

Microangioarchitecture of haemorrhoids

A scanning electron microscopy study of vascular corrosion casts

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Keywords

Corpus cavernosum recti, haemorrhoids, microvascular anatomy, vascular castings, SEM

Summary

Haemorrhoids are normal physiological structures (vascular cushions), which lead to haemorrhoidal disease when they enlarge pathologically. These vascular cushions are located in the submucosa of the lower rectum and are an important part of the continence organ. The cushions contain enormously dilatable veins which, when filled, enable a gas- and water-tight closure of the anal canal. As vascular cushions require rapid filling and haemorrhoid-associated anorectal bleeding is bright red, it is suggested that dilated veins are filled with arterial blood delivered via arteriovenous (AV) anastomoses. To specify blood supply and microvascular anatomy of the anorectum we analyzed vascular corrosion casts of human recta made from excised post mortem specimens. We found wide dilated veins and venules in the submucosa. Dilatations were often associated with sphincters capable to block venous outflow. Though careful analyses of completely filled vascular corrosion casts we could not confirm the existence of large AV-anastomoses in the anorectum postulated by previous authors. **Conclusion:** From our findings we conclude that the swelling of the dilatable veins is rather the result of blockage of venous outflow from these veins by the sphincters found than their filling via arteriovenous anastomoses as proposed so far.

Schlüsselwörter

Corpus cavernosum recti, Hämorrhoiden, Mikrogefäßarchitektur, Gefäßausgusspräparate, SEM

Zusammenfassung

Hämorrhoiden sind normale physiologische Strukturen (Gefäßpolster), die bereits bei Neugeborenen vorkommen. Bei pathologischer Vergrößerung führen sie zur Hämorrhoidal-erkrankung. Diese Gefäßpolster sind im unteren Rektum lokalisiert und tragen einen wichtigen Teil zur Feinkontinenz bei, indem sie als in der Submukosa gelegene, stark erweiterungsfähige Venen nach ihrer Füllung zu einem luft- und wasserdichten Abschluss des Analkanals führen. Aufgrund ihrer Fähigkeit zu schwellen, werden diese Gefäßpolster – in Analogie zum corpus cavernosum penis – als corpus cavernosum recti bezeichnet. Da die Gefäßpolster schnell gefüllt werden müssen und Hämorrhoiden-assoziiertes anorektales Bluten hellrot ist, vermutete man, dass in den genannten Venen arterielles Blut fließt, das über arteriovenöse (AV) Anastomosen dorthin gelangt. Um diese Anastomosen und die Mikrogefäßarchitektur der Gefäßpolster zu untersuchen, wurden post mortem Anorekta des Menschen präpariert, eine Kanüle in die obere Rektalarterie (in manchen Fällen zusätzlich auch in die mittlere Rektalarterie) eingebunden, und das Blutgefäßsystem unter manuellem Druck mit physiologischer Kochsalzlösung blutfrei gespült. Sobald der venöse Efflux klar wurde, wurden 20 ml flüssiger Kunststoff Mercox-Cl-2B® (4 + 1 verdünnt mit monomerer Methylmethacrylsäure

wobei 4 ml Monomer 0,3 g Härter MA enthalten) injiziert. Nach Aushärtung des Kunststoffes wurden die injizierten Anorekta in 7,5%iger Kalilauge mazeriert, in fließendem Leitungswasser und destilliertem Wasser gereinigt, und gefriergetrocknet. Die trockenen Gefäßausgusspräparate wurden auf Präparateträger aufgebracht, mit Kohlenstoff und Gold bedampft und nach einer ersten stereomikroskopischen Betrachtung im Rasterelektronenmikroskop untersucht. Bereits die stereomikroskopische Betrachtung der Präparate zeigte, dass in der Submukosa auffällig weit dilatierte Venen und Venolen vorhanden sind, die Durchmesser bis zu einem Millimeter besitzen und starke Kaliberschwankungen aufweisen. Die rasterelektronenmikroskopische Untersuchung zeigte, dass diese Gefäße um vieles kleinere Venolen aufnehmen und an vielen Stellen ringförmige Einengungen zeigen, die auf das Vorhandensein von Sphinkterstrukturen hinweisen. Trotz sorgfältiger Analysen von vollständig gefüllten Gefäßausgusspräparaten konnten die von früheren Autoren im Anorektum postulierten AV-Anastomosen nicht gefunden werden. **Schlussfolgerung:** Unsere Befunde sprechen dafür, dass das Anschwellen der venösen Gefäßpolster des corpus cavernosum recti durch die Blockade des Blutausstromes aus den weiten Venen der Submukosa durch die Sphinkteren bedingt ist und nicht durch deren Füllung über AV-Anastomosen wie bisher angenommen.

