediate segment which consisted of 3 or 4 layers of vascular smooth muscle cells arranged in a circular fashion around the anastomosis. The intermediate segment was more cellular than the artery and the nuclear staining contributed to the characteristic appearance of the AVA (Fig 2).

Size of anastomoses

In perfused material the internal diameter of arteries and veins was often greater than the internal diameter of the epithelioid

segment of AVAs which varied from 16 µm in terminal papillae and laminae to 50 µm at the base of the laminae.

Electron microscopy

Endothelium: A distinctive feature of AVAs in the horse, more so than in other species, was the height and surface complexity of the endothelial cells. They were of irregular size and varied from flattened cells typical of those lining most blood vessels to prominent cells which protruded deeply into the lumen, and contacting one another formed undercut and tunnel-like areas (Figs 9 and 10). The complexity of the luminal surface of the endothelial cells varied from the presence of numerous microvilli (Fig 11) to long cytoplasmic processes which extended into the lumen and encircled dense homogeneous vesicular material (Fig 14) or finely floccular membrane-bound material (Fig 13). The structure of the endothelium suggested cells with a high metabolic activity. The luminal cytoplasm contained many vesicles of various sizes; some were electron-lucent, others contained finely granular or membranous material while those close to the surface appeared to open into the lumen (Figs 11 and 14). The cytoplasm contained a

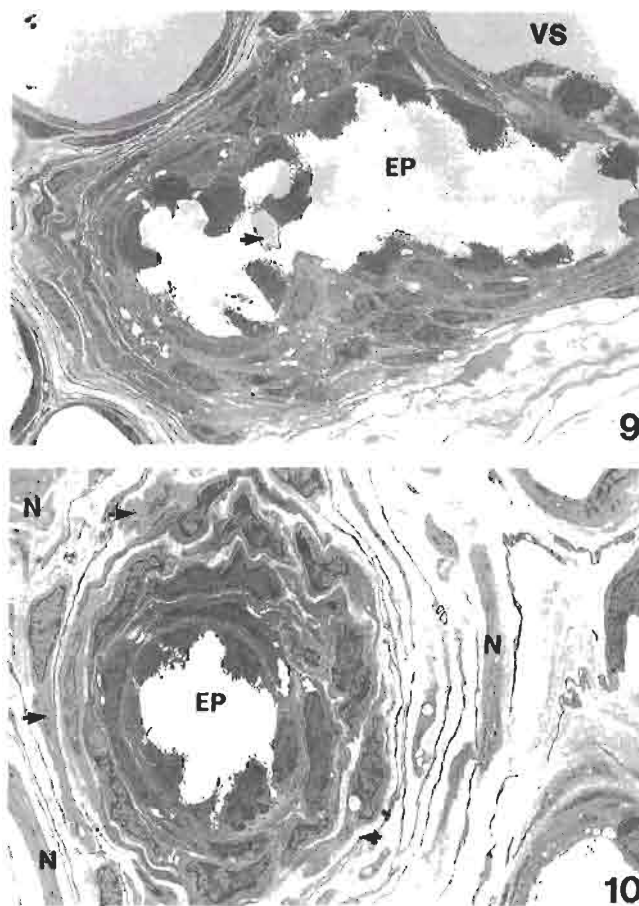


Fig 9: Electronmicrograph of a longitudinal section through the intermediate or epithelioid segment (EP) of an AVA at the base of the dermal laminae. The tissue was fixed by perfusion and vessels are widely open. Compare the smooth surface and height of endothelium of vessels adjacent to the anastomosis with the larger cells of the AVA endothelium which have prominent microvilli and protrude in the lumen. An extension of endothelial cell cytoplasm encloses material in the lumen (arrow). Note the thickness of the AVA wall; spaces resulting from the extraction of glycogen are present in the endothelial muscle cells. The section is close to the junction of the epithelioid segment with the venous segment (VS) x2000. Fig 10: Electronmicrograph of a transverse section through the epithelioid segment (EP) of an AVA. Nerve bundles (N) and axons (arrows) contribute to the dense innervation of the anastomosis. x2800.

