Scanning Microscopy

Volume 1 | Number 1 Article 19

10-17-1986

Vasospastic Phenomena on the Luminal Replica of Rat Brain Vessels

Enrico D. F. Motti
University of Zürich

Hans-Georg Imhof
University of Zürich

Jesus Martinez Garza
University of Zürich

Gazi M. Yasargil
University of Zürich

Scanning Microscopy, Vol. 1, No. 1, 1987 (Pages 207-222)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0981-7035/87$3.00+.00

VASOSPASTIC PHENOMENA ON THE LUMINAL REPLICA OF RAT BRAIN VESSELS

Enrico D.F. Motti*, Hans-Georg Imhof, Jesus Martinez Garza
and Gazi M.Yasargil

Laboratory of Microsurgery of the Institute of Neurosurgery,
University of Zürich, Rämistrasse 100, CH-8091 Zürich, Switzerland

(Received for publication March 26, 1986, and in revised form October 17, 1986)

Abstract

Strikingly localized ring-like constrictions (other than sphincters, cushions and offshoot furrows) have appeared on the casted vessels of some of our preparations. Morphology of the phenomena reveals diverse degree of active contraction of the vessel wall, ranging from corrugation of the luminal surface to near severance of the acrylic cast. Distribution of the vasospastic phenomena reveals as particularly affected the arteries in the diameter range between 25 and 75 μm, that belong either to intra-arterial anastomoses between the branches of each of the three major cerebral arteries or to their terminal junctions in the border zones (inter-arterial anastomoses). Among the possible causes for the occurrence of the observed vasospasms, we indicate the susceptibility to raised intraluminal pressure during injection, producing contraction of the smooth muscle cell. Reactivity appears heightened in the anastomotic districts of the circulation. The evidence of so-called "plastic strips" clinging to constricted sections of affected vessels prompts re-examination of their proposed origin as "plastic wrapping". Rather, they appear to be remnants of dynamic elements of the vascular wall (smooth muscle cells) that resisted corrosion.

KEY WORDS: Arterial anastomoses, autoregulation, border-zones, brain, corrosion-casting, methylmethacrylate, microcirculation, rat, smooth muscle cells, vasospasm

*Address for correspondence:
Via della Chiusa 8
I-20123 Milano, Italy

Phone No. (0039-2)8376290

Introduction

The history of the study of cerebral microcirculation has largely been the history of techniques of microscopic observation and of the varying interpretations the obtained images offered the investigators. Disagreement was reached, on occasion, on the distinction between arteries and veins (Campbell, 1938). In the course of the even longer history of corrosion casting methods, concerns about the artifactual appearances caused by the casting medium have surfaced from time to time (Dobson, 1970). Currently the introduction of SEM observation of microcorrosion casts, while allowing new understanding of angioarchitecture, has moved interpretative concerns to the world of the smaller. Caution is still required against quick interpretation of appearances and dynamic conclusions based on corrosion casts (Hodde and Nowell, 1980; Lametschwandtner and Lametschwandtner-Albrecht, 1983).

It is then with a good amount of uncertainty that we at first treated the hitherto undescribed phenomenon of widespread constrictions on the luminal replicas of cerebral vessels. We encountered them in one of our specimens in the course of SEM investigation of microcorrosion casts of the brain vascularization in rats. During continuing observations entailing the painstaking search for capillary arterio-venous connections over the whole accessible surfaces of the cerebral hemispheres in a large series of specimens (Motti et al., 1984, 1986a), the microcorrosion casts in 2 other rat brains also showed ring-like constrictions. The scope of the present paper is to describe the morphology and distribution of the original chance finding and offer a tentative explanation for its occurrence in the 3 cases.

Materials and Methods

ZBZ-Cara rats (University Hospital of Zurich breed of white rats) of both sexes were used

weighing an average of 250 g. The rats, anesthetized with intramuscular Hypnorm (combination of fluanisone and phentanylcitrate; Philips-Duphar BV, Amsterdam, Holland (0.1 to 0.2 ml/100g)), were restrained supine on a standard rat operating board. A transverse cervical incision at the level of the clavicle was extended to the sternocleidomastoid muscle bilaterally. Using microsurgical technique, we dissected free a large segment of the common carotid artery (CCA) bilaterally without injury to the accompanying vessels and nerves. The external jugular vein (EJV) was also exposed bilaterally. Threads were placed around each vessel in preparation for the ensuing stages. A proximal ligature was placed around the left CCA with distal application of a temporary clip followed by incision and cannulation with a 19 gauge intravenous catheter (Deseret Co., Sandy, Utah) secured by a ligature. The catheter was prefilled and connected to a 250 ml bottle of wash-out solution (see below) which was allowed to flow on release of the vascular clip. Next, the right CCA was cannulated using a catheter in parallel to the contralateral catheter and connected to the same bottle by means of a three-way junction. Among the three specimens considered in this paper, a three-way "Y" junction was used in rat Y and a three-way disposable stopcock was used for rats X and Z.

After completion of the cannulation procedure, both EJVs were divided. The height of the bottle of wash-out solution was adjusted to about 1 meter above the heart. The rats cephalic vasculature was then washed with ca. 100 ml of a solution having the following composition (Gannon, 1978):

Dextran 70.000.....3%
NaCl.....0.9%
Papaverin HCl.....1.10^{-7} g/ml
Heparin.....10 IU/ml

The solution was prepared by the central pharmacy of the University Hospital of Zurich and filtered in order to eliminate all particles exceeding 0.2 µm. The solution was also warmed to 38°C immediately before use. The infusion of dextran solution was followed by infusion of a small quantity (ca.30 ml) of isotonic saline. Effluent was normally clear at the end of perfusion with only minimal streaks of blood. During the wash-out stage the corrosion-casting compound (slightly modified from the description of Nopanitaya et al., 1979) was prepared in two different mixtures:

1) Batson's N.17
component: A(monomer).....4.17 ml
B(catalyst).....1 ml
C(promoter).....0.08 ml
(D)Sevriton.....4 ml
(De Trey AG, Zürich,Switzerland)
+ a small quantity of Sudan Black B
(C.I.No.26150, Eastman Kodak,Rochester N.Y.)

2) Batson's N.17
component: A(monomer).....4.17 ml
B(catalyst).....1.5 ml
C(promoter).....0.08 ml
(D)Sevriton.....1 ml
+ a small quantity of red colored paste provided with the Batson's N.17 kit
(Polysciences, Inc.,Warrington PA)

The components A+D were mixed with the pigments beforehand and the components B+C were added to each mixture and stirred 60 seconds before injection. The second compound was mixed and injected just prior to completion of the injection of the first compound mixture. Injection of each compound was by hand, and performed (using a 10 ml syringe), applying pulsations (Bradley and Sacks, 1981) and the total quantities injected averaged 6 ml for the first compound and 2 ml for the second. The rationale for using manual injection (Meiselman and Cokelet, 1975) and the two compounds of different viscosity was entirely empirical, as preliminary trials produced the best results with the above procedure.