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Mikroarchitektur der Hämorrhoidalgefäße – Eine rasterelektronenmikroskopische Studie an Gefäßausgusspräparaten

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Haemorrhoids are anal vascular cushions representing normal physiological structures. They can be found in newborns and are an important part of the continence organ (1). The anal cushions can swell and thus close the anal canal gas- and water-tight (2). Because of the ability to swell Stelzner et al. (3) – in analogy to the corpus cavernosum penis – termed this structure corpus cavernosum recti (CCR).

The rectum and the anal canal are the distal portions of the colon and regulate defaecation. Anatomically the rectum begins between the 2nd and 3rd sacral vertebra. It is about 12 cm long and is lined with a mucosa. The anal canal is approximately 3–4 cm long. It is lined with mucosa proximally and perianal skin distally. At the proximal part of the rectum three transverse folds are located with Kohlrausch's plica being the most prominent one lying 6–7 cm above the anus (5). After this fold an expandable area, the rectal ampulla follows, which stores faeces (6). At the caudal part of the ampulla 8–10 longitudinal folds are present. They are also part of the anorectal line, which demarcates the borderline between the rectal mucosa and the perianal skin of the anal canal (5). At the anorectal line, also termed dentate line, a transitional epithelium is present (7).

One of the first descriptions of the blood supply of the anorectum was given by Miles (8). He stated that the superior haemorrhoidal artery has a constant branching pattern and that each of its branches supplies a defined area of the anorectum. He also argued that the site where haemorrhoids develop is predetermined by the incoming branches of the superior haemorrhoidal artery. Later this was disproved by Thomson (1) and Aigner et al. (9), who performed an in-depth study on the nature of haemorrhoids. They found that the branching pattern of the superior haemorrhoidal artery is very variable and branches do not follow a defined route. In addition, Thomson (1) also stated the importance of the middle and the inferior haemorrhoidal artery. Like Miles (8) he also pointed out the constant appearance of the anal cushions and haemorrhoids at the left lateral, right anterior and right posterior position.

In 1962 Stelzner et al. (3) suggested that the dilated veins within the CCR are perfused with arterial blood derived from direct tiny communications between arterioles and dilated veins, which could explain both, the bright red anorectal bleeding and the high oxygen saturation of 98% of the anorectal blood (10). This finding led to the description of two separate circulations (11), namely a

- functional circulation which guarantees rapid filling of the venous dilatations and the swelling of the anal cushions and
- a nutritive circulation which supplies the anal region with arterial blood.

Haemorrhoidal disease is very common. The generally accepted prevalence is 50% in people who are older than 50 years (12). Scientists and health professionals studied the development of haemorrhoids for centuries, but still little is known about the aetiology of haemorrhoidal disease. At present, three theories about the development of haemorrhoids are discussed:

- varicose vein theory,
- vascular hyperplasia theory, and
- sliding anal lining theory.

For more details see Thomson (1).

Varicose vein theory

This theory states that dilated veins are a pathological feature. However, Thomson (1) claimed that dilated veins are normal structures making the varicose vein theory invalid. Furthermore, it was suggested that –because of the existence of free communications with the portal system – haemorrhoids are caused by portal hypertension. This suggestion was later disproved by studies in patients suffering from portal hypertension (13, 14). Hosking et al. (13) found in 65% of patients with liver cirrhosis a prevalence of haemorrhoidal disease, which is not significantly different from the prevalence of haemorrhoidal disease in the normal population (12).

Vascular hyperplasia theory

This theory assumes that the submucosal vessels enlarge and thus haemorrhoidal disease develops. Stelzner et al. (3) stated that the cause of haemorrhoidal disease is CCR hyperplasia.