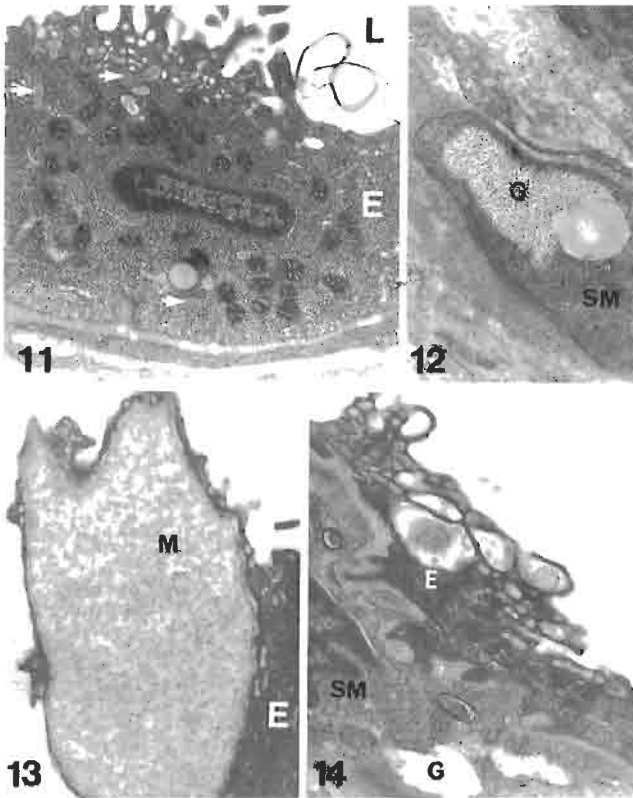


Fig 11: Electronmicrograph of an AVA endothelial cell (E). Microvilli are conspicuous on the cell surface. The luminal cytoplasm contains clear vesicles some of which open into the lumen (L). Dense lysosome-like vesicles (arrows) occur throughout the cell but are concentrated in the luminal cytoplasm: they contain homogeneous granular, vesicular or membranous material. The cytoplasm contains numerous free ribosomes and some strands of RER. Pinocytotic vesicles containing homogeneous material are present on the abluminal cell surface, x30,000. Fig 12: Electronmicrograph of part of smooth muscle cell (SM) in wall of AVA. Tissue was processed to preserve glycogen deposits (G) by not staining 'en bloc' with uranyl acetate, x20,000. Fig 13: Electronmicrograph. Membrane bound floccular material (M) in lumen of AVA is shown enclosed by cytoplasmic process of endothelial cell (E) (compare with Fig 9), x23,000. Fig 14: Electronmicrograph showing cytoplasmic processes of AVA endothelial cell (E) extending from cell surface to enclose homogeneous and vesicular material. Similar material is present in vesicles within the cell cytoplasm. Smooth muscle cell, SM; space occupied by glycogen deposit, G, x30,000.

high concentration of free ribosomes, membranes of rough endoplasmic reticulum, many mitochondria and lysosome-like bodies. Pinocytotic vesicles were prominent along the basal plasma membrane but were not conspicuous throughout the cytoplasm.

Media: The smooth muscle cells of the AVA wall were typical of vascular smooth muscle and did not show the epithelioid modification, such as polygonal shape and low microfibrillar density observed in anastomoses of other species. Areas of glycogen were prominent in the cytoplasm (Fig 12) and because glycogen had been largely extracted in *en bloc* staining, the cells had a 'moth-eaten' appearance (Figs 9, 10 and 14).

Innervation: As in other cutaneous anastomoses, those in the equine foot were more densely innervated than were contiguous vessels. Nerve bundles and single axons were readily observed around the anastomoses (Fig 10).