In each of the three rats considered in this paper, the brain was removed after standing for 2 hrs at room temperature and then placed in 4% formalin. After one month brain Y was cut along two coronal planes respectively through the rostral and caudal portions of the lateral ventricles. The resulting blocks and the separate diencephalon with exposed choroid plexuses, were then placed in a KOH 40% solution to corrode away brain tissue. Brain X and Z were left 10 months in formalin and then placed in the KOH 40% solution. The corrosion solution was frequently changed with distilled water. Each specimen was suspended into a bottle, containing the above fluids and a magnetic stirring bar, taking care a safe distance was present between movement of the bar and the lowermost portion of the cast. The bottle was then placed onto a magnetic stirrer (operated at low speeds so as to avoid turbulence) in order to ensure a continuous delicate stream and tease away the corroded tissue. Once macroscopically complete corrosion of all organic matter was obtained (3 months for brain Y and 2 and 1 months respectively for brains X and Z), the casts were washed in numerous successive distilled water baths and then dried in an oven at 60°C. Following gold-palladium sputter-coating, the casts were observed in a JEOL-25 scanning electron microscope operating at an accelerating voltage of 25 kV.

Results

General

All three specimens were of very good

"casting" quality: full injection of the cerebral vasculature being ascertained by the frequent possibility to follow the entire circulatory route from artery to vein through capillaries. Interruptions in the casts were limited to the largest venous vessels (likely due to the mixing of the casting medium that takes place after crossing the capillary segment of the circulation,in the presence of the flush solution) and have presented an advantage, allowing inspection of the fields underlying the very large venous collecting vessels. The quality of "corrosion" was satisfactory except for amorphous patches of undigested tissue debris clinging mostly to the larger vessels. Uncommon, deep ring-like furrows were visible in the casts of arterial vessels of the pial distribution.

Morphology of constrictions

The ring-like furrows occurred along the course of the arteries and resembled neither wrinkled "bendings" of the cast by external forces as occur in muscle (Garbett and Gibbins, 1980; Potter and Groom, 1983), nor handling of the cast before setting of the resin, nor exposure to excessive temperatures during drying or coating. Neither were offshoot imprints left by "cushion" or "side-branch sphincter" responsible (Legait, 1946; Rhodin, 1967; Kojimahara and Ooneda, 1980; Nakai et al., 1981; Casellas et al., 1982; Jokelainen et al., 1982). Resemblance was closer to some of the vasoconstrictions noted by Schmidt et al. (1983) in contracted spleen.

Morphology of the furrows ranged from the isolated deep "strangulation" groove shown in Fig.1, (the cross sectional area of which was less than a fifth that of the unconstricted lumen a few μm away) to corrugations, with deep nuclear imprints, left by a contiguous series of constrictions that made the surface wavy in a discrete segment of the artery (Phelps and Luft, 1969) (Fig.2). Intermediate aspects included segments of sequential constrictions and bulges mimicking the "sausage effect" (Fig.3). This was recognized *in vivo* (Byrom, 1954; Meyer et al., 1960; Rodda and Denny-Brown, 1966a,b; Auer, 1978) and *in vitro* (Vinall and Simeone, 1982). "Dense packing of nuclear imprints" (Hodde and Veltman, 1979) was also observed in corrugated segments (Figs.2,3). A fine longitudinal endothelial pleating(Greensmith

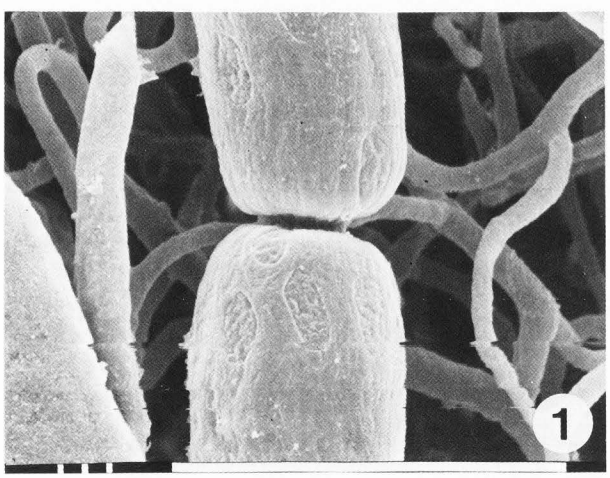


Fig.1. Localized circular constriction on the replica of a pial vessel. Note the imprints of elongated arterial nuclei and the finely pleated surface in the depth of the furrow, suggesting active constriction on part of the dynamic elements of the vessel wall. Bar=100 μm.

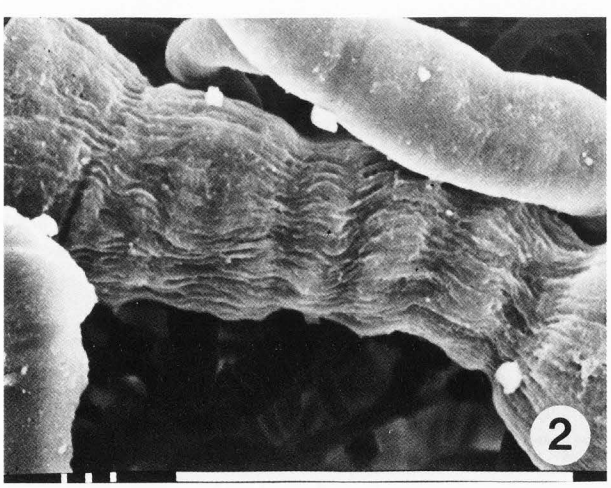


Fig.2. Corrugation of the endothelial surface is evident in this larger diameter pial vessel (≅ 100 μm). Note the localized rows of rod-like deep nuclear imprints. Bar=100 μm.

