The Vascular Architecture of the Porcine Small Intestine

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ABSTRACT

The vascular anatomy of the porcine small intestine was studied by injection of intestinal vessels with India Ink. Examination of transverse and longitudinal serial sections of the injected intestine facilitated a three-dimensional interpretation of the vascular pattern. An artery from the mesentery penetrated the tunica muscularis, supplied muscular branches and passed on to the submucosa where it formed an arterial rete. From the submucosal arteries, arterioles arose and followed a direct axial course to the tips of villi where they ramified into a subepithelial capillary plexus. Some of the capillaries, at the midpoint of the villus, fused into paraxial venules, which emptied into a "transverse venule" at the base of the villus. Other villus capillaries were continuous with those of the crypts. The pericryptal capillary plexus received a few arterial branches from the submucosal arteries. The transverse venule and the pericryptal capillary plexus emptied into large, segmentally dilated veins in the submucosa. The submucosal veins formed an extensive anastomosing network drained by large venous trunks which passed through the muscle layers to the mesentery.

The observations suggest possible relationships between the vascular pattern and intestinal fluid movement.

RÉSUMÉ

Les auteurs ont étudié l'anatomie vasculaire de l'intestin grêle du porc en injectant de l'encre de Chine dans les vaisseaux intestinaux. L'examen de séries de coupes transversales et longitudinales de l'intestin, à la suite de l'injection, facilita l'interprétation tridimensionnelle de son organisation vasculaire. Une artère venant du mésentère pénétrait la musculeuse, lui fournissait des branches et continuait jusqu'à la sous-muqueuse où elle formait un réseau artériel. Les artères de la sous-muqueuse donnaient naissance à des artérioles qui suivaient un trajet axial direct jusqu'au sommet des villosités où elles se ramifiaient en un plexus capillaire sous-épithélial. A la partie moyenne de la villosité, certains capillaires se fusionnaient en des veinules para-axiales; ces dernières se déversaient dans une "veinule transversale", à la base de la villosité. D'autres capillaires de la villosité étaient continus avec ceux des cryptes. Le plexus capillaire situé à la périphérie des cryptes recevait quelques branches des artères de la sous-muqueuse. La veinule transversale et le plexus capillaire de la périphérie des cryptes se déversaient dans des veines de la sous-muqueuse, plus volumineuses et présentant des dilatations segmentaires. Les veines de la sous-muqueuse formaient un vaste réseau pourvu d'anastomoses et drainé par de gros troncs veineux qui traversaient la musculeuse et se dirigeaient vers le mésentère.

Ces observations laissent supposer l'existence de relations entre l'arrangement vasculaire et le mouvement du fluide intestinal.

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INTRODUCTION

The early reports of the vascular anatomy of the intestinal villus, in man and animals, classified three forms (24). Several authors described (6, 10, 27) a "step-ladder" circulatory arrangement for man and rabbits. In this system, an arteriole

was said to ascend on one side of the villus and a venule descend on the other, and with interconnecting capillaries, suggested the ladder shape. Mall (15), and Bohm and Davidoff (3) described a "fountain" pattern with an arteriole passing up the centre of the villus to the tip where it arborized into a subepithelial capillary network. These vessels converged near the base of the villus to form efferent venules. The "tuft" concept of villus circulation has been proposed and supported by several workers (19, 21, 25, 26). In this pattern the arteriole at the villus base gave off a tuft of capillaries which anastomose with each other at the tip of the villus to form an efferent axial venule.

More recent investigations have failed to confirm the "step-ladder" arrangement of vessels. Instead, modifications of the "fountain" pattern in the rabbit, dog, opossum and man (11, 16, 17, 2) and the "tuft" pattern in the monkey (20) have been described.

There are two characteristics of the submucosal vessels which differ somewhat between species. Horses, carnivores and pigs are reported to have arteriovenous anastomoses in the submucosa (28). Certain species, including the cat and dog, possess thin-walled, highly dilatable submucosal veins called "Venenbällchen" (24). These have not been demonstrated in man (14).

