Scanning Microscopy

Volume 3 | Number 1 Article 32

3-17-1989

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Scanning Microscopy, Vol. 3, No. 1, 1989 (Pages 305-314)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0891-7035/89$3.00+.00

THE VASCULARIZATION OF THE SKIN OF THE ATLANTIC HAGFISH, MYXINE GLUTINOSA L. AS REVEALED BY SCANNING ELECTRON MICROSCOPY OF VASCULAR CORROSION CASTS

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(Received for publication May 1, 1988, and in revised form March 17, 1989)

Abstract

The vascularization of three different (A, B, C) skin regions (from the level of the heart to the cloaca including dorsal, lateral and ventral skin areas) of the Atlantic hagfish, Myxine glutinosa L. was studied by scanning electron microscopy of vascular corrosion casts. Vessel variables were measured either from semithin sections (diameters) or from vascular corrosion casts (diameters, lengths) and total blood capacities as well as vessel surfaces per unit skin area (mm^2) were calculated. There are no significant differences in the number of subepidermal capillary meshes (ranging from 164 to 185 meshes per micrograph) in areas A, B or C nor in vessel lengths. The average vessel length per mm^2 is 32 mm. Assuming an average diameter of 22.3 µm these vessels have an average surface of 2.24 mm^2 and a volume of 12.5 nanoliters (nl). In contrary weighing two pieces (5 mm times 5 mm in size) of the whole skin vascular bed - knowing the density of the casting medium - results in only one fifth of that volume. Overestimation of vessel lengths and diameters by measuring casted structures from micrographs on the one hand and inaccuracies in weighing or dissection of casted skin pieces on the other hand are discussed as sources of observed differences.

Key Words: Myxine, skin, vascularization, corrosion cast, scanning electron microscopy

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Introduction

The skin of lower vertebrates is highly vascularized. It therefore is considered to be an auxiliary to gills and lungs in respiration. Most studies done on the anatomy of skin vascularization use light microscopy of India-ink injected cleared specimens (Spalteholz, 1914). Because of the lack of depth of focus this otherwise excellent technique is unable to demonstrate complex microangioarchitectonic patterns. To the contrary scanning electron microscopy (SEM) of vascular corrosion casts can do so (Murakami, 1971; Hodde and Nowell, 1980; Lametschwandtner et al., 1984). Presently only the amphibian skin vascularization has been studied this way (Jasinski and Miodonski, 1978; Miodonski and Jasinski, 1979).

The present study intends - beside demonstrating the general microangioarchitectural patterns of the myxinoid skin - (1) to give quantitative data on blood vessel parameters important for respiration physiology, i.e., vessel diameters and vessel lengths enabling one to estimate skin respiratory surfaces and exchange areas as well as blood volumes capable for delivery and removal of skin metabolic substances and (2) to test the assumption that according to the hagfish mode of life - Myxine spends a lot of time burrowed in the mud of the bottom of the sea in depths around 100 meters with the tip of the nose slightly protruding above the surface mud layer - that there should be a rather uniform vascularization all over the body skin with the exception of the areas around the ducts of the metabolic highly active slime glands (Lametschwandtner et al., 1986).

Materials and Methods

Preparation of corrosion casts

A total of 12 female hagfish, Myxine glutinosa L. with body lengths ranging between 26 and 46 cm was studied. Animals were caught with baited traps in the Oslo

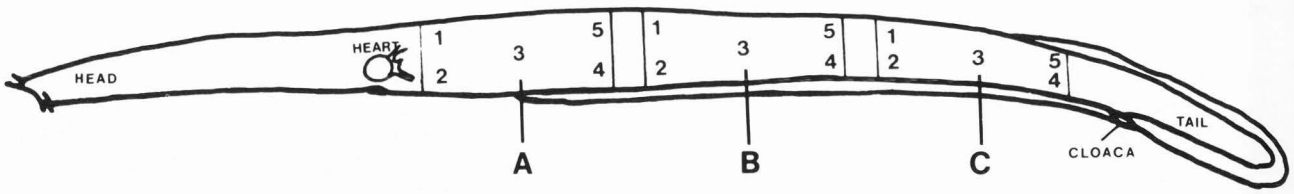


Fig.1. Myxine glutinosa L. Topography of skin areas A, B and C studied. Micrographs were taken from regions 1 - 5.

Fjord near the Biological station of the University of Oslo at Dröbak at depths between 80 and 120 meters and kept in seawater-tanks at +4 °C till they were transported by air mail to Salzburg, Austria. After a recovery period of a week in the aerated transport sea water at the same temperature animals were anaestethized by immersion in MS 222 or by intraperitoneal injection of urethane (2 animals) and the impar dorsal aorta was exposed by transecting the lateral body wall by a 4 cm longitudinal section at the level of the liver. Then a plastic cannula (Braunule, Braun-Melsungen, FRG) was tied in and rinsing with cold filtered sea-water started (perfusion pressure: 40 cm water column; perfusion volume: 20 - 50 ml) whereby the animals tail was positioned slightly above the niveau of the injection site and the blood sinus spaces lying between body musculature and skin were stripped off the blood. After clear reflux from the opened ventricle 10 ml MercoxCL-2B (mixture after Hodde, 1981) were injected with moderate hand pressure so that at the end of the injection curing of the resin started. Casted animals were processed as described elsewhere (Lametschwandtner et al., 1984, 1986). From clean dry casts, pieces of skin (1cm x 1cm to 5cm x 2 cm) were excised from defined body regions (Fig. 1), mounted onto copper foils soldered to Al-stubs using the conductive bridges method (Lametschwandtner et al., 1980), evaporated with carbon and gold, sputtered with gold and examined with a scanning electron microscope Stereoscan 250 (Cambridge Instruments) using 5 kV accelerating voltage. After a first inspection of the epidermal aspects of the skin casts were remounted upside down in order to visualize hypodermal and dermal vascular layers.