Sliding anal lining theory

This theory describes haemorrhoidal disease as being caused by degeneration of supportive tissue, i. e. musculature and connective tissue, and by a displacement of the anal cushions towards caudal caused by chronic obstipation and shear forces (1).

New insight

These three theories are solely based on anatomical and histological findings. To gain new insights into the cause of haemorrhoidal disease Chung et al. (15) studied the correlation between haemorrhoidal disease and neovascularisation. They found that in 54% of patients with haemorrhoidal disease endoglin, an angiogenesis promoting protein, is overexpressed in haemorrhoidal tissue. The authors concluded that neovascularisation plays a role in the development of haemorrhoidal disease. Aigner et al. (16) focused on the superior rectal artery (SRA) of patients suffering from haemorrhoidal disease. They reported correlations between haemorrhoidal disease and both, an increase in the calibre of the SRA and an increase in arterial blood flow. Scheyer et al. (17) considered as one cause for the increased blood pressure within the anorectal vessels an imbalance between arterial inflow and venous outflow.

Symptoms

Pathologically enlarged haemorrhoidal cushions are graded into four degrees (18).

- In the first degree haemorrhoids are considered as enlarged anal cushions which do not prolapse.
- Haemorrhoids which prolapse during defaecation, but spontaneously return

to their original position after defaecation are staged second degree.

- Third degree haemorrhoids do not return without manual help after prolapse and
- fourth degree haemorrhoids remain prolapsed permanently.

Secondary symptoms associated with haemorrhoidal disease are inflammations, thromboses and anorectal bleedings (19). These symptoms do not correlate with the grade of haemorrhoidal disease, i. e. low grade haemorrhoids can possibly cause

more severe secondary symptoms than high grade haemorrhoids (20).

Though many studies were performed it is still unknown how haemorrhoidal disease develops and where exactly haemorrhoidal associated anorectal bleeding occurs. Stelzner et al. (3) suggested that venous dilatations are the source of bleeding. Other authors considered the capillary bed within the submucosa as the source (1, 19).

Because haemorrhoidal disease associated anorectal bleeding is due to traumata of the rectal mucosa, the correlation between eating habits and development of

haemorrhoidal disease is commonly accepted. Low fibre diets cause hard faeces and obstipation leading to increased pressing during defaecation (2). This leads to the enlargement and dislocation of the haemorrhoidal cushions. But also diarrhoea is considered to be an important challenge for the anal cushions (21).

Techniques used in the study of the vascular anatomy of the CCR had a limited ability to display the 3D-topography and the shape of the CCR microvascular bed. Here we used scanning electron microscopy (SEM) of vascular corrosion casts (VCCs) with its high depth of focus and great spatial resolution to

- demonstrate the AV-anastomoses postulated to feed the CCR (3), and
- study the topography of the wide venous vessels of the CCR and their spatial relations with other CCR vessels to gain a better understanding of how swelling of the CCR occurs.

Material and methods

Segments of the human rectum were excised 12–48 hours post mortem. A cannula was inserted into the SRA (in some cases also in the middle rectal artery too) and the blood vascular system was rinsed with physiological saline (0,9% NaCl; 20°C; manual pressure) until clear reflux escaped from opened rectal veins. Then 20 ml of Mercox-Cl-2B (diluted 4+1 with monomeric methylmetacrylic acid, Fluka, Basle, CH) were injected the same route until resin reflux become highly viscous. After hardening of the injected resin, specimens were tempered (water bath, 60°C; 12 hours), macerated (7.5% KOH; 40°C), rinsed and frozen in distilled water, and finally freeze-dried.

Dry specimens were mounted onto specimen stubs by using the conductive bridge method (22), evaporated with carbon and gold, sputtered with gold, and examined in a scanning electron microscope (ESEM XL-30; FEI company, Netherlands) at an accelerating voltage of 10 kV. For further details of vascular corrosion casting see Aharinejad and Lametschwandner (23) and Motta et al. (24).