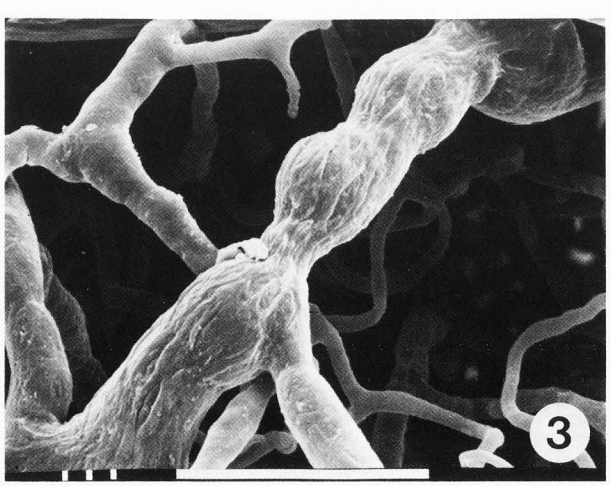


Fig.3. "Sausage effect" ("rosary-like") in a constricted segment of a pial arterial vessel. Crowding of nuclear imprints and a smooth muscle cell remnant are visible. Bar=100 μm.

and Duling, 1984) was present in the groove of most constrictions (Figs.1,4). Hereafter the ring-like furrows and corrugated segments will likewise be referred to either as "constrictions" or as "vasospastic events".

Repeated examples were recorded of smooth muscle cell (SMC) remnants (Rosenbauer and Kegel, 1978), the so-called "plastic strips", or "vascular cuffs" of previous reports (Anderson and Anderson, 1978; Kardon and Kessel, 1979; Hodde and Nowell, 1980; Castenholz et al., 1982), clinging to the constricted site (Figs.5,6).

Constrictions in the pial distribution

SEM investigation has limited access to vessels deeper than a few hundred μm from the surface of intact corrosion casted brains and the overwhelming majority of the vasospastic events we observed are in the pial distribution. A total of 471 sites of constriction (represented by either a single vasospastic event or a "corrugation") have been found in the 3 specimens object of this paper: 242 in brain Y, 213 in brain X, 16 in brain Z (Tab.1).

Constrictions overwhelmingly affected "intra-arterial" and "inter-arterial" anastomoses (Figs.6, 7,8,9), which are found respectively within the

Distribution of the 471 vasospastic sites in arterial vessels of diverse caliber

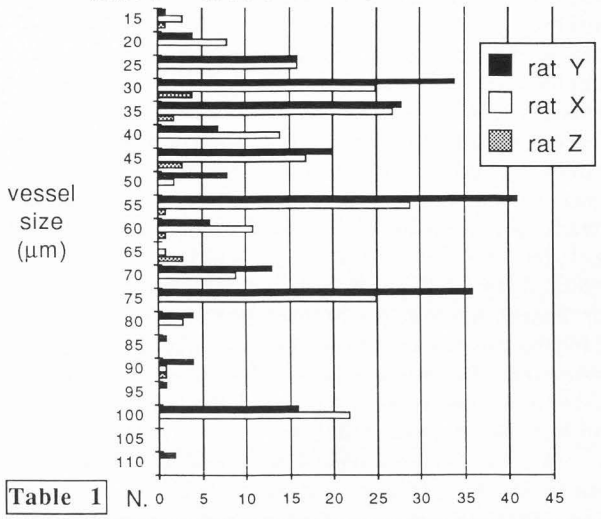
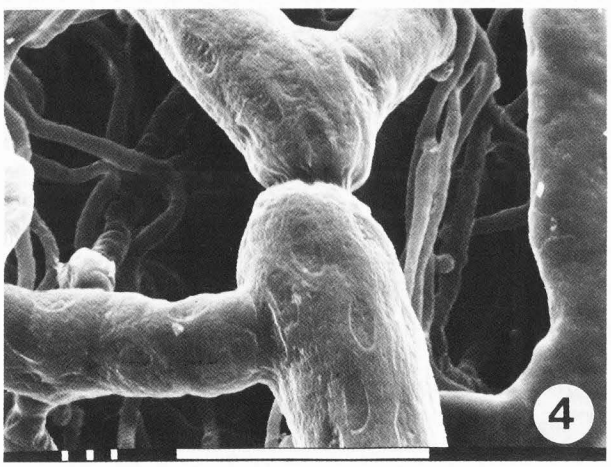


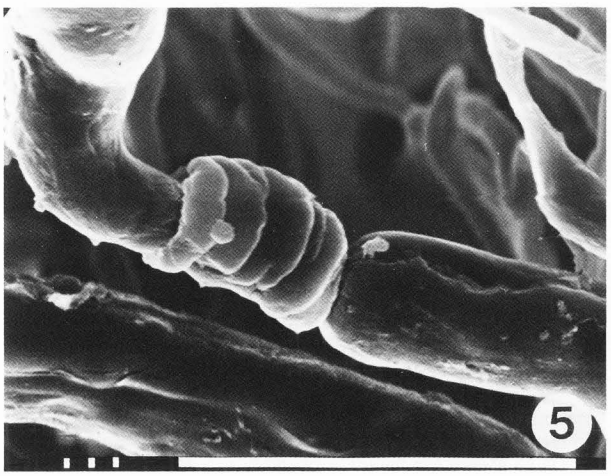
Table 1

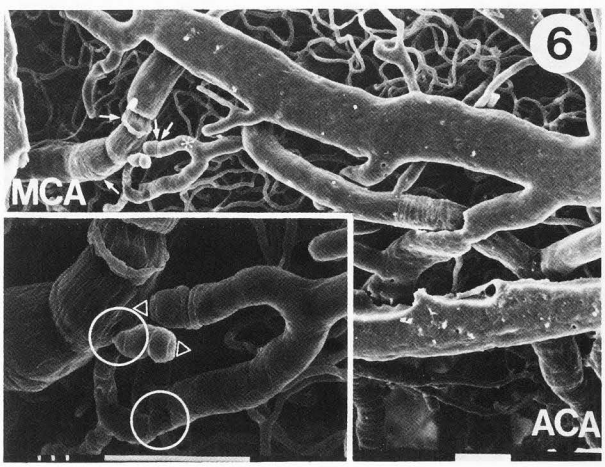
Fig.4. Common example of constriction occurring in the proximity of an arterial bifurcation. Longitudinal endothelial pleating is displayed in the groove. Bar=100 μm.