Quantitative measurements

Measurements from semithin sections. Semithin sections (1µm thick) of defined skin regions of Myxine fixed by immersion in 2.5 % buffered glutaraldehyde (sodium cacodylate, 0.15 M; pH 7.2), dehydrated in a graded series of ethanol and propylene-oxide and embedded in Epon 812 (Serva) were used. Measurements on skin vessel diameters were made using a calibrated ocular micrometer.

Calculation of percentages of subepidermal capillaries. Camera lucida drawings of SEM-micrographs of casted skin preparations from dorsolateral body regions were analyzed by an image analysis system (Quantimet 720; Reichert-Imanco) and percentages of capillaries per mm² of skin were calculated.

Calculation of vessel diameter, length and surface.

Point-counting method. Scanning electron micrographs of casted skin areas (7 micrographs from dorsolateral skin

Table 1. Diameters of skin vessels (in µm) in Myxine glutinosa L.

layer	subepidermal capillaries	mid-dermal vessels	deep-dermal vessels	subcutis arterioles
number of vessels measured	28	18	6	12
diameter in semithin sections	16.1 ± 0.9*	15.0 ± 1.3	16.0 ± 2.0	24.5 ± 1.8
diameter in casts	22.3 ± 0.9	20.0 ± 0.9	21.0 ± 2.4	31.6 ± 2.1
diameter expansion in casts	28%	25%	24%	22%

* mean +/- standard error of the mean

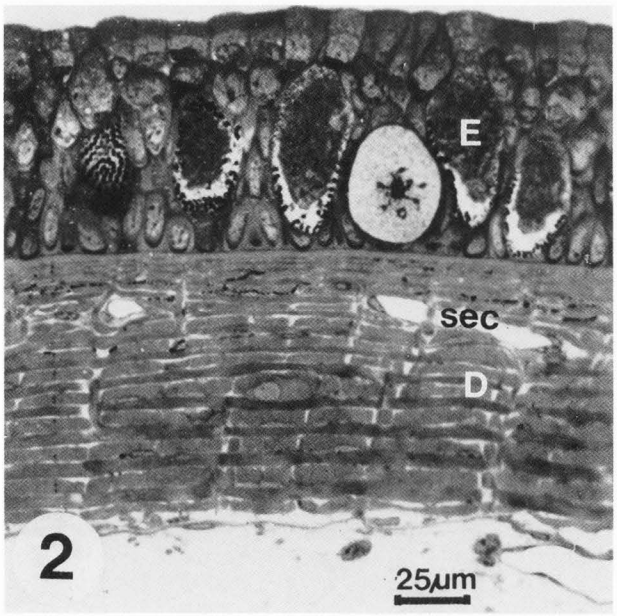


Fig.2. Histology of the skin of Myxine. Transverse section. 7 µm. Azan-staining. D = dermis, E = epidermis, sec = subepidermal capillaries.

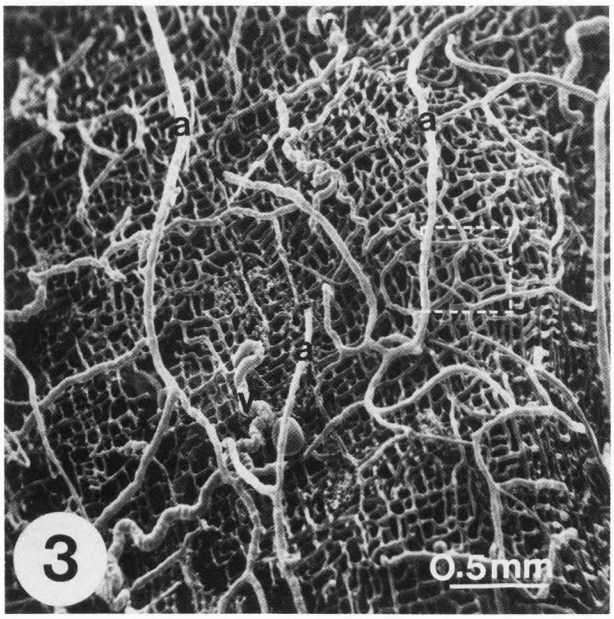


Fig.3. Vascular corrosion cast of the skin of Myxine as seen from the hypodermal side (= hypodermal view). Note supplying arterioles (a) and draining venules (v).

areas corresponding to area 2 of region B; see Fig. 1) were overlaid with a "stereological test grid" consisting of 64 squares, 8 mm x 8 mm each and 25 points for vessel measurements were randomly designed. From vessels the diameter and the average angle enclosed between joining vessels of the meshwork were recorded. Additionally the number of subepidermal capillary meshes per micrograph was counted directly and extrapolated to 1 mm² of skin.

Measurements with a digitizing pad. 160 scanning electron micrographs (size: 11.5 cm x 7.6 cm) revealing a specimen area of 0.48 mm x 0.32 mm were used. From at least 6 completely filled animals vessel lengths in 3 different regions of body skin (A,B and C; for topography see Fig. 1) each of which was subdivided into 5 defined areas (see Fig. 1) were measured using a digitizing pad (Houston Hipad) connected to an Apple IIe microcomputer. In detail, subepidermal vessels were traced with the light-pen along their midline. From the resulting drawings the number of capillary meshes per micrograph (Fig. 13) was counted directly and extrapolated to 1 mm² of skin surface. To count the number of meshes, 9 micrographs randomly selected from each of the 5 areas of the 3 regions (see Fig. 1) were used.