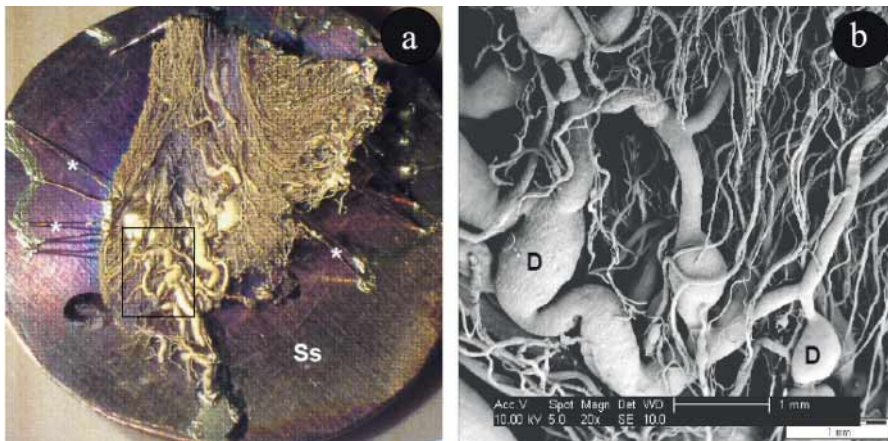


Fig. 1 Microvasculature of the human lower rectum (vascular corrosion casts)
a) longitudinal section (macrophotography): Note the wide dilated veins located in the submucosa and that veins frequently interconnect.
b) detail of a)

*: conductive bridges; Ss: specimen stub.; D: wide dilated veins

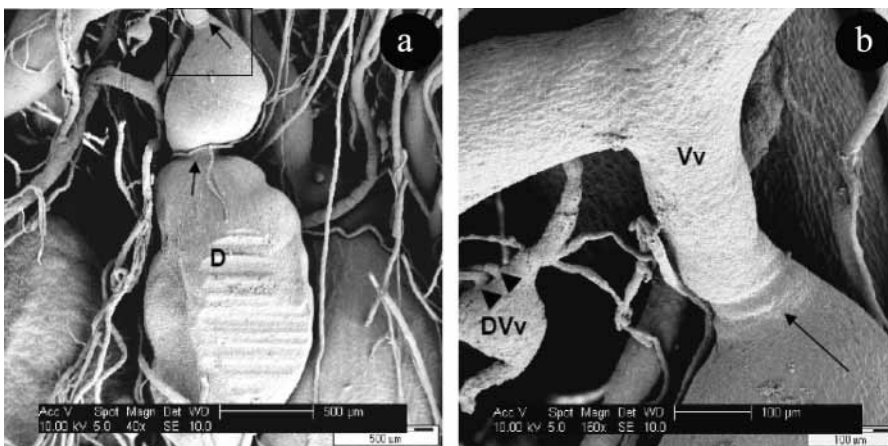


Fig. 2 Dilated vein (D) within the submucosa of the human lower rectum (vascular corrosion casts)
a) note the sphincter structures at the begin and the middle of the dilatation (→)
b) sphincter at the begin of a dilated vein (→); detail of a); note the small venules (arrowheads) emptying into a dilated venule (DVv); Vv: venule.

Results

In vascular corrosion casts of the human rectum wide, dilated vessels are seen already at stereomicroscopic inspection (► Fig. 1a). Scanning electron microscopy (SEM) of the same areas convincingly shows that these wide vessels are veins as indicated by the round to oval endothelial cell nuclei impressions seen on the cast surfaces (► Fig. 1b). These veins frequently connect with each other (► Fig. 1b). The shapes of venous dilatations vary greatly from wide stretched ones (► Fig. 1b and ► Fig. 2a) to spherical ones (► Fig. 1b and ► Fig. 2b). Diameters of these dilated veins can reach up to 1 mm (► Fig. 2b). Besides the endothelial cell nuclei imprint patterns typical for veins also merging patterns confirm their venous nature (► Fig. 1b).

On casts of the wide dilated veins circular narrowings were found at the beginning, in the middle, and at the end of dilated segments (► Fig. 2). These narrowings reflect the site of sphincters made up by an accumulation of vascular smooth muscle cells indicating venous sphincters.

A common observation were small venules which emptied directly into wide dilated veins (► Fig. 4). Capillaries either changed into postcapillary venules or shunted into a nearby vein (► Fig. 5). No arteries were found which directly fed into dilated veins. Endothelial cell nuclear imprints (► Fig. 4b, arrows) and the shape of the vessels clearly confirm their venous nature. Arterioles feeding directly into dilated veins were not found.