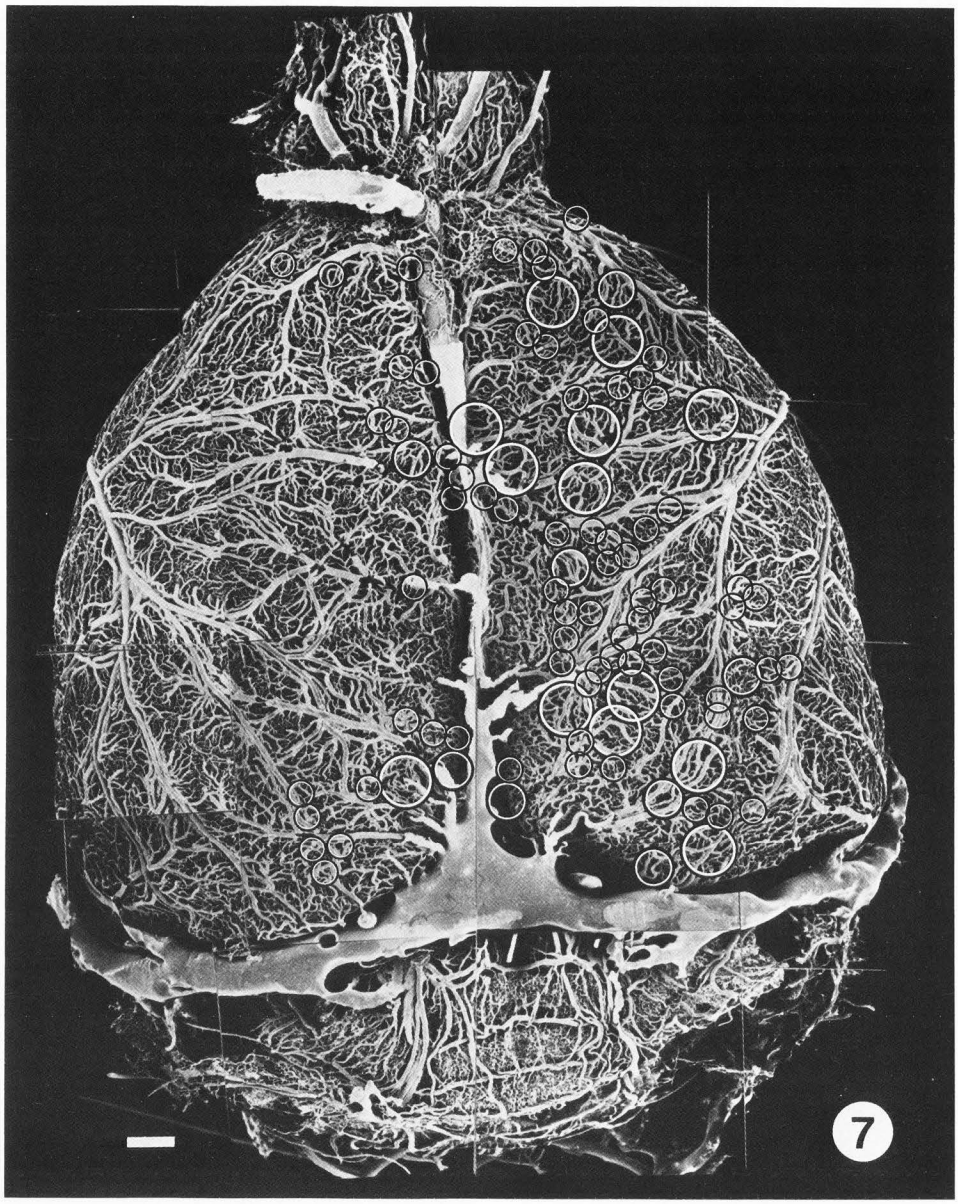
Fig.5. Several smooth muscle cell remnants adhering ring-like to an abrupt constriction along the course of a pial vessel. Bar=100μm.

Fig.6. Cluster of constrictions occurring in a border-zone where interhemispheric Anterior Cerebral Artery (ACA) branches (emerging from the sagittal fissure, below) anastomose with right frontal Middle Cerebral Artery (MCA) branches. The spasm furrow represents a weak locus and the cast of the anastomotic vessel (asterisk) has broken in one site (in the inset: empty arrowheads mark the displaced stumps). Instances of smooth muscle cell remnants adhering to constricted sites are observed (small arrows).Bar=100 μm. In the inset the imprints of side-arm sphincters (circles), which appear exaggerated, are also displayed. Bar=100 μm.









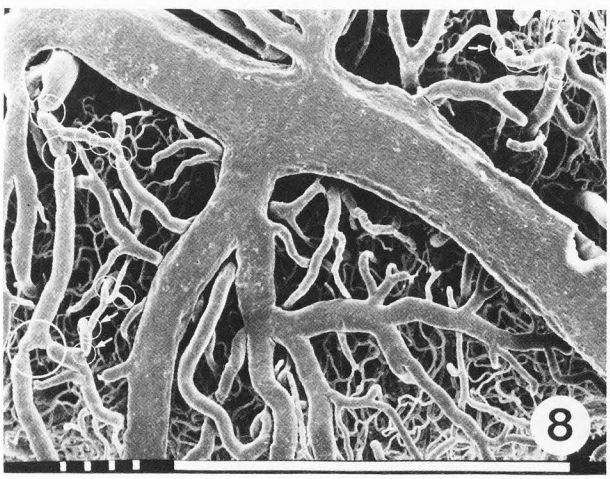
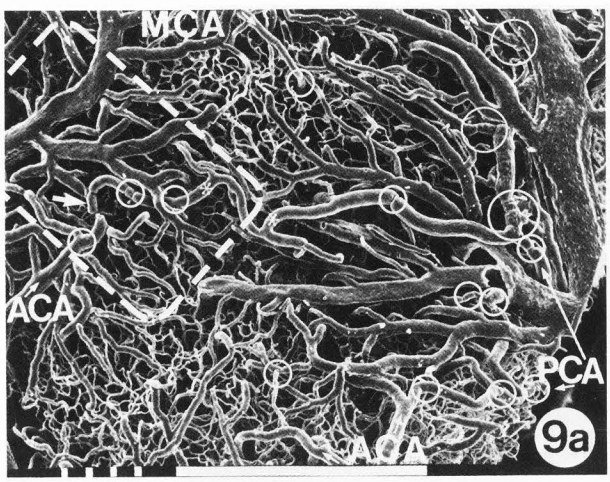


Fig.7. Brain of rat X seen from above. The approximate location of the vasospastic imprints, object of this report, is indicated by circles. Larger circles represent clusters of numerous vasospastic events. See also Table 4. Bar = 1mm.

Fig.8. Numerous constrictions are visible in the arterial surface distribution in this parietal cortex area. Most striking spasms involve bifurcation sites. Large circle indicates constrictions on both downstream limbs of an arterial bifurcation. Smaller circles indicate single instances of vasospasm: most are seen on the intra-arterial anastomotic polygon at left. Three small arrows indicate constrictions at branching points. Bar= 1 mm.