Estimation of vessel volume. Beside mathematical calculation of vessel volume using diameter data from direct measurements from histological sections (see above) and lengths from planimetry from SEM micrographs (see above) vessel volume was calculated by weighing casts. For first experiments two obviously completely filled skin pieces (5 mm x 5 mm), as evidenced by inspection of the specimens under the dissecting microscope were weighted with an analytical balance to micrograms. The volume then was calculated according to the formula

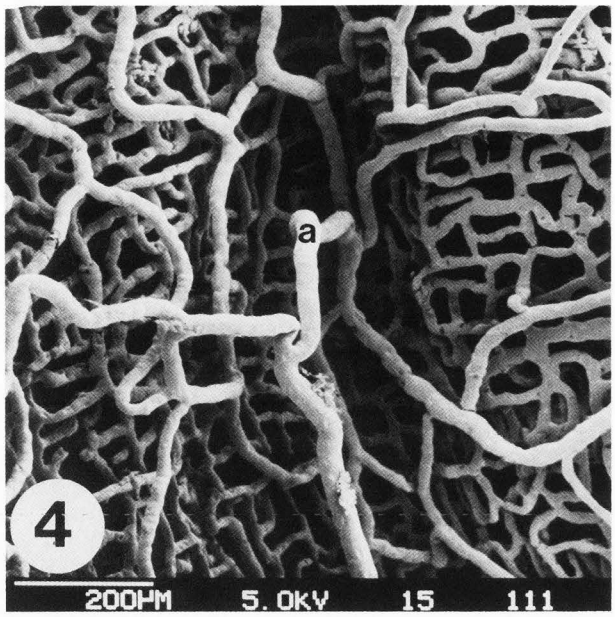
$$\text{volume} = \text{mass}/\text{density}$$

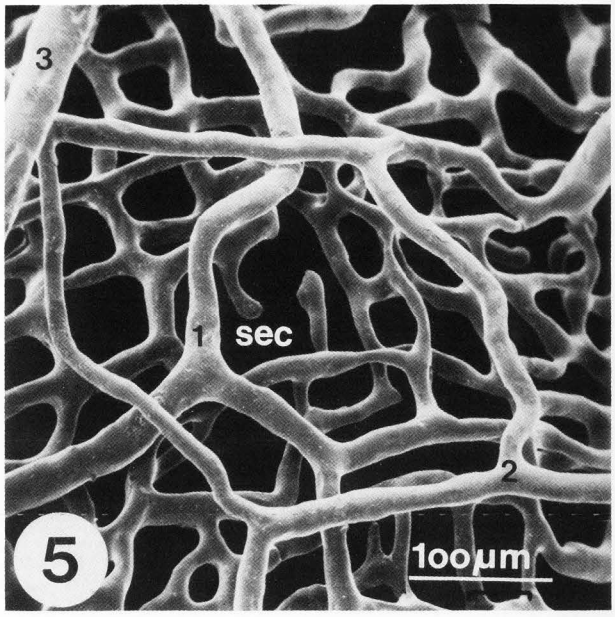
whereby the density equals the specific weight of the casting media used and the mass equals the weight of the cast. From another experiment of ours (Weiger et al., 1986) the density of the casting media used is known to be 1.16 grams per ml of polymerized resin.

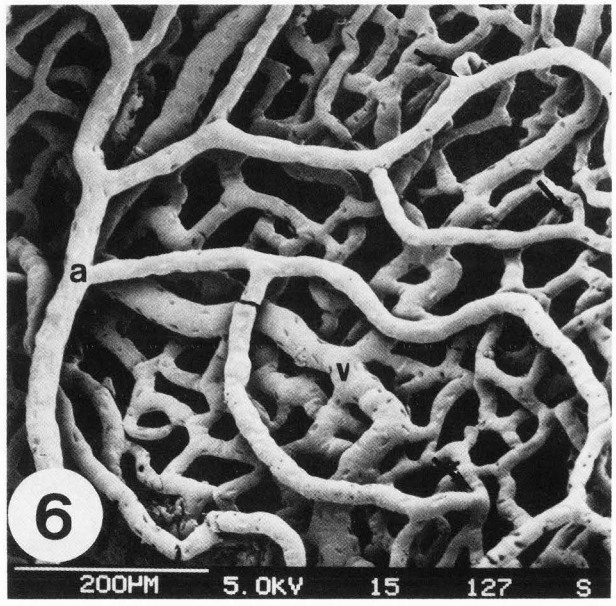
Results

Supply, microangioarchitecture and drainage

The skin of Myxine glutinosa L. consists of a multilayered non keratinized epidermis composed of four different cell types (Fig. 2). This epidermis rests upon a mighty dermis formed by thick bundles of collagen fibers crossing each other in characteristic angles. Depending on the body region a hypodermis of various thicknesses follows which is separated from the underlying body musculature by extensive blood-sinus spaces traversed by vessels and nerves. For further details on skin structure we refer to Blackstad (1963).