Veins located in the anal region often changed their caliber abruptly as did small venules. These venules then reduced their calibre from e. g. 100 µm to 35 µm. Locally, vessels coiled around each other (► Fig. 6a).

A phenomenon often seen on arterioles were plastic strips, which here imitate vascular smooth muscle cells (myocytes) (► Fig. 6).

Signs of angiogenesis were rarely seen and were indicative of the non-sprouting type, i. e. intussusceptive microvascular growth (IMG) (► Fig. 7).

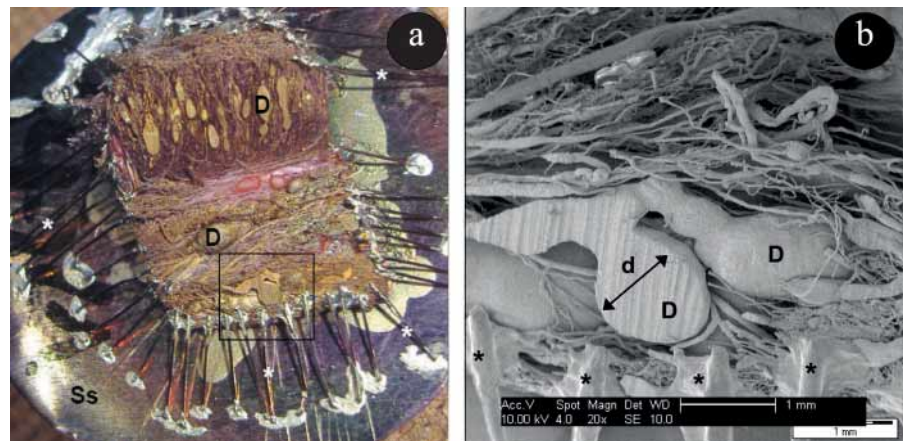


Fig. 3 Microvasculature of the human lower rectum (vascular corrosion casts)

a) longitudinal section (macrophotography): note the enlarged vessels (D).

*: conductive bridges; Ss: specimen stub.

b) dilated vein within the submucosa; detail of a) in the enboxed area: Note that one of the dilatations (D) reaches a diameter (d) of approximately 1 mm. Asterisks mark conductive bridges.

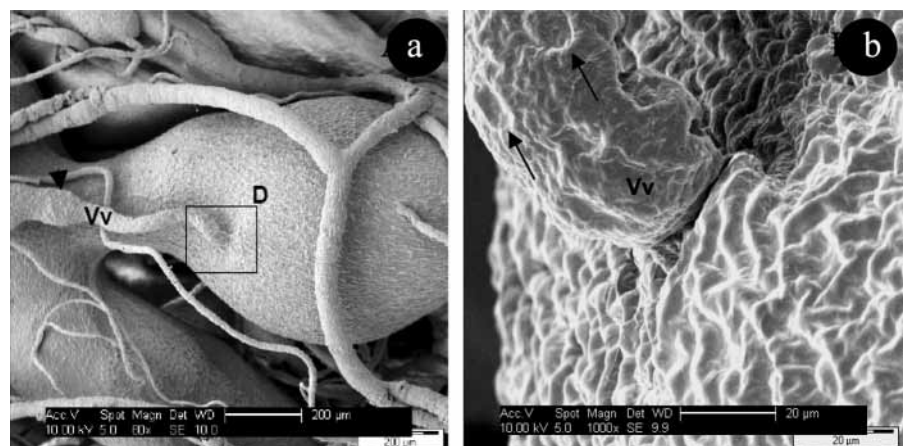


Fig. 4 Dilated vein (D) within the submucosa

a) A small calibre venule (Vv) emptying into a dilated vein (D): Note that the venule enlarges distally (arrowhead).

b) Detail of a): Venule (Vv) joins a large venous dilatation; note the venular endothelial cell nuclear imprints (→).