Axons containing small dense-cored vesicles, typical of those in noradrenergic nerve (NA) terminals (Fig 15), occurred

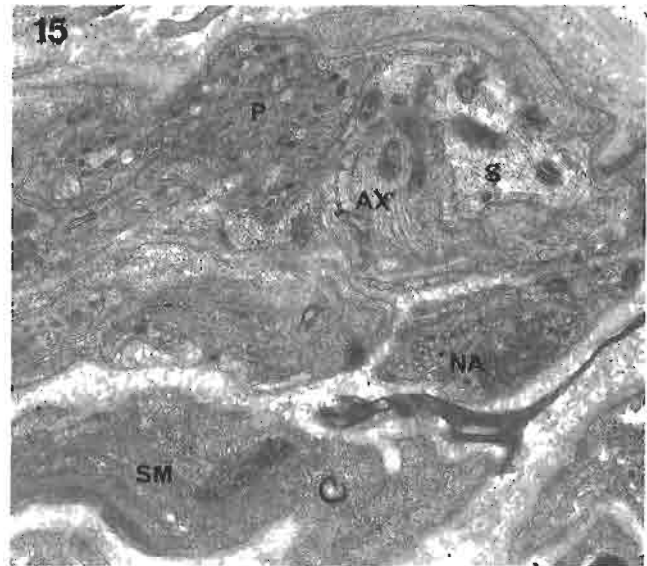


Fig 15: Electronmicrograph showing types of nerves present in wall of equine AVA. Peptidergic nerve profiles, P, contain large dense granules; adrenergic profiles, NA, contain small clear and dense-cored vesicles. Axons, Ax; smooth muscle, SM; Schwann cell cytoplasm, S, x35,000.

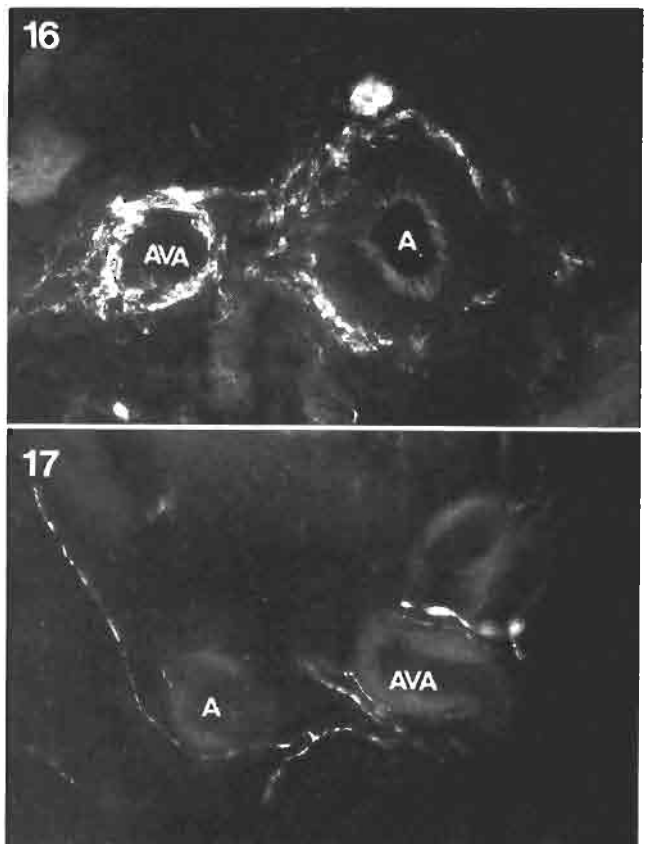
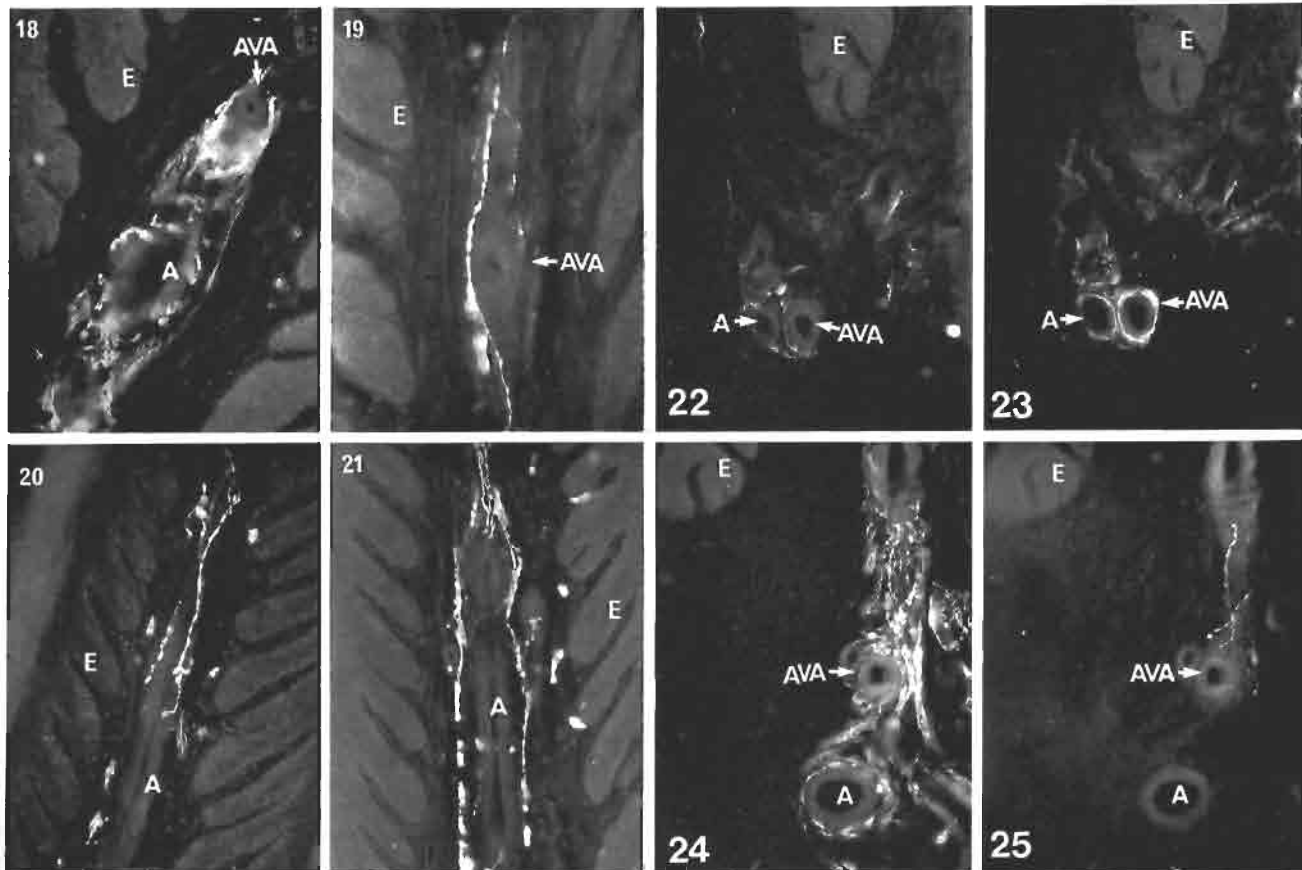