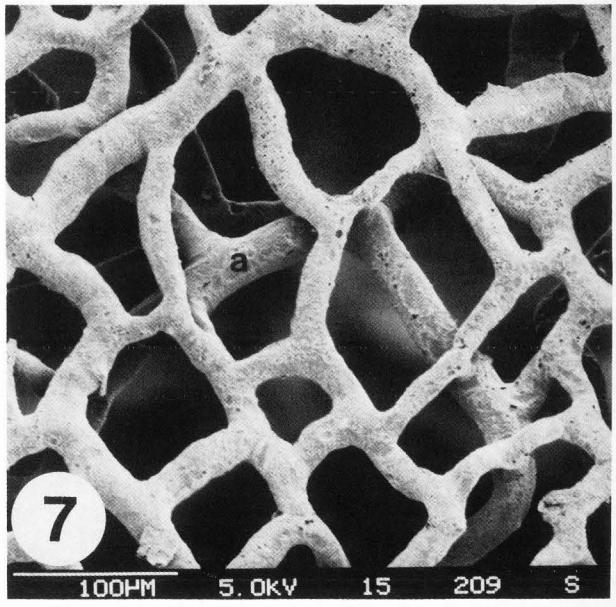


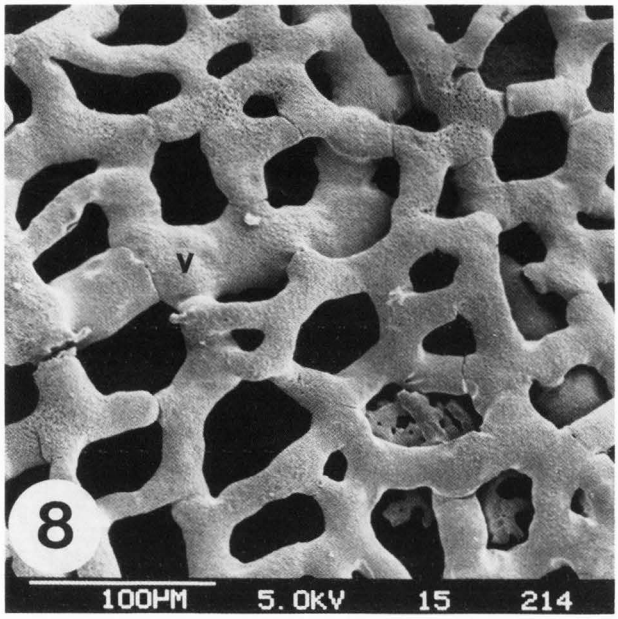
Fig.4. Branching pattern of a supplying arteriole (a) within the border region of two myomers. Hypodermal view.

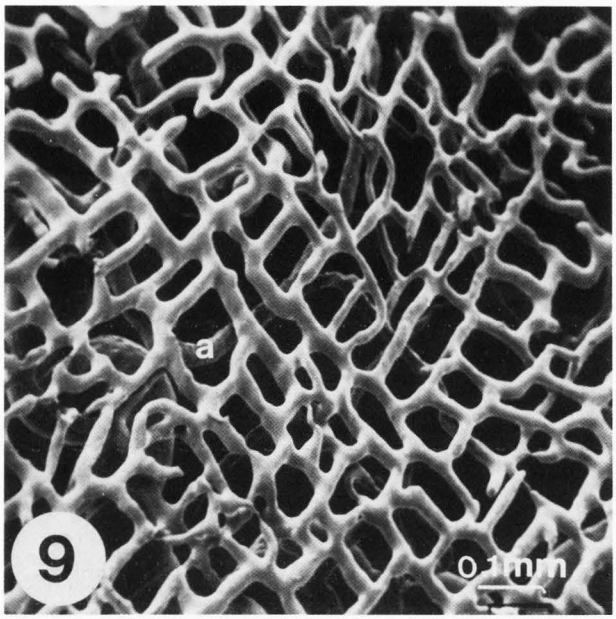
Fig.5. Detail of Fig. 3 (enclosed area). Note the dense subepidermal capillary layer (sec), mid-dermal (1), deep dermal (2) and hypodermal (3) vessels. Hypodermal view.

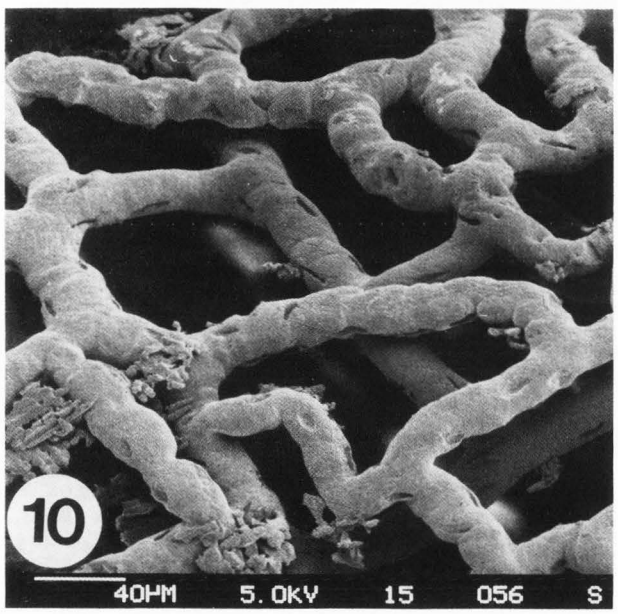
Fig.6. Arrangement of dermal arterioles (a) and venules (v). Note the transition of arterioles into capillaries (arrows). Hypodermal view.

Fig.7. Transition of an arteriole (a) into the subepidermal capillary network as seen from the epidermal side. Epidermal view.

The skin of Myxine glutinosa L. is supplied by ventral branches of the segmental arteries (Fig.3). At the borderline between two myomers these branches traverse the blood sinus space to split off into arterioles in hypodermal and deep dermal layers (Fig. 4). Arterioles either form polygonal meshes (Fig. 5) or run just parallel to give rise to the subepidermal vascular network (Figs. 6 and 7). This network is composed of meshes ranging from almost round to polygonal, rhomboidal, rectangular or square in shape (Figs. 8 and 9). In head and dorsal skin regions only some tendency to a prevalence of a regular







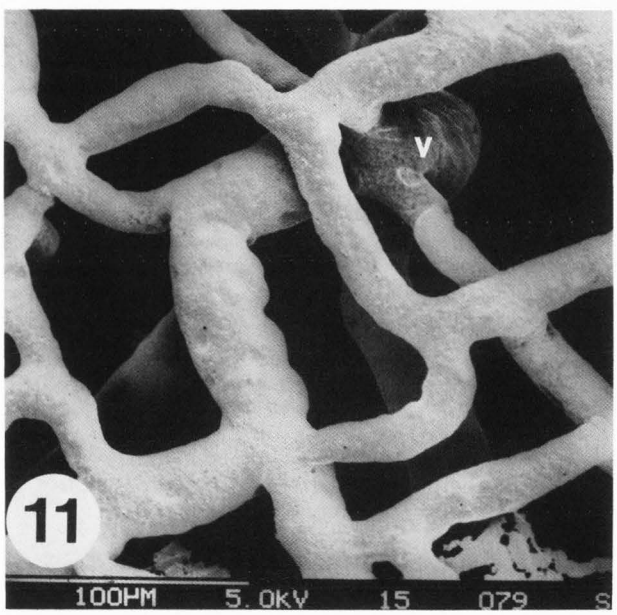


Fig.8. Venule (v) integrated in the subepidermal capillary network consisting of wide sinusoid capillaries. Epidermal view. Note the round to polygonal meshes.