Discussion

Reported prevalence of haemorrhoidal disease ranges from 10% to 86% (2, 25). According to the current knowledge dilated veins within the anal cushions are found in fetuses and thus represent a physiological phenomenon and are by no means indicating a pathology (1), but are an important functional component of the anorectal continence organ. Our findings confirm that in the submucosa and in subepithelial layers of the anorectum many dilated veins

are present. The arrangement of these vessels is very uncommon as far as they frequently interconnect with nearby veins. Interestingly, the thin proximal portions of the connecting vessels enlarge abruptly at some distance from the dilated vein (e. g. ► Fig. 4). If these thin portions of the venules reflect the ability of the vessels to actively narrow their lumina and so to assist the sphincters in filling and swelling of distinct portions of haemorrhoidal veins has to be the subject of further studies. The shape of these vessels clearly reflects their

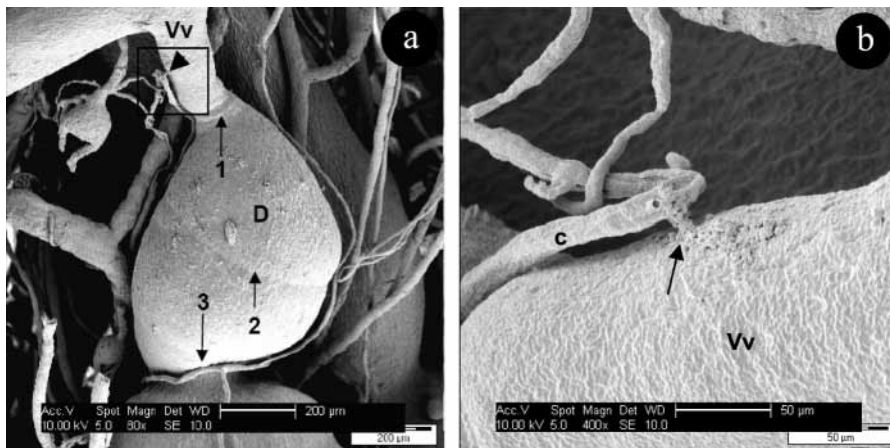


Fig. 5 Dilated veins

- a)** venous dilatation (D) with sphincter structures (→) at the begin (→ 1), at the middle (→ 2) and at the end of the dilatation (→ 3); note the small connection between the venule (Vv) and a nearby small vessel (arrowhead)
b) detail of a); shunting (→) of a capillary (c) to the nearby venule (Vv)

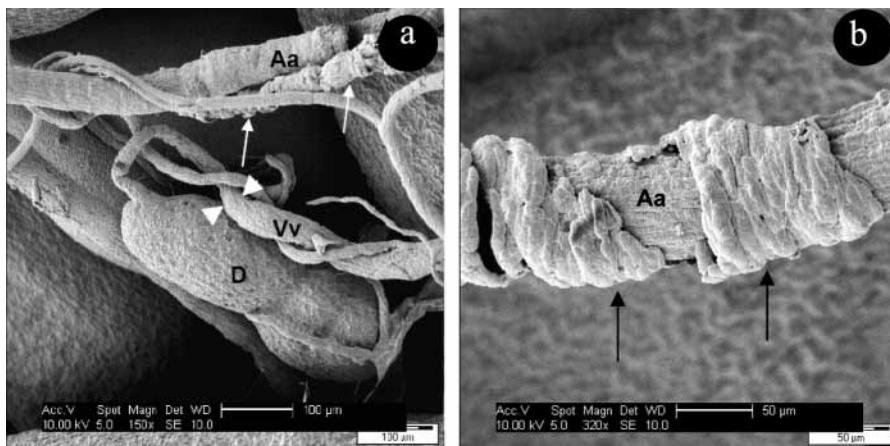


Fig. 6 Dilated venules (D) within the submucosa of the human rectum

(→: plastic strips; Aa: arteriole)

- a)** Note that the small venule (Vv) which empties into the dilated venule enlarges some 200 μm distally (arrowheads) and that vessels twist around each other.
b) Detail from a); plastic strips (→) around an arteriole (Aa)

ability to swell and consequently to narrow the lumen of the rectum ensuring the gas-tight closure of the anal canal.