Fig 16: Transverse section through artery (A) and anastomosis (AVA) at the base of dermal laminae showing catecholamine fluorescence. Note the more intense fluorescence in the plexus of nerves around the AVA compared to that of the artery, x250. Fig 17: Artery (A) and anastomosis (AVA) in dermis at base of laminae showing immunofluorescence for VIP-like reactivity. Note that VIP immunoreactivity occurs in single nerve fibres and is more dense around anastomosis, x200.



Figs 18–21: The peptidergic innervation of arteries (A) and anastomoses (AVA) along the laminae is shown by immunofluorescence. E = epidermal lamina. Fig 18, NPY-like reactivity, $\times 200$; Fig 19, VIP-like reactivity, $\times 250$; Fig 20, SP-like reactivity, $\times 150$; Fig 21, CGRP-like reactivity, $\times 150$. Note the dense plexus of nerves around AVA labelled for NPY compared with the single nerve fibres labelled for the other peptides.

Figs 22–25: The location of peptidergic nerves around arteries (A) and anastomoses (AVA) is compared by double immunofluorescent staining of single sections. E, epidermal lamina, $\times 150$; Figs 23 and 24 show CGRP- and NPY-like reactivity respectively; Figs 25 and 26 show CGRP- and VIP-like reactivity respectively.

in close proximity to the vascular muscle of the AVA wall and formed the great majority of nerves around the anastomoses. Catecholamine fluorescence confirmed the adrenergic nature of the innervation (Fig 16).