Fig.9. Subepidermal capillary network of the skin of the head region. Note the rhomboid, rectangular and even square meshes. (a) arteriole. Epidermal view.

tetragonal pattern exists (Fig. 9). Capillaries themselves reveal endothelial cell nuclei imprints, slight outpouchings, constrictions and small pits and holes (Fig. 10). While only a few arterioles, which gradually decrease in diameter feed the capillaries of the subepidermal network (Figs. 4, 6 and 7) venules very abruptly drain with also an initially large caliber (Fig. 11).

Fig.10. Subepidermal capillary network. Note endothelial cell nuclei imprints, slight outpouchings and constrictions locally leading to a faint "pearl-string-like" appearance. Epidermal view.

Fig.11. Venule draining the subepidermal network. Note the characteristic round endothelial cell nuclei imprints upon the slightly lobated venule (v). Epidermal view.

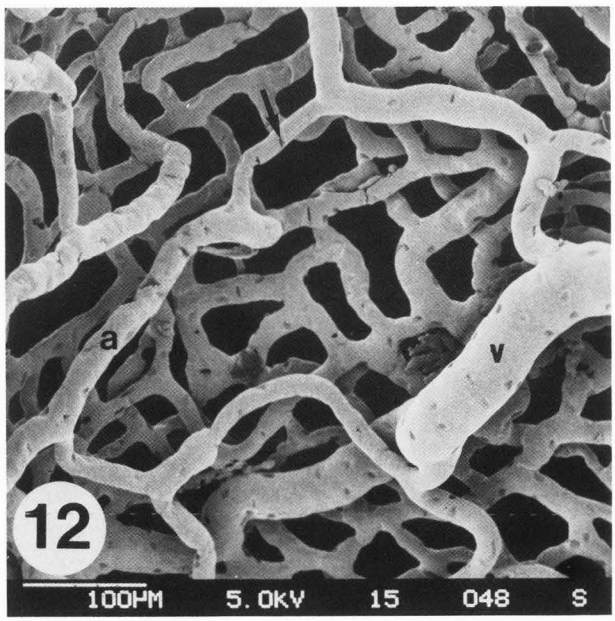


Fig.12. Vascular patterns within dermal areas. Note the rapid transition from arterioles (a) to venules (v) by short capillaries (arrow). Hypodermal view.

Draining venules may integrate over a considerable distance into the subepidermal capillary meshwork or lay close beneath before descending to deeper dermal layers (Fig. 8). Dermal venules and veins finally drain via segmental veins laying between two myomers housing segmental arteries (Fig. 3). In dermal layers the transitional zone from arterioles to venules can be very short (Fig. 12).

Quantitative data

The subepidermal network. Angles between subepidermal vessels forming a rhomboidal meshwork were found to range from 78 to 84 degrees when measured from corrosion casts. Diameters of vessels of the subepidermal network (as well as of mid- and deep dermal layers and in hypodermal areas) are listed in table 1.

By the point-counting method the number of meshes per mm² of dorsolateral skin areas (corresponding to area 2 of region B; see Fig. 1) was found to range from 164 to 185; by planimetry from 186 (region B, area 2) to 248 (region C, area 1). The actual number of capillary meshes per micrograph (area: 0.1536 mm²) is given in Fig. 13. There are no striking differences between the 3 regions nor within the 5 areas within a distinct region. The smaller standard deviation in the number of meshes, however, indicates area B as having the most regular meshwork structure.

The average skin area enclosed by a vascular mesh comprises 1500 to 6000 µm². The percent coverage of a unit subepidermal skin area by the subepidermal network is 34%.

Data from planimetry of corrosion casts reveal an average vessel-length of 32 mm per mm² of skin. The actual lengths of subepidermal capillaries measured per micrograph (area: 0.1536 mm²) within the 3 regions are given in Fig. 14. There are no obvious differences in vessel lengths in the regions and areas studied. Assuming an average diameter of 22.3 µm (see table 1) these vessel lengths have an average surface of 2.24 mm².

Blood volumes

Subepidermal network. Using average length (32 mm) and diameter from casted subepidermal capillaries (22.3 µm) reveals them to have a maximal blood capacity of 12.5 nanoliters (nl).

Whole skin preparations. The average weight of two corrosion casts measuring 5 mm x 5 mm each was found to be 54 micrograms. Since the density of the polymerized resin is 1.16 grams per ml and the shrinkage of the resin during hardening is 17.8 percent (volume) (Weiger et al., 1982, 1986) the weight of the casts reflects only 82.2 percent of the actual blood bearing capacity of the whole skin vasculature. According to the formula

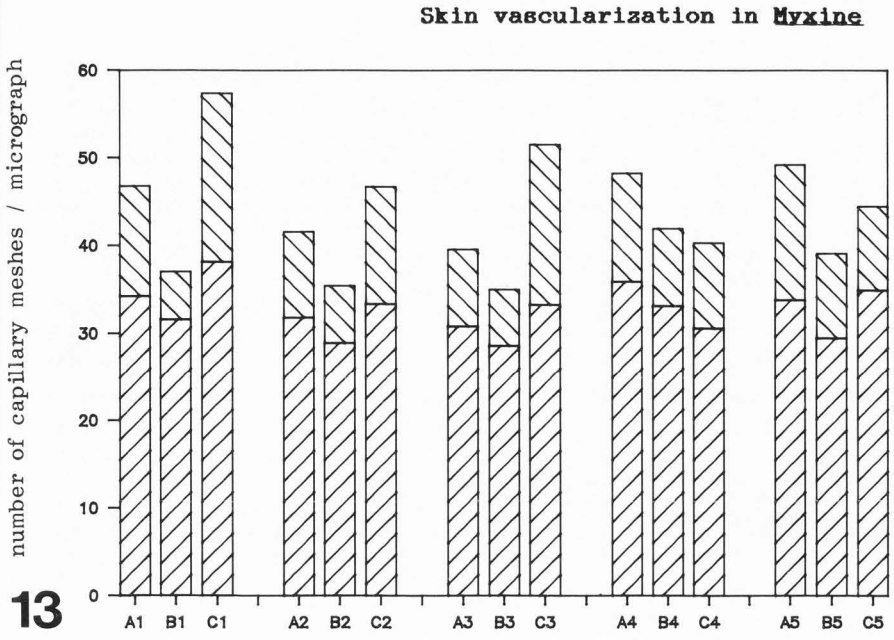
V(cast in mm³) = weight of the cast (5mm x 5mm)/ 25 density

the volume of 1 mm² of casted whole skin (dorsal body region) without shrinkage correction was found to be 1.862 nl, and respectively , 2.265 nl after correction.