Careful study of the interrelationships between the various anatomic components of the intestinal wall should provide insight into physiological and pathological intestinal function. With this view in mind, the present investigation was undertaken in an effort to describe the vascular anatomy of the porcine intestine, compare it to that observed in other species and suggest implications for fluid movement across the intestinal mucosa.

MATERIALS AND METHODS

Seven weanling Yorkshire-cross pigs, weighing from 6 to 12 kg, were used in this study. The pigs were deeply anaesthetized with intravenous pentobarbital sodium. Heparin (3,000 U.S.P. units) was administered with the anaesthetic to prevent intravascular coagulation. Cannulae were inserted and secured in the thoracic aorta and the portal vein. Clamps were placed on the brachiocephalic artery and on the aorta just proximal to the renal arteries. Perfusion fluid, consisting of 10 mg of ammonium oxalate and 10 mg of sodium nitrate made up to five litres with physiological saline, was pumped through the aorta at 90-120 mm Hg pressure until the intestine became blanched. The injection media, consisting of four parts India Ink and three parts bovine plasma, was injected into the arterial and venous vessels alternately, using a pressure of 90-120 mm Hg for the arterial system and 40-60 mm Hg for the venous system. After the injection, the vessels were ligated and portions of the small intestine were fixed in Bouin-Hollande fluid for 18 hours. The tissues were stored in 70% alcohol until they were processed for sectioning.

For the preparation of histological sections, the intestinal tissues were dehydrated in alcohol, cleared with xylene, and embedded in paraffin. Serial sections (50-150 microns) were mounted on glass slides.

Fig. 1. Transverse section of jejunum showing an artery (A) and a vein (V) at a point in their passage through the tunica muscularis. X30.

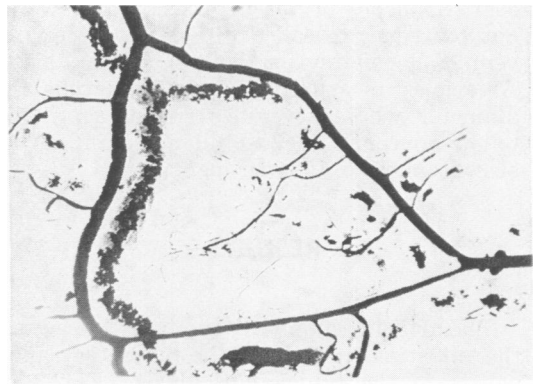

Fig. 2. Longitudinal section through the small intestinal submucosa showing anastomotic branches of the arterial rete. X90.

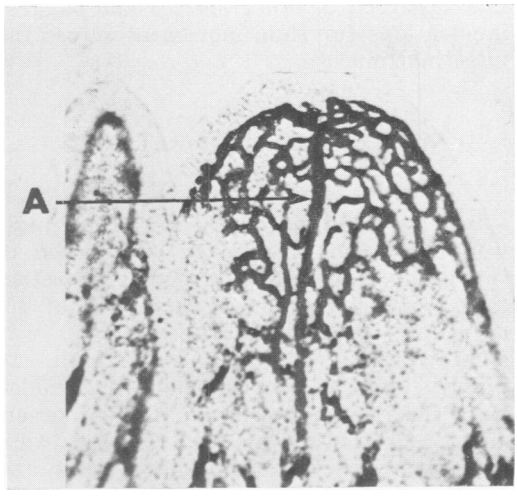

Fig. 3. Longitudinal section through a duodenal villus. The central arteriole (A) can be seen to arborize into an extensive capillary plexus. X375.