Axon profiles containing large granular vesicles (P) were typical of those in peptidergic nerves (Fig 15). They occurred amongst, but were less numerous than, the adrenergic axons. Immunofluorescence showed that axons associated with arteries and AVAs reacted for the presence of the peptides SP, CGRP, VIP and NPY (Figs 18–25). They occurred as single fibres in nerve plexuses and in nerve bundles around blood vessels at the base of the laminae and extended as single fibres along the laminae.

Nerves showing immunofluorescence for NPY were numerous around anastomoses where the density of reaction suggested branching of nerves and plexus formation (Figs 18 and 23). CGRP immunoreactive nerves (Figs 21, 22 and 24) were numerous around arteries, AVAs and in nerve bundles but occurred more as single fibres in contrast to the plexus-like reactivity observed with NPY. Both SP (Fig 20) and VIP (Figs 17, 19 and 25) occurred in single fibres which extended along the laminae. Few immunofluorescent fibres were observed in relation to veins.

The location of peptidergic nerves and their density, relative to each other, were shown by double immunolabelling of the same section (Figs 22–25).

Discussion

Arteriovenous anastomoses have been identified in the equine foot in the dermis of the coronary band, in the coronary and terminal papillae, in neurovascular bundles at the base of the dermal laminae and at the entrance to and along the length of the dermal laminae. The anastomoses were of the epithelioid type and consisted of small, simple direct shunts similar to those in Weddell seals (Molyneux and Bryden 1978) in contrast to the complex 'glomeral' type AVAs observed in other species such as the sheep, monkey and man (Molyneux and Bryden 1981).

A particular characteristic of AVAs in the equine foot was the height and complexity of the endothelial cells which extended into the lumen forming undercut and tunnel-like areas. This feature was considered responsible for the prominent marking of AVAs in vascular cast preparations described by Pollitt and Molyneux (1987, 1990). Similar but less pronounced marking of casts by AVAs in the rabbit ear has been reported by Morris and Bevan (1984), Amevo and Molyneux (1985) and Kishi *et al.* (1988).

There is insufficient evidence in this study to determine the functional significance of the AVA endothelium but the number of microvilli, the extent of cytoplasmic processes apparently enclosing material in the lumen, the concentration of luminal vesicles, mitochondria, ribosomes and lysosome-like bodies suggest that these cells are more metabolically active than

endothelial cells lining contiguous vessels. Endothelial cells are known to synthesise and secrete several macromolecules that function in the regulation of haemostasis such as the factor VIII complex (Hoyer 1982), plasminogen activators (Loskutoff *et al.* 1982) and thrombospondin (Mosher and Doyle 1982), while prostacyclin (Jaffe *et al.* 1982; Moncada and Vane 1982) and endothelium-derived relaxing factor (EDRF) (Furchgott *et al.* 1981) have been implicated in vasodilatation. EDRF is now known to be nitric oxide (NO), the endogenous nitro-dilator (Moncada *et al.* 1991) and is produced for long periods when endothelial cell NO synthase is induced, by circulating cytokines and endotoxin. NO induced by this pathway is cytotoxic to invading microorganisms and tumour cells but is also known to cause pathological vasodilatation and tissue damage (Moncada *et al.* 1991) as occurs in laminitis.

Receptors for substance P, a known vasodilator, have been detected autoradiographically on endothelium in dog renal arteries (Stephenson and Summers 1987) and serotonin and substance P were localised by electron microscopic-immunocytochemistry on some endothelial cells in rat femoral and mesenteric arteries (Loesch and Burnstock 1988). In view of the importance of dilatation of AVAs in thermoregulation (Hales and Molyneux 1988) and the distinctive structure of AVA endothelium, it could be expected that the endothelium of AVAs in the equine foot would be involved in similar secretory and metabolic functions.

Direct observation of AVAs in rabbit ear chambers (Clark and Clark 1934; Sonomoto 1953; Molyneux 1985a, b) has shown that AVAs are autonomous in action and open and close more vigorously than the surrounding vessels. The amounts of glycogen within the smooth muscle cells of the AVA wall (Figs 9, 10, 12 and 14) in the equine foot are consistent with such high energy requirements.