Discussion

In lower vertebrates the skin represents an auxiliary organ in respiration. Studies done have shown that in urodela the respiratory capillaries of the skin contribute 36.9 % of the total surface of respiratory capillaries, lungs contribute 62.2% and the palate 0.9% (Czopek, 1955a, b; Czopek and Sawa, 1971; Jasinski and Miodonski, 1978). In anurans skin capillaries represent 23.9% of the total respiratory surface (Jasinski and Miodonski, 1978). In the river lamprey skin capillaries represent 8% of the total respiratory surface (Czopek and Sawa, 1971).

Comparing the vascular patterns of the subepidermal capillary network of amphibians and myxinoids it becomes evident that in amphibians meshes are more irregular and are more variable in size (Jasinski and Miodonski, 1978; Miodonski and Jasinski, 1979) while in river lampreys (Lampetridae) regular tetragonal capillary meshes dominate (Czopek and Sawa, 1971). In Myxine glutinosa L. both patterns coexist with the latter found in head regions.



Figures 13 and 14.

For terms A, B, C and numbers 1-5 on the abscissa see Figure 1.

Mean

Standard deviation

Fig. 13. Mean value of meshes. Number of capillary meshes (per micrograph) of the subepidermal network of Myxine.

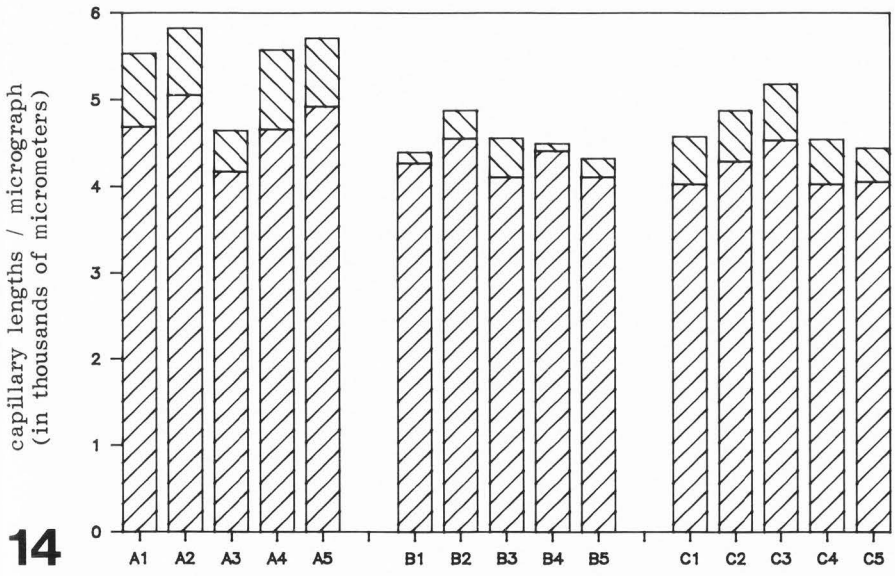


Fig. 14. Mean value of capillary lengths. Total length of subepidermal vessels of the skin of Myxine.

In the river lamprey the regular tetragonal pattern of capillary meshes is explained by the typical crossing-over of subsequent collagen bundle-layers forming the dermis. These layers cross each other at angles between 76 and 85 degrees. In Myxine angles range from 78 to 84 degrees.

The number of capillary meshes found in this study is about the same as reported by Hans and Tabencka (1938) in Myxine.

The diameter of subepidermal capillaries of Myxine as measured from semithin sections (16 $\pm$ 1 µm) equals that of the river lamprey (Czopek and Sawa, 1971). Thus they are about twice as wide as the capillaries in the comparable skin layer of the eel and of other fishes having respiratory capillary diameters of 8 µm (Jakubowski, 1958). In the frog subepidermal capillaries with a diameter of 9.1 µm are reported (Jasinski and Miodonski, 1978).

In Myxine the large capillaries (diameter in corrosion casts: 22.3 $\pm$ 0.9 µm) have to be termed sinusoid capillaries. We do not think that immersion of hagfish in MS 222 (tricaine methanesulfonate) for anesthesia or an inappropiate perfusion or injection pressure have caused vessel with large diameters. We assume them to be real existing structures rather than artifacts caused by the technique used.

Concerning the total length of

subepidermal capillaries Hans and Tabencka (1938) report 34.24 mm per mm² of skin surface. Their data are similar to ours revealing 32 mm. With a capillary length of 32 mm per mm² Myxine lies in the range of the pond loach having 27.49 to 33.28 mm (Jakubowski, 1958). The lengths are, however, above those of the carp having 20.87 to 27.30 mm (Jakubowski, 1960b) and the white amur with 21.91 mm per mm² of skin surface (Jakubowski, 1982). The capillary length found per mm² of skin Myxine is about twice that of the eel having 15.39 to 21.35 mm (Jakubowski, 1960a).

Concerning the calculated subepidermal capillary lengths in the present study, because the blood vessels were traced on micrographs, data obtained should be considered as overestimated. This overestimation increases with an increasing number of meshes per unit skin area and also leads to an overestimation of surface- and volume- data based upon these length measurements. The more than five times higher volumes of subepidermal capillaries per mm² of skin as received from planimetry data in comparison to those of a 1 mm² cast of a whole skin vascular bed cannot be attributed only to this overestimation. It also has to be considered that capillaries not always show a round profile as used for calculation but that they are sometimes rather flat structures (see Fig. 8). Furthermore, inaccuracies in weighing or in dissection of casted skin pieces have to be taken into account.