The vasculature was most easily studied if the sections were cleared with xylene and left unstained. Some sections were stained lightly with hematoxylin and eosin to study the relationship of vessels to other tissues. Serial sections were cut both transversely and longitudinally in respect to villi and crypts, in order to facilitate a three dimensional interpretation of the vascular pattern. Each section revealed only segments of any particular vessel. It was therefore necessary to follow a vessel through several serial sections in order to determine its course and origin. Several criteria were used to identify the vessel types. Large vessels which were paired in close association to one another were considered arteries and veins, arteries being distinguished from veins by a smaller lumen and a thicker muscular coat. Branches of these vessels which were not paired were called either arterioles or venules depending on their origin and size. Arterioles measured 10 to 15 microns in diameter whereas venules ranged from 15 to 35 microns. The cappillaries ranged in size from 5 to 10 microns.

RESULTS

The arteries supplying the intestine enter the intestinal wall from the mesentery and penetrate the tunica muscularis (Fig. 1). Branches given off the artery pass between the muscle layers where they subdivide supplying both the longitudinal and circular muscle layers. The main arterial trunk enters the submucosa and ramifies into many branches some of which anastomose with one another forming a submucosal arterial rete (Fig. 2). From the submucosal arteries, arterioles arise and follow an almost direct axial course to the tips of the villi above.

The submucosal arteries send a few branches to the crypt plexus, the tunica muscularis, and the duodenal glands as well. In the villus, the central arteriole arborizes into a dense capillary net at the villus tip (Fig. 3). This plexus runs immediately beneath the epithelial cell layer (Fig. 4). The capillaries which extend below the base of the villi are continuous with those of adjacent villi.

At a point approximately midway between the base and the tip of the villus, some of the capillaries converge to form venules (Fig. 5). There are one or two venules on either side of the central arteriole which empty into a large venous channel running transversely along the bases of the villi. This subvillus "transverse venule" has not been described in other species. Large veins leave this vessel at intervals to connect with the submucosal veins. Beneath the "transverse venule" is an extensive plexus in the area of the crypts of Lieberkühn. Some of the capillaries of the villus plexus are continuous with those of the crypt plexus. The crypt plexus empties into the submucosal veins by small venous branches at the base of the crypt area. Serial cross-sections through the crypts indicated an extensive cryptal capillary plexus. Vessels are arranged in a "honeycomb" fashion with each crypt surrounded by capillary rings throughout its length (Fig. 6).

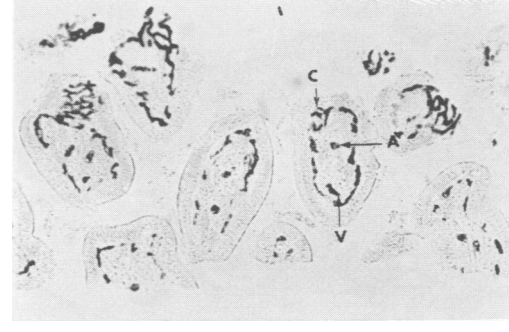

Fig. 4. Transverse section through several villi showing central arterioles (A), paraxial venules (V) and subepithelial capillaries (C). X215.

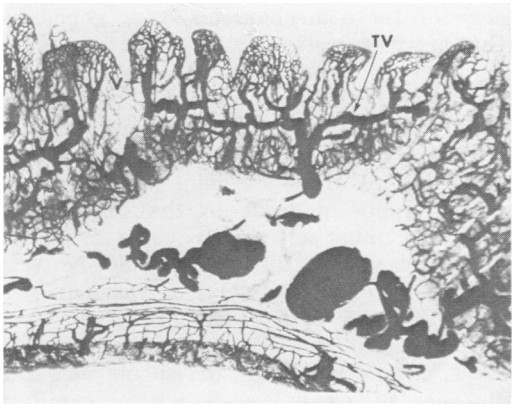

Fig. 5. Transverse section through the jejunal wall to show the paraxial venules (V) draining into the "transverse venule" (TV) and on into a larger venous vessel to the submucosa. X85.

Submucosal veins combine to form the main venous trunk which penetrates the tunica muscularis to the mesentery, collecting branches from the muscle layers as it passes. The submucosal veins form an anastomosing plexus. The veins are often very tortuous with what appear to be segmental dilated portions.