The density of AVAs in the foot of the horse has been conservatively estimated at 500/cm² (Pollitt and Molyneux 1987, 1990), a value which is significantly higher than in the rabbit ear (78/cm²: Harmon and Molyneux 1981) and sheep leg skin (72 cm²: Molyneux 1965) but less than in the skin of the Weddell seal (1000/cm²: Molyneux and Bryden 1978). However, it appears from this microscopical study and the microvascular cast study of Pollitt and Molyneux (1990) that the number of AVAs in the equine foot and their location, particularly at the base of, and along the length of the dermal laminae, indicates their strategic placement to influence peripheral blood flow and perhaps the nutrition and integrity of the dermal-epidermal attachment. Epidermal necrosis which occurs in acute laminitis (Roberts *et al.* 1980) is consistent with ischaemia of the laminar tissues resulting from a decrease in the capillary circulation. Direct observation of the microcirculation in implanted rabbit ear chambers has shown that preferential blood flow through AVAs can effect a decrease in flow to arterioles and capillaries (Molyneux 1985a, b).

The role of cutaneous AVAs in thermoregulation is well established (review, Hales 1985). It is dependent upon the large volume of warm arterial blood that can be shunted into superficial veins, thus promoting heat loss by radiation. In the horse heat loss through the hooves would not be substantial, but AVAs may contribute to the maintenance of tissue metabolism by allowing perfusion of the feet when the horse is in a cold environment, such as standing in cold water or on ice. Such a mechanism of cold induced vasodilatation (CIV) occurs in the rabbit ear (Grant *et al.* 1933) and human finger (Grant and Bland 1931) and has been suggested to occur in the feet of arctic birds (Murrish and Guard 1977). There is evidence from ²⁴Na clearance studies (Edwards 1965) that CIV involves the intermittent opening of AVAs so that warm arterial blood is shunted to the peripheral tissue and prevents freezing. In the horse AVAs may also act as vascular safety valves which can dissipate the high hydrostatic pressures generated in the enclosed foot circulation, particularly

during galloping and jumping (see discussion by Pollitt and Molyneux 1990).

The innervation of AVAs in the foot of the horse was more dense than that of the arteries and consisted of adrenergic and peptidergic nerves. The pattern of innervation was similar to that of epithelioid-type anastomoses in the extremities of other species (Molyneux and Bryden 1981). Adrenergic nerves were identified by the presence of typical NA nerve profiles (Fig 15) and by CA fluorescence (Fig 16) while P-type nerve profiles containing large granular vesicles (Fig 15) and the presence of immunofluorescence showing NPY-, VIP-, CGRP- and SP-like reactivity (Figs 18, 19, 20 and 21) was evidence of a peptidergic innervation.

Noradrenaline is an established sympathetic vasoconstrictor of vascular smooth muscle in peripheral vessels and NPY is known to potentiate NA evoked vasoconstriction (Wahlestedt *et al.* 1985) and to coexist with NA in peripheral adrenergic nerves, particularly those surrounding blood vessels (Lundberg and Hokfelt 1986). In this study the similar distribution and density of CA fluorescence and NPY-like immunofluorescence around AVAs (compare Figs 16 and 23) was consistent with the coexistence of NA and NPY in adrenergic nerves.

Both SP (von Euler and Gaddum 1931) and CGRP (Fisher *et al.* 1983) are known vasodilators and coexist in primary sensory neurones in trigeminal ganglia (Lee *et al.* 1985), in spinal ganglia and sensory nerves (Lundberg *et al.* 1985) and in nerves around arteries and AVAs in the sheep tongue (Molyneux and Haller 1988). Nerve fibres showing SP-like immunoreactivity have been described around large peripheral arteries and veins in the guinea-pig (Furness *et al.* 1982) and rat (Barja *et al.* 1983) and around pial arteries of the cat (Chan-Palay and Palay 1977; Edvinson *et al.* 1981; Bevan *et al.* 1984).