In the present study for the first time an attempt is made to get an estimation of the blood volume, the whole vascular bed of a unit of a regular structured organ, i.e., 1 mm² of skin of Myxine, can maximally contain. Because only two specimens were used the data themselves have to be considered as very preliminary. Nevertheless, it becomes evident that fully filled corrosion casts can be used as a tool to determine the maximal content of any particular microvascular bed or of ductal systems in general without laborious and time consuming reconstruction techniques. This has become possible because data on density and shrinkage for defined casting media are now available (Weiger et al., 1986).

Acknowledgements

This work was supported by the Stiftungs- und Förderungsgesellschaft der Paris Lodron Universität Salzburg and the Jubiläumsfonds der Oesterreichischen Nationalbank (Project 1868). The authors thank Miss Synnöve Tholo and Mrs. Marianne Fliesser for technical assistance. The excellent photographic work of Mrs. Karin Bernatzky and Mr. Rudolf Hametner is greatly acknowledged.

References

1.Blackstad TW (1963) The skin and the slime glands. In: Brodal A., Fänge R. (Eds.) The biology of Myxine. 195 - 200. Universitetetsforlaget, Oslo.

2.Czopek J (1955a) The vascularization of respiratory surfaces in some Salientia. Zool. Pol. 6, 101 - 134.

3.Czopek J (1955b) Vascularization of respiratory surfaces in Leiopelma hochstetteri Fitzinger and Xenopus laevis Daudin. Acta Anat 25, 346 - 360.

4. Czopek J, Sawa O (1971) The structure and vascularization of the skin and of the gills in the river lamprey (Lampetra fluviatilis L. Acta anat 79, 305 - 320.

5.Hans M, Tabencka Z (1938) Über die Blutgefässe der Haut von Myxine glutinosa L. Bull. Int. Acad. Pol. Sci.Ser. B II Zool. 69 - 77.

6.Hodde KC (1981) Cephalic vascular patterns in the rat. Academic Proefschrift.94p. Rapodi, Amsterdam.

7.Hodde KC, Miodonski A, Bakker C, Veltman WAM (1977) SEM of microcorrosion casts with special attention on arteriovenous differences and application to the rat's cochlea. Scanning Electron Microsc.1977;II: 477 - 484.

8.Hodde KC, Nowell JA (1980) SEM of micro-corrosion casts. Scanning Electron Microsc.1980;II: 88 - 106.

9. Jakubowski M (1958) The structure and vascularization of the skin of the pond-loach (Misgurnus fossilis L.). Acta biol. crac. zool. 1, 113 - 127.

10. Jakubowski M (1960a) The structure and vascularization of the skin of the eel (Anguilla anguilla L.) and the viviparous blenny (Zoarces viviparus L.). Acta biol. crac. zool. 3, 1 - 22.

11. Jakubowski M (1960b) The structure and vascularization of the skin of the leather carp (Cyprinus carpio L.var nuda) and flounder (Pleoronectes flescus luscus Pall). Acta biol. crac. zool. 3, 139 - 162.

12. Jakubowski M. (1982) Size and vascularization of the gill and respiratory surfaces in the white amur, Ctenopharyngodon idella (Val.) (Pisces, Cyprinidae). Acta biol. crac. zool.24, 93 - 96.

13. Jasinski A, Miodonski M (1978) Model of skin vascularization in Rana esculenta L. Scanning electron microscopy of microcorrosion casts. Cell Tiss. Res 191, 539 - 548.

14. Lametschwandtner A, Miodonski A, Simonsberger P (1980) On the prevention of specimen charging in scanning electron microscopy of vascular corrosion casts by attaching conductive bridges. Mikroskopie 36, 270 - 273.

15. Lametschwandtner A, Lametschwandtner U, Weiger T (1984) Scanning electron microscopy of vascular corrosion

casts - technique and applications. Scanning Electron Microsc. 1984;II: 663 - 695.

16. Lametschwandtner A, Lametschwandtner U, Patzner RA (1986) The different vascular patterns of the slime glands in the hagfishes, Myxine glutinosa Linnaeus and Eptatretus stouti Lockington. A scanning electron microscope study of vascular corrosion casts. Acta Zool. 67, 243 - 248.

17. Miodonski A, Jasinski A (1979) Scanning electron microscopy of microcorrosion casts of the vascular bed in the skin of the spotted salamander, Salamandra salamandra L. Cell Tiss. Res. 196, 153 - 162.

18. Murakami T (1971) Application of the scanning electron microscope to the study of the fine distribution of blood vessels. Archvm histol. jap.32, 445 - 454.

19. Spalteholtz W (1914) Über das Durchsichtigmachen von menschlichen und tierischen Präparaten. Leipzig.

20. Weiger T, Lametschwandtner A, Adam H (1982) Methylmethacrylate and Mercox-Cl in scanning electron microscopy of corrosion casts. Mikroskopie 39, 187 - 197.

21. Weiger T, Lametschwandtner A, Stockmayer P (1986) Technical parameters of plastics (Mercox CL-2B and various methylmethacrylates) used in scanning electron microscopy of vascular corrosion casts. Scanning Electron Microsc.1986;I: 243 - 252.

Discussion with Reviewers

C.W. Kirscher: Fig. 9 shows a portion of the subepidermal capillary network in which there appear to be abrupt endings. Again, in figure 5 (and perhaps in figure 3) there appear to be abrupt endings on the arterial side. Is this artifact or real? If real, could you comment on a possible physiological (or biological) consequence?
Authors: We consider these abrupt endings as artifacts caused by incomplete filling.

C.W. Kirscher: Have you wounded these areas and layers to observe the revascularization? If so, is a similar or the same network reconstituted? If not, I feel you have an excellent model to look at the significance of revascularization to restitution of skin function.
Authors: We have done no wounding experiments and so we cannot comment upon skin revascularization in Myxine.