The villi of the small intestine vary in shape from broad "leaf-like" villi to narrow "finger-like" villi as reported previously (22). The pattern of vessels was similar in all types of villi except the drainage of the villus plexus of the narrower villi was usually accomplished by a single venule.

The extent of the crypt capillary plexus is proportional to the amount of crypt tissue. Therefore, the plexus was most extensive in the duodenum, slightly less so in the jujenum with a looser mesh in the ileum.

Figure 7 is a diagram representing a composite view of the porcine intestinal vasculature as interpreted from serial sections.

DISCUSSION

The blood vascular pattern of the porcine intestinal mucosa (Fig. 7) more closely conforms to the classical "fountain" rather than the "tuft" concept. In this respect, it resembles that described for the rabbit, dog (11), man (2, 11) and rat (16, 17), but differs from that in the monkey (20).

Precapillary arteriovenous anastomoses, previously described at the villus tip in man and rabbits (11), could not be demonstrated in the pig. Submucosal arteriovenous anastomoses which have been reported in the pig (28) could not be demonstrated with any certainty in this study. Longitudinal sections through the submucosa occasionally gave the impression of an anastomosis between a vein and an artery, but it usually appeared to be an artery passing under or over a vein. The segmental dilated portions of the submucosal veins may correspond to the "Venenbällchen" reported by Spanner (24).

The fact that the capillary plexuses in the pericryptal and villus stroma are connected suggests the possibility that the base of the villus depends to some degree on this route for arterial blood in addition to that of the submucosal artery. Another suggestion, which appears more likely functionally, is that the interconnection between the villus and cryptal plexuses represents an alternate route for venous return. Blood may flow from the villus capillaries directly into collecting venules and on to the submucosa or it may pass through the crypt plexus on its way to the submucosa. The existence of these alternate circulatory routes suggests that the distribution of blood to the intestinal mucosal compartments is probably controlled by some type of regulatory mechanism. It has been reported that there are local mechanisms which aid in regulating the distribution of blood to the various compartments of the intestine (5, 12, 13).

The mucosal vascular arrangement of the pig may be pertinent to an understanding of the production of intestinal fluid normally as well as in diarrhea. Thirty years ago, the suggestion was advanced that during digestion a constant secretion of fluid from

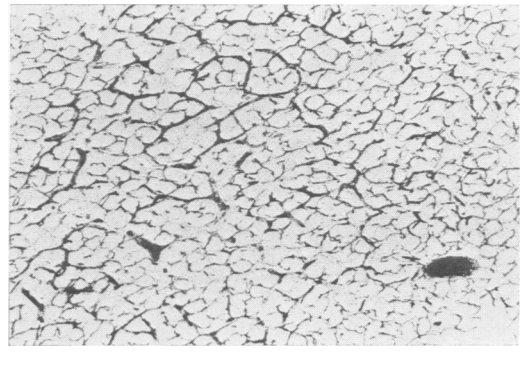

Fig. 6. Longitudinal section through the duodenal mucosa at the level of the crypts of Lieberkühn showing the extensive capillary network in this area. X40.

the crypts of Lieberkühn could keep food particles in suspension thereby facilitating enzyme action and absorption (4). Florey *et al* (4) state:

> One may envisage a circulation of fluid during active digestion, the secretion passing out from the crypts of Lieberkühn into the lumen and back into the villi.

Little attention has been given to this suggestion until recently (9). In their review on intestinal secretion, Hendrix and Bayless (9) have summarized the three proposed mechanisms . . .

> to explain fluid and electrolyte movement into the intestinal lumen: 1. transfer by filtration generated by increased tissue pressure; 2. transfer generated by electrical and chemical gradients across a semi-permeable membrane composed of the intestinal mucosa; and 3. transfer by active secretion by the crypts of Lieberkühn.