According to Stelzner et al. (3), dilated veins contain arterial blood, which derives from arterio-venous communications. As filling of these dilated veins has to be quick it is reasonable to think that this best is done by an arterial input. Stelzner et al. (3) reconstructed the 3D structure of the CCR from serial sections of paraplast embedded specimens by light microscopy and de-

scribed arterio-venous anastomoses. In contrast, we could not find any such arterio-venous anastomoses, which is puzzling as they should be numerous in order to fill and thereby dilate veins up to diameters of 1 mm. But an arterial inflow alone could not explain the swelling of the haemorrhoids if one assumes a permanent venous outflow. Swelling thus must also depend on a temporary venous outflow blockade. This blockade could be done by clusters of vascular smooth muscle cells

(sphincters) which upon contraction reduce the luminal diameter of venous outflow vessels. As a result of this venous outflow blockade veins will dilate/balloon and make the anal cushions swelling.

The circular impressions which we found on casts at the proximal and distal ends of dilated venous vascular segments clearly demonstrate that the structural prerequisites for blocking venous outflow are present.

During defaecation sphincters relax, blood drains off the veins, cushions collapse and stool can pass easily.

The finding that anorectal bleedings are highly saturated with oxygen (10) is in favour with the hypothesis that the dilated veins contain arterial blood. However, as these findings were gained by puncturing the CCR where beside dilated veins also numerous arterial vessels are present, these results have to be questioned as long as it was not excluded that arterial vessels could have been punctured, too. Although anorectal bleedings as symptoms of haemorrhoidal disease are very common, the exact site of bleeding still remains unclear. According to Stelzner et al. (3) anorectal bleedings are due to lesions of the dilated veins which are filled with arterial blood. These lesions are most likely caused by shear forces generated during defaecation. In our study we found dilated veins with diameters up to 1 mm. Lesions of these vessels would lead to massive bleedings causing even anaemia in certain cases (21). In contrast to Stelzner et al. (3) Thomson (1) considered the sub-epitelial capillaries as the site of bleeding what also would explain the bright red bleedings.

In vascular corrosion castings lesioned vessels are detectable by masses of resins which escaped from the opened vessels. Another source of leakage of resin out of a vessel could be due to autolysis of vascular wall components making the vessel highly permeable for the resin if casting is done hours post mortem. Thus, both extravasated resin (extravasates) related to pre-existing lesions and extravasates related to autolysis of vascular walls have to be taken into account. But extravasates due to autolysis will reveal an overall random dis-

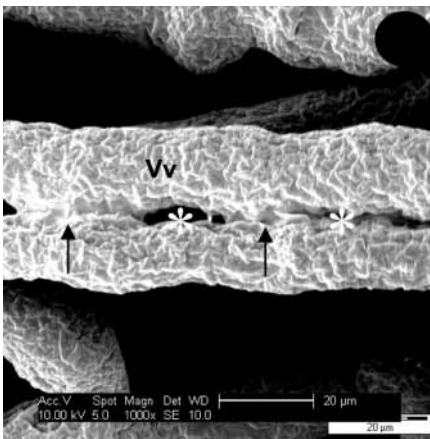


Fig. 7 Venule from the submucosa of the human rectum showing ongoing intussusceptive microvascular growth

*: Sites where transluminal growth of tissue pillars separates the venule (Vv) into two venules; → sites where the venule is still a single vessel.

tribution in the specimen while extravasates due to lesions will show local preferences. As the specimens used in the present study were casted 12–48 h post mortem autolysis will not yet have made blood vessels so permeable to let the resin escape transmurally to form similar large extravasates as is the case in lesioned (opened) vessel.

In 2002 Aigner et al. (16) focussed their interest on changes of branches of the superior haemorrhoidal artery (SRA). They found that in patients with haemorrhoidal disease calibre and blood flow within the SRA significantly differed from a control group. While in the control group diameters of branches of the SRA ranged from 0.6 mm to 1.2 mm and blood flow was between 5.0 cm/s and 21.0 cm/s, in the patient group the calibres of the SRA ranged from

0.6 mm to 3.6 mm and blood flow was between 10 cm/s and 125 cm/s.

Conclusion

Although SEM analysis of vascular corrosion casts has a very high spatial resolution, and artefacts can be differentiated from real vascular structures, we were not able to find any arterio-venous anastomoses which could be in favour of an arterial inflow into the wide dilated veins dominating the vascular bed of the corpus cavernosum recti.

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