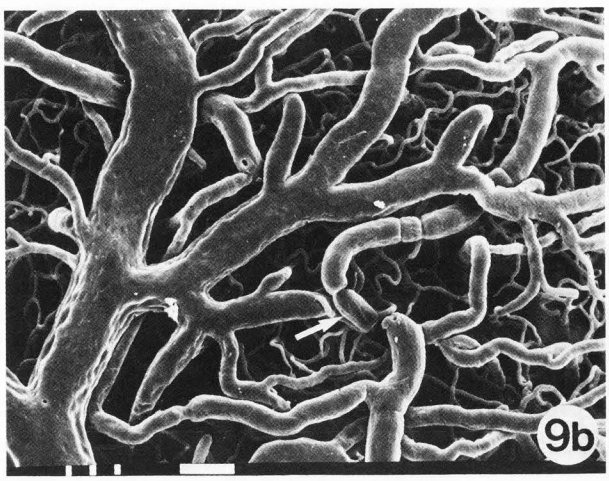
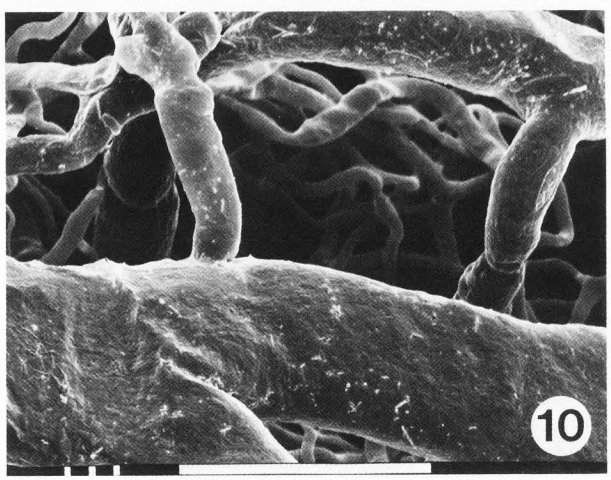


Fig.9a,b. Right parasagittal parietal area showing convergence of branches of Middle Cerebral Artery (MCA), Posterior Cerebral Artery (PCA) and Anterior Cerebral Artery (ACA). The branches of each major artery are indicated by small arrows suggesting direction of flow. Many sites of spasm (circles) are visible along the course of the richly anastomosing arteries. See two "cornuate" terminations (asterisks) mirroring each other in the border zone. The arteries building a quadrilateral anastomotic pattern framed in (9a. Bar=1 mm) and enlarged in (9b. Bar=100 μm), display a cluster of constrictions. Vasospasm on an ACA branch has been so intense as to pinch off a section (larger arrow in 9a,b) of a fully perfused vessel. Vibration, possibly produced by handling, severed this section from its location in continuity with both stumps as recognized in early observations of the site.



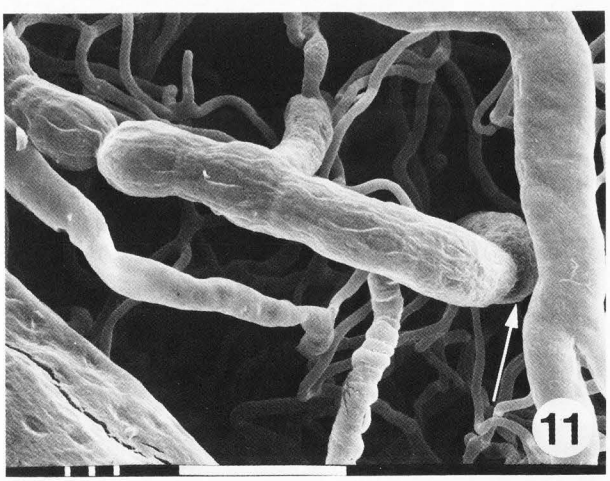


Fig.10. Radial arteries displaying ring constrictions on penetrating the cortex: the diameter of the perforators appears to increase as they course deeper into parenchyma. Bar=100 μm.

Fig.11. A constriction is displayed on a perforator artery just after curving into the cortex: the vessel appears to dilate inside parenchyma (arrow). The surface vessel (with dense nuclear imprints) is not maximally constricted as revealed by another site of spasm along its course. Bar=100 μm.

territory of a major cerebral artery and between peripheral branches of the three major cerebral arteries in the watershed or border zones (Coyle and Jokelainen, 1982). Local special morphological details set the latter areas apart: see the "bicornuate" wide-open bifurcations the arteries frequently show in their terminal end-to-end inosculation (Fig.9a). No preferential relation has been found between location of the spastic events and site of branchings: some were located where we would expect side-arm sphincters to be (Figs.6,8),