B. Forslind: Is there a risk of dilatation or constriction to diameters larger/smaller than the functional ones during the procedure of vessel preparation?
Authors: Since hagfish anesthetized by immersion in MS 222 and by subcutaneous injection with urethane (2 animals) revealed no significant differences in vessel diameters the mode of anesthesia is not likely to influence vessel diameters. Injection pressure is also very unlikely to change vessel calibers at the capillary level.

B. Forslind: Are there any data to indicate a difference in the morphology of the capillaries on the arterial and the venous side of the capillary bed, i.e., have you any indications that such a change in the vessel diameter would be more conspicuous on the venous side of the capillary network?
Authors: Our micrographs do not show a clear arterial or venous side of the capillaries. There are no distinct endothelial imprint patterns as reported for the arterial and venous side of capillaries in the rat endometrium by Christofferson (1988) (Christofferson R. H.: Angiogenesis as induced by trophoblast and cancer cells. Ph.D. Thesis. pp 83. Uppsala, 1988).

B. Forslind: You have given data to show that there is a relative expansion of the vessels at microcasting compared to thin section data on vessel diameter. Are there any in-vivo (vital microscope) studies made on hagfish (or lamprey or other fish) vessels that would indicate an effect of specimen preparation on vessel diameter (on the arterial vs the venous side). Not being a marine biologist it appears to me that fish gills would be suitable for this kind of studies.
Authors: Specimens used for semithin sectioning were fixed by immersion resulting in decreased diameters as compared to resin perfused vessels in corrosion casts. We consider the latter to be more close to the in-vivo situation then the former. We agree that gills would be a good model to study these question.

A.J. Miodonski: The significant morphologic feature regulating diffusion of the respiratory gases is not the thickness of the skin but the distance between the skin and the blood flowing through it. In amphibians, the skin is thin (10-40μm), but in cyclostomes it is thick and limits cutaneous gas exchange (Czopek J.:Surviving ability in river lamprey (Lampetra fluviatilis L.) under conditions of partial or complete elimination of gill respiration. Bull. Acad. Polon. Sci. Ser.Biol. 18, 237 - 240 (1970)). How thick is the skin of Myxine? Please comment on its cutaneous gas exchange in relation to the cyclostomes taking into consideration other factors. The

cyclostomes have about 1,4 x 10^5 nucleated, spheric erythrocytes per mm^3; each has a volume of 1530 μm^3. The cyclostomes have a total hemoglobin content of 318 grams. Myxine probably has a mixture of hemoglobin of molecular weights from 17 to 34 kdal that have high oxygen affinity and lack the Bohr effect. These parameters may be an adaptation of Myxine to penetrate into the host bodies.
Authors: In the skin areas examined the epidermis is about 75 µm (see Fig. 2) to 100 µm thick. This "thin epidermis" - as compared with the eel, the pond-loach and even the river lamprey (for references see Czopek and Sawa, 1971) - together with the high number of capillary meshes per mm^2 of skin are in favour of a good cutaneous respiration. This together with the parameters you have pointed out enable this hagfish to have its specific mood of behaviour and ecology, i.e., to live at depths around 100 meters below sea level buried almost totally in the mud.

A.J. Miodonski: The distal segments of the arteries and the proximal segments of the veins of frog skin appear to be divided into a mosaic of small patches. Is this found in the skin of Myxine?
Authors: In Myxine the distribution of distal segments of skin arteries and proximal segments of veins is different from that in Rana esculenta described by you. In Myxine distal segments of arteries very closely approach the subepidermal capillary layer before they capillarize (see Fig. 7); proximal segments of veins are even integrated into the subepidermal capillary network (see Figs. 8 and 11). A subdivision of the subepidermal capillary bed into small patches as described by you in Rana esculenta (Jasinski and Miodonski, 1978) and in Salamandra salamandra (Miodonski and Jasinski, 1979) - at least in the skin areas examined - cannot be made.

A.J. Miodonski: Are in your opinion the surface features observed on casted arteries and veins (and capillaries as well), figs.: 5, 6, 11, 12, virtually different (in accordance to their classification proposed by Miodonski A, Hodde KC, Bakker C (1976) Rasterelektronenmikroskopie von Plastik-Korrosions-Präparaten: Morphologische Unterschiede zwischen Arterien und Venen. Beitr. elektronenmikroskop. Direktabb. Oberfl. (BEDO) 9, 435 - 442) or are they not as it was supposed for fishes by Syed Ali?
Authors: In Myxine vascular casts also reveal clear imprints of endothelial cell nuclei the patterns of which are however not as distinct "arterial" or "venous" as those of mammalian blood vessels. To differentiate casted arteries from casted veins with sufficient reliability to the criteria described by you (Hodde et al., 1977; see references) additional information, like general morphological appearance and branching patterns have to be considered too.

A. Castenholz: The high number of blood capillaries and "sinusoidal capillaries" respectively in Myxine appearing in your cast preparations is referred to the respiratory function of the skin capillary system by you. Is there other evidence for such a particular function considering the semithin sections? What exhibit these tissue preparations about the structural differentiation of the capillary wall? Is there a continuous endothelial layer, and are the capillaries furnished with a basement membrane and pericytic cells? These questions cannot be answered from figure 2 due to the small magnification of the figure.
Authors: Subepidermal capillaries of Myxine own a continuous endothelium (0.03 µm thick), are furnished with a basement membrane (with lamina rara and lamina densa) and pericytes (see Georgieva-Hanson V (1980) Ergebnisse einer licht-, raster- und transmissionselektronenmikroskopischen Untersuchung der Haut von Myxine glutinosa L. (Cyclostomata): Cytoarchitektur der Epidermiszellen, Sinnesknospen und sensorische Innervation, Mikrovaskularisation. Thesis. pp 117. University of Salzburg/Austria).

D.E. Schraufnagel: Do not foreshortening errors make angle measurements untrustworthy?
Authors: Not in this study because a flat vascular network was examined and no angles in 3-D were measured.