Substance P in nerves around blood vessels has been implicated in sensory and vasomotor functions. It may be selectively involved in thermally induced nociception and may represent a pain transmitter or modulator (Henry 1982). The action of SP as a vasodilator has been proposed in local and reflex responses to injury (Lembeck and Gamse 1982) and there is evidence for its vasomotor action from the direct observation of the effect of SP on blood vessels. In the cat perivascular microinjection of SP effected dose-dependent increases in arteriolar calibre (Edvinson *et al.* 1981) and in the hamster intravenous administration of SP caused a significant dilatation of subcutaneous arterioles with a concomitant drop in mean arterial pressure (Gerstberger *et al.* 1987). However, the lack of correlation between the degree of innervation of blood vessels and their reaction to exogenous SP, and the depletion of SP-like immunoreactivity from such nerves with capsaicin, suggested their sensory rather than vasomotor nature (Barja *et al.* 1983).

In the hoof of the horse SP and CGRP nerves had a similar distribution and extended along vessels to the periphery of the laminae. CGRP nerves were more plentiful along arteries and around AVAs than were SP nerves. If co-existence of CGRP and SP occurs in the horse as in other species, the results suggest that not all CGRP nerves contain SP at the same time.

VIP is a potent vasodilator (Said and Mutt 1970) and is widely distributed in the peripheral vascular system (Hakanson *et al.* 1982). VIP satisfies a number of the classical criteria as a neurotransmitter and is released from vesicles of terminals of peripheral nerves associated with blood vessels (Fahrenkrug 1982). In the innervation of exocrine glands VIP and acetylcholine co-exist and are released concomitantly from the same nerve endings; acetylcholine mainly stimulates secretory cells whereas VIP is more potent in increasing blood flow by causing relaxation of vascular smooth muscle (Hokfelt *et al.* 1980; Lundberg *et al.* 1980). In the cat tongue electrical stimulation of the chordal-lingual nerve causes a biphasic vasodilatory response with the release of acetylcholine and VIP, the latter having the effect of maintaining atropine-resistant

vasodilatation (Lundberg *et al.* 1982). VIP is also implicated in the control of blood flow through AVAs. Under heat stress conditions it can be calculated from the blood flow data of Hales (1985) that ~70% of total limb blood flow in the sheep passes through AVAs and there is immunocytochemical evidence that acetylcholine and VIP co-exist in the dense plexus of nerves around AVAs (Molyneux and Haller 1988).

In the foot of the horse nerves showing VIP-like immunoreactivity occurred as single fibres accompanying blood vessels and in some cases were more prominent around anastomoses (Fig 18). VIP-containing nerves have not previously been reported in the foot of the horse and their significance in the control of blood flow to the laminae awaits the results of pharmacological and blood flow studies now in progress. However, because VIP induces vasodilatation in most vascular beds, including splanchnic, coronary, cerebral, pial, extracranial and peripheral systemic vessels (Said 1982), it is likely that it acts in a similar fashion in the foot of the horse and causes increased blood flow through arteries and AVAs.

The development of laminitis is associated with both ischaemia of the peripheral circulation (Coffman *et al.* 1970) and an unchanged or increased total blood flow to the foot (Robinson *et al.* 1976) and there is evidence, from the results of radionuclide studies, that the shunting of blood through open AVAs may explain this paradox (Hood *et al.* 1978; Trout 1987). The results of this study support such an hypothesis and suggest that the distribution and size of AVAs in the equine foot correlate with their involvement in the pathogenesis of laminitis.

The factors which initiate and maintain the opening of AVAs in laminitis are not known but could involve the release of VIP from nerves around AVAs in a mechanism similar to that in the cat's tongue where neural release of VIP maintains active vasodilatation. Moreover, in laminitis damage also occurs in tissues such as the gut, uterus and lung and it is tempting to speculate that vasodilatory peptides released from such regions may enter the circulation and act on peripheral AVAs as target organs, causing and maintaining vasodilatation during the development of acute laminitis. A similar sequence occurs in the horse during prolonged exercise when there is a significant rise in circulating VIP (Hall *et al.* 1982) which has general vasodilatory effects and evokes atropine-resistant dilatation of blood vessels in skeletal muscle (Jarhult *et al.* 1980).

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