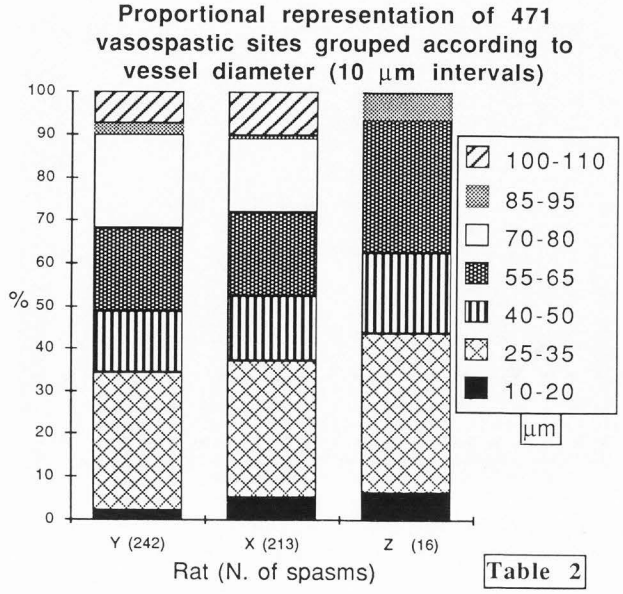


Table 2

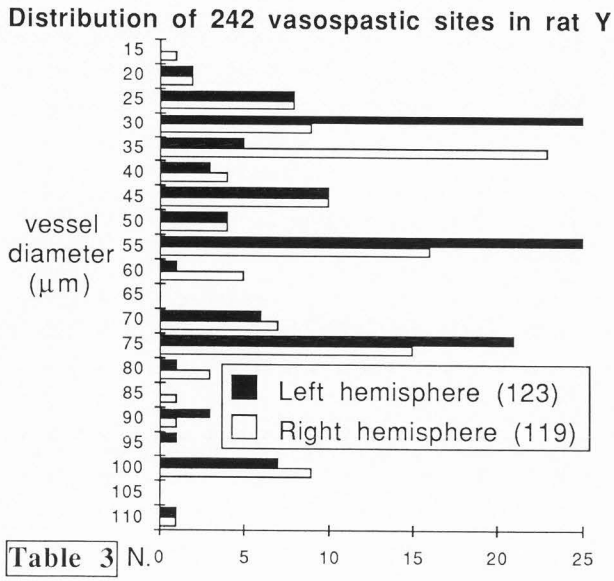


Table 3

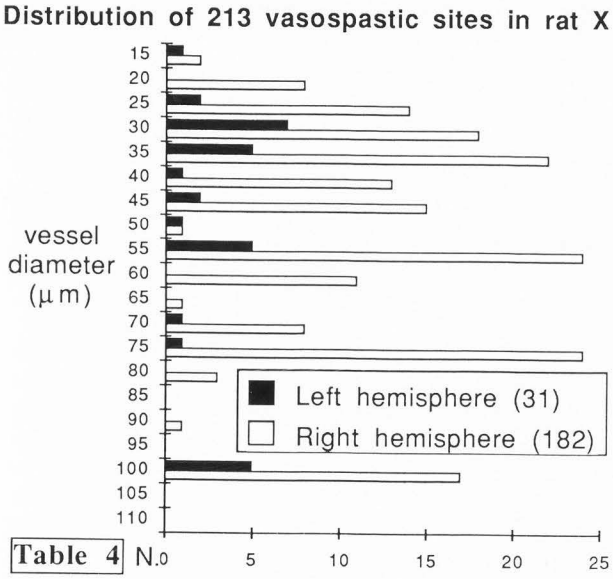


Table 4

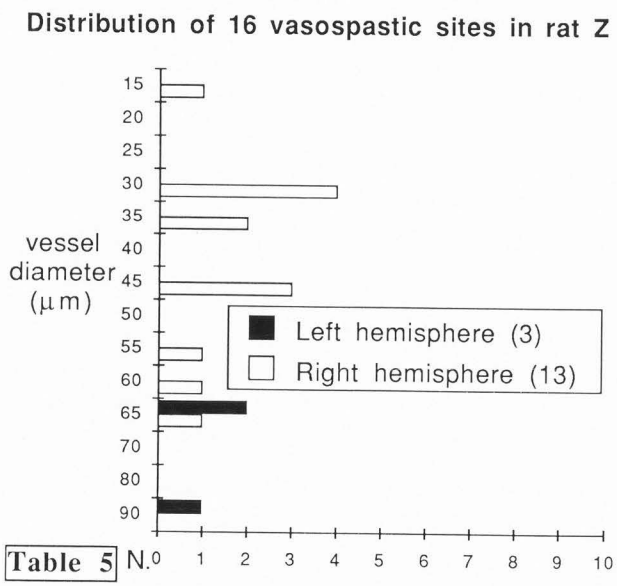


Table 5

most however were removed from the origin of offshoots. Constrictions were never found "on" bifurcations, but commonly immediately upstream on the parent trunk (Fig.4) or on both downstream limbs (large circle in Fig.8).

Most spastic sites, out of a total of 471, were in arteries having diameters (measured in non-constricted segments) of 25 to 35 μm (152) and of 50 to 60 μm (99). Another peak occurrence was observed grouped about the 70-80 μm diameter range (90) (Tables 1,2). The single most represented diameter was 55 μm with 71 occurrences (Tab.1).

In two casted brains (X and Z) the vasospastic events occurred with striking lateralization: the right hemisphere showed 4 times more spasms than the left hemisphere. In brain Y the number of spastic events was practically equal on both sides. Side distribution and size of vessels affected in each instance of spasm is graphically presented in Tables 3,4,5.

Constrictions in non-pial locations

We found circular grooves also on some visible radial arteries after their downward curve into parenchyma, apparently always at the same depth, about 30-40 μm intracortically (Figs.10,11).

Deep groove constrictions and sausage vessels were also represented in the arteries in the thela of the choroid plexus of the lateral ventricles, exposed and observed in rat Y. Concentration of

the vasospastic sites on afferents to the anastomotic limbs of the Ant. and Post. Choroidal arteries and on the bridge anastomoses themselves was striking (Fig.12).

Discussion

Vessel wall diameter regulation is central to overall cerebral circulation and function. Diseases which, through impaired autoregulation or vasoconstriction, disrupt brain function carry particularly grave consequences for affected individuals and society. Vascular narrowing is possibly the foremost alteration produced in cerebral blood vessels by hypertension, among the structural and functional changes that predispose to stroke (Meyer et al., 1985). Microvascular constriction has been reported to occur in diverse models of cerebral ischemia (Waltz and Sundt,1967; Hart et al., 1978) and has also been the suggested mediator of alcohol intoxication (Altura et al., 1983). "Radiographic" vasospasm of the main arterial trunks (thought to be caused by endogenous blood-derived substances) is a feared complication of subarachnoid hemorrhage (Heros et al.,1983) as well as of severe blunt head trauma in 10 to 57% of cases (Wilkins, 1975).

The corrosion-casting method is admittedly fraught with too many uncertainties about the preservation of physiological conditions to offer meaningful functional findings, as pointed out by Lametschwandtner and Lametschwandtner-Albrecht, (1983). Our present findings cannot expect to add substantially to the understanding of microcirculatory physiology. They may, however, suggest new methodological tools aimed at uncovering some of the derangements of lumen diameter regulation and of cerebral circulation suffered in disease. We have tried to understand the mechanisms leading to production of the vasospastic phenomena observed in our specimens and possibly gain in this process some insight in the functional reactivity of rat brain vessels in the agonal stage.

The particular alterations shown in the vessel walls do not appear to be artifactual: trauma (due to manipulation of incompletely polymerized casted specimens) and plastic retraction (on setting) cause distinctly different changes. Vasoconstrictive reactions to various casting media have been cited by Hodde and Nowell (1980). Diverse authors have mentioned casting compounds as the cause of muscle contraction (Garbett and Gibbins, 1980; Potter and Groom, 1983). We are not aware of any reported evidence of acrylates causing vasospasms in microcorrosion casts studied at SEM. A rosary-like deformation, produced by successive bulges and constrictions, has been documented by Duvernoy et al. (1981) in corrosion casts of the human cerebrum. Extreme arteriolar constrictions have been noted by Schmidt et al.(1983) in microcorrosion casts of the spleen, some (as in their Fig.3-c) closely mimic the one in our Fig.1.