After discussing the evidence supporting each of these mechanisms, they concluded that active secretion by the crypts has more evidence in its favour, but that all three processes probably contribute to intestinal fluid formation and that further investigation should decide their relative importance. All three of these mechanisms depend on blood supply at least to the extent that the fluid must ultimately come from the capillaries; but, filtration would be very dependent on blood volume, vascular permeability and hydrostatic pressure.

In a recent study (8), it was found that loops, twists, windings, off-shoots and confluences of small vessels within capillary beds are places of preferential passage of injected fluorescent dye. The fact that these types of formations comprise the entire pericryptal capillary plexus suggests that these vessels may be especially suited to transcapillary fluid transport normally. The potential venous return route from villus capillaries to the dense crypt plexus of looped and winding vessels supports the

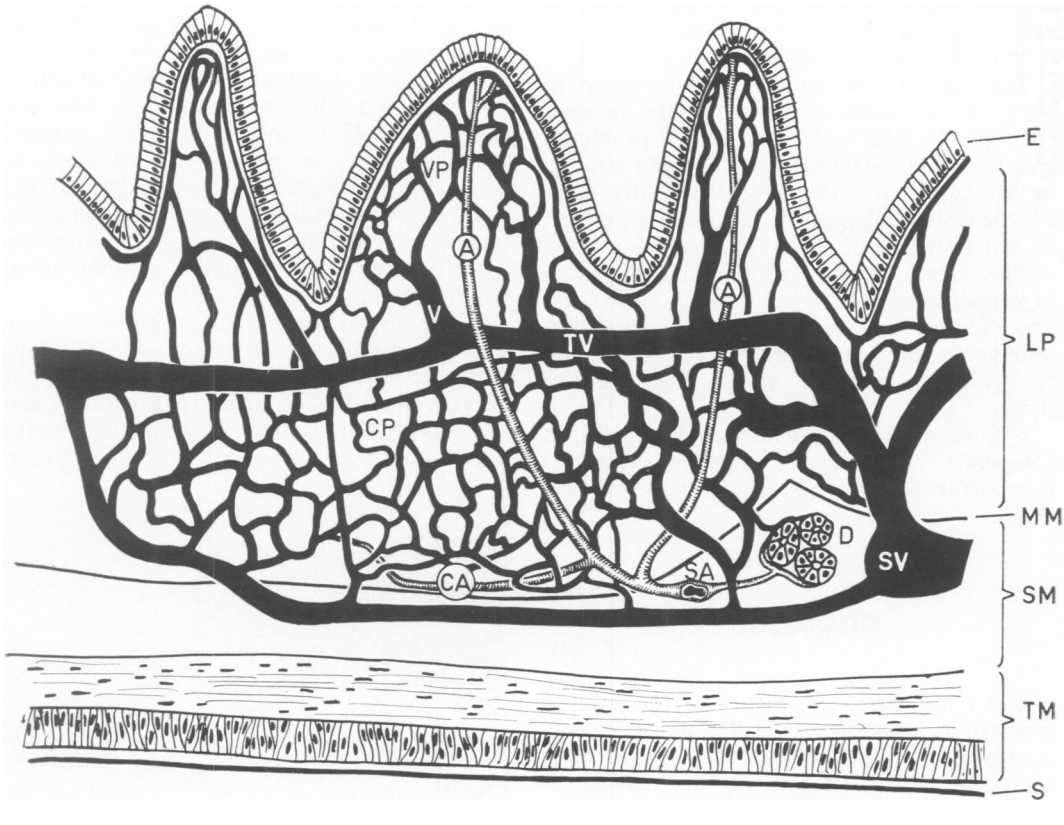

Fig. 7. Diagram of the intestinal vascular pattern of the pig. SA-submucosal artery; SV-submucosal vein; A-central villus arteriole; V-paraxial villus venule; TV-"transverse venule"; VP-villus capillary plexus; CP-pericryptal capillary plexus; CA-artery supplying the pericryptal capillary plexus; D-duodenal glands; E-epithelium; LP-lamina propria; MM-muscularis mucosa; SM-submucosa; TM-tunica muscularis; S-serosa. (The epithelium of the crypts is not shown.)