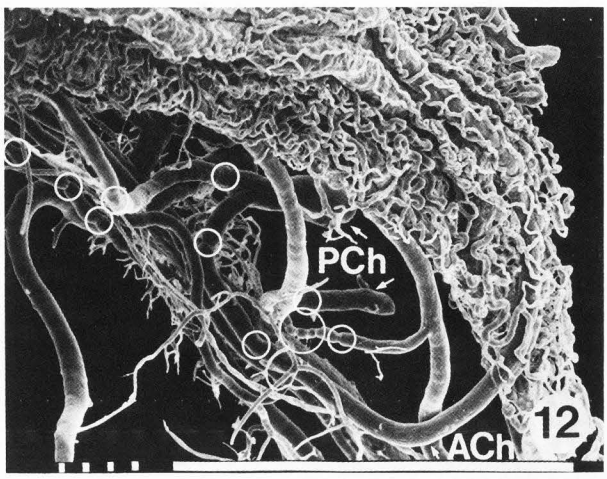


Fig.12. Choroid plexus of the lateral ventricle, rat Y. Numerous constrictions (circles) on the course of anastomotic bridges (and on the arterial branches leading to them) between ramifications of Anterior Choroidal (ACh) and Posterior Choroidal (PCh) arteries. Bar= 1 mm.

In our experimental series only three rat brains out of 80 showed widespread occurrence of vasospasms, thus apparently excluding both eventual pharmacological effects of substances employed and a host of conditions common to all of our experimental animals: narcosis, casting medium, raised intracranial pressure, agonal spasms, etc. The striking lateralization of vasospasm in two of our specimens also does not support any "generalized" cause.

The dynamic constrictive events (that in some cases pinch off the cast (Fig.9b)) cannot conceivably antedate the injection procedure (due to disease or any other cause) since the three rat brains are among our best fully perfused specimens. Time then becomes an important consideration if we think our overall injection procedure (from start of wash-out to early solidification) takes less than 20 min and consider also that vasospasms must set-in after most of the vascular bed is injected and before polymerization has progressed, a likely time span limited to a few minutes.

We have also noted heightened susceptibility of the arterial anastomotic communications and vessels in the 25-75 μm diameter range being most affected. Autoregulation of cerebral vessels (defined as maintenance of adequate cerebral blood flow within a range of changes in perfusion pressure) sustained by an intrinsic miogenic response (Bayliss, 1902; Johnson, 1980), is thought to be

actuated by segments of the vascularization, connected in series, which resist excess pressure by contracting and falling pressure by dilating.

We offer to explain the observed phenomena by suggesting hand-injection of the casting medium raised intraluminal pressure causing critical distention of the vessels which was resisted by equally dramatic contraction. Such extreme myogenic response would affect principally sites where pressure and susceptibility are supposedly maximal as in border zones at "null-points" (Gannushkina et al., 1977). These occur where two concurrent arterial inflows meet with only capillaries to provide outflow. Dinsdale et al.(1971) have drawn attention to extreme vasospasm affecting intra-arterial anastomotic branches following acute hypertension. The heightened susceptibility of border zones has attracted attention in brain-blood-barrier disturbances, focal hyperemia, hemorrhage and small infarcts. Several published studies in different species show the respective border zones (and foremost the parieto-occipital one, in the rat) as a predilection area for the disorder object of the investigation. This is clearly recognized in the illustrations of Robertson et al., (1970) and Johansson (1984) that differ very little from our Fig.7.

The striking concentrations of vasospastic sites also on interarterial anastomoses in the thela of the choroid plexus of the lateral ventricles in rat Y (Fig.12), corroborates the above interpretation.

Increase in the vascular volume of the part being injected has been noted by Sobin (1966) and Rhodin (1973) has indicated that injection pressure produces expansion of the vascular walls which prevents accurate measurement of luminal diameters. Dilatation of cerebral vessels, also during injection, has been reported by Walter et al. (1983) and Hodde et al.(1984) and confirmed in our observations (unpublished) in the course of skull-window experiments (Motti et al., 1983). Vasoconstriction reactive to vasodistention during injection of casting media has been mentioned as a possibility by Hodde and Nowell (1980).

We did not gauge the pressure produced by hand injection during the experiments and we surmise *a posteriori* the gradient of pressure could have been uncommonly steep (just short of producing extravasations) in the three rats considered. Alternatively, heightened physiological susceptibility to intraluminal distention in the three specimens cannot be excluded.

It could also be inferred that occurrence of vasospasm is much more frequent in microcorrosion casts than currently recognized. In fact their presence could arguably be the common underlying cause of both most frequent, if apparently opposite, and puzzling flaws haunting corrosion casting procedures; namely rupture/extravasations (reportedly a frequent occurrence in border zones) and partial filling. Poor quality specimens are eventually discarded before reaching SEM observation. They however do not present favorable observational conditions to allow recognition of fine vasoconstrictive phenomena, due to the opposite conditions of excessive rarefaction and excess of the casting medium. These circumstances are respectively aggravated by fragility and hindrance to corrosion. The pictures in the report by Anderson and Anderson (1978) appear nontheless to confirm this hypothesis, as the casts, derived from poorly injected specimens, show diffuse vasospastic features that abruptly reduce arteries of sizable luminal diameters to a trickle of casting medium.

We further suggest right side lateralization (in two rats) could have been caused by the mentioned alteration of our customary procedure represented by exchange of the Y piece (an effective equalizer of injection pressure between the two carotids) with a three-way stopcock due to temporary unavailability of the former. A simple test, conducted with segments of catheters and resin injection procedure closely reproducing the real experimental setting, has confirmed the three-way stopcock as a poor equalizer of pressure (compounded by the added resistance produced by a longer catheter on the left side). Consistently the straight-port gauge rose much more quickly and recorded pressure values about twice as high as the side-port gauge (Fig.13).

Corroboration of the suggested explanation of reactive myogenic contraction comes from the striking similarity readily apparent when comparing the morphology of our spastic sites with the iconography of diameter alterations of pial vessels that accompanies reports on the cerebrovascular consequences of arterial hypertension. Evidence of muscular constriction according to some authors (Byrom, 1954; Meyer et al., 1960; Rodda and Denny-Brown, 1966a,b; Strandgaard et al., 1976), is interpreted as segmental vasodilation by others (Auer, 1978; Kontos et al., 1981). The issue is still unresolved (Werber and Heistad 1984).

In our material, as well, the alternate viewpoint that the observed phenomena actually represent extensive dilatation of arterial vessels interrupted by localized narrower segments of normal (i.e., inside autoregulative range) radius would be difficult to counter. This is because of the absence of documentation of normal resting diameters of individual arteries previous to the injection procedure.