concept of a fluid circuit like that suggested by Florey *et al* (4). As the blood enters the many subepithelial villus capillaries from one central arteriole, its hydrostatic pressure will be greatly diminished thus facilitating absorption of fluid into the capillaries. The absorbed fluid would then flow in the capillaries to the crypts to be partly filtered and/or secreted back into the intestinal lumen. By local regulation of blood flow, this circuit may be active only during digestion. At other times, the crypt-villus circuit would be "functionally" bypassed; each unit would have its own arterial supply and venous return systems. This type of vascular arrangement would permit the efficient recyling of water and solutes required for digestion and efficient provision to the crypt cells of many of the raw materials required for "secretion" (Fig. 8).

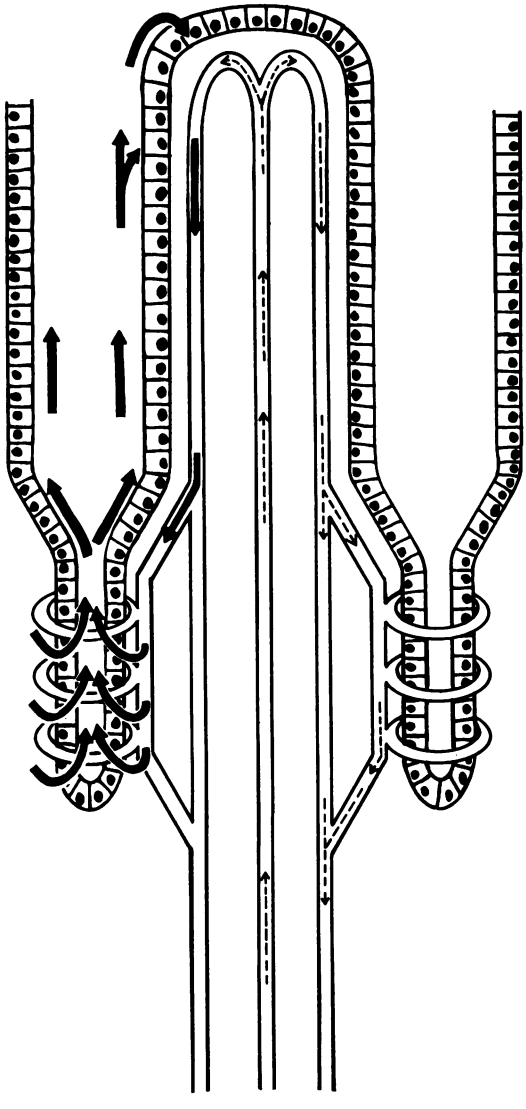

Fig. 8. Schematic illustration of the proposed fluid circuit in relation to the intestinal vascular anatomy as described in the text. The direction of blood flow is indicated by broken arrows and the fluid circuit by solid arrows. (The direct arterial supply to the pericrypta plexus is not shown.)

Colibacillary diarrhea is a disease entity of small pigs in which a pathogenic strain of *Escherichia coli* produces an enterotoxin which somehow induces fluid movement into the intestinal lumen (1, 7, 18, 23). Several possible explanations for this have been discussed (18); however, the exact mechanism responsible for the excess fluid production is unknown. Present evidence suggests that enterotoxin acts on crypt cells to increase active secretion. If vascular permeability or local control of blood supply are also affected by enterotoxin, it is possible that fluid production in enteric colibacillosis and similar diseases may be also in this way at least partly related to the mucosal vasculature. It may be that the toxin has several sites of activity. Until these sites are determined, the pathogenesis of fluid production in colibacillary diarrhea remains a matter of conjecture. Nevertheless, it is very likely that the unique vascular arrangement of the intestinal mucosa plays a significant role in this and other intestinal phenomena.

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