We already noted that increase in vascular

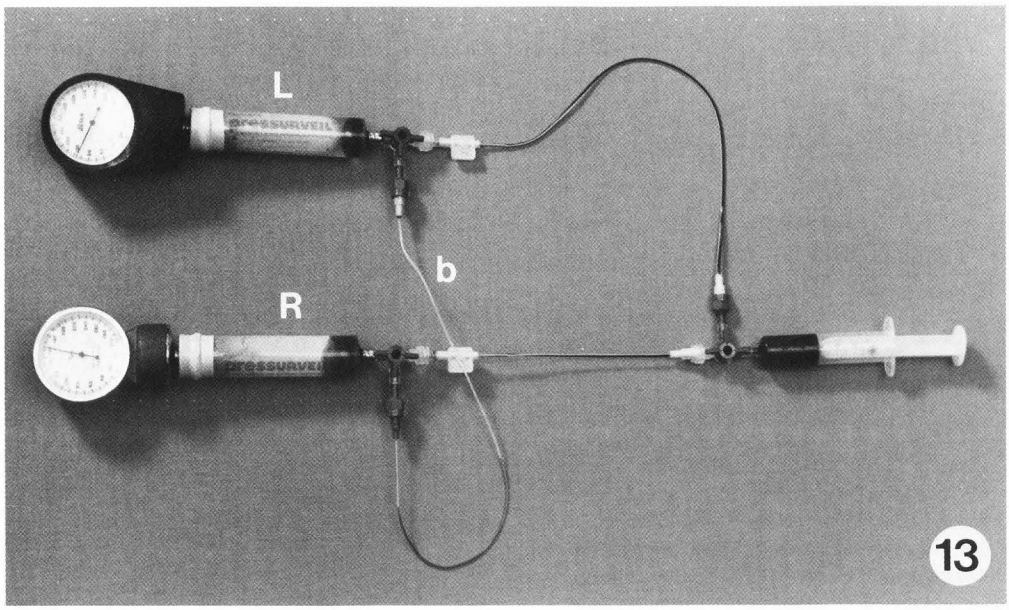


Fig.13. Two intravascular-pressure gauges and disposable pressure transfer units (calibrated in mm Hg. Concept Inc, Clearwater, FLA) have been connected to two catheters fashioned similarly to the ones in the experimental setting and to a three-way stopcock attached to the syringe containing the resin mixture. The catheter bridging the two pressure transfer units (b) was introduced as a measure of simulation of the arterial anastomotic circles at the base of the brain. Right (R) and left (L) side as in the supine rat. On injection much higher readings have consistently been obtained on the right (straight-port) side.

volume and pial vasodilation takes place during casting medium injection. We think vaso-distention in our rats would be only the first causative factor in a chain of events leading to reactive spasms. We note, in fact, that passive distention of the vessels during injection is mostly a transient phenomenon simultaneous with the "systole" of the manually applied pulsations. We also note that outflow of the casting medium through divided veins continues after termination of the injection procedure, due to the resiliency of the stretched vascular walls (among other possible factors) that slowly restores lower vascular volumes. The surface features of the casted vessels in constricted sites support our opinion that we are witnessing extraordinary spastic events. Deep grooves and corrugated surfaces (with nuclear crowding and fine endothelial longitudinal pleating) predominate, as in histological and TEM studies of vessel wall contraction (Phelps and Luft,1969; Greensmith and Duling, 1984). The ordinary smooth endothelial replica (with the well-known nuclear and cell-border imprint patterns) appears on the unconstricted segments, as in supposedly normal vessels of corresponding diameter.

Closer verification of the proposed intrinsic myogenic explanation for the occurrence of the observed phenomena could of course have been obtained had we provided a means of objective pressure monitoring of the injection procedure in the course of our experimental series (to be associated with skull window observation). We however feel justified in pursuing the pulsating-hand-injection technique as it has provided us with casted specimens of optimal quality, in regard to the capillary segment of the circulation, as far as we can judge from published documentation.

The set of observations and propositions discussed above requires a close-knit succession of events that leaves little room for the interpretation of "plastic-strips artifacts" (Fig.5) as anything else but the effectors of constriction themselves, the SMCs. The detailed morphology of SMCs, conclusively documented in SEM of non-corrosion casted vessels (Holley and Fahim, 1983), all but coincides with so-called plastic rings in microcorrosion casting studies.

SMCs are surely able to strangulate, by the amazing force of their contraction, the (still fluid) acrylic mixture as they have been demonstrated to generate 14,000 times their weight in tension (Allen et al., 1976). It is, moreover, puzzling to contemplate the view advanced by Castenholz et al.,(1982) of myocites being annihilated and displaced by the casting resin precisely at this time and place of fierce constriction.

Acknowledgments

The authors would like to thank both Cecile Thalman and Rosemarie Frick for their technical assistance and also Klaus Marquardt for the expert collaboration in the electron microscopy laboratory headed by Dr.S.Geroulanos of the Dept. of Surgery.

E.D.F.Motti worked on this project while on leave from the Institute of Neurosurgery of the University of Milano, Italy. J.Martinez Garza was on leave from the Dept. of Neurosurgery of the University of Monterrey, Nuevo Leon, Mexico.

This study was supported by: the Erziehungsdirektion des Kantons Zürich and the EMDO and Emil Barell foundations.

The experiments have been conducted with authorization of the project in accordance to Swiss animal protection law (Schweizerische Tierschutzgesetz (TschG) und Tierschutzverordnung) within centralized facilities for animal experimentation, staffed and equipped for professional keeping of animals under the control of a supervising commission.

References

Allen GS, Gross CJ, French LA, Chou SN. (1976) Cerebral arterial spasm. Part 5. In vitro contractile activity of vasoactive agents including human cerebrospinal fluid on human basilar and anterior cerebral arteries. J.Neurosurg., 44, 594-600.

Altura BM, Altura BT, Gebrewold A. (1983) Alcohol-induced spasms of cerebral blood vessels: relation to cerebrovascular accidents and sudden death. Science, 220, 331-333.

Anderson BG, Anderson WD. (1978) SEM of microcorrosion casts; intracranial and abdominal microvasculature in domestic animals. Am.J.Anat., 153, 523-536.

Auer LM. (1978) The pathogenesis of hypertensive encephalopathy. Acta Neurochir. Suppl.27, Springer, Wien, NY, 26-57.

Bayliss WM. (1902) On the local reactions of the arterial wall to changes of internal pressure. J.Physiol., 220-231.

Bradley M, Sacks AH. (1981) A technique for casting and fabricating hollow slide models of the microcirculation. Microvasc.Res., 22, 210-218.

Byrom FB. (1954) The pathogenesis of hypertensive encephalopathy and its relation to the malignant phase of hypertension. Lancet, ii, 201-211.

Campbell CAP. (1938) The vascular architecture of the cat's brain, in: The Circulation of the Brain and Spinal cord, Cobb S, Frantz AM, Penfiel W, Riley HA. (eds), Williams&Wilkins,Baltimore, MD., 69-93.

Casellas D, Dupont M, Jover B, Mimran A. (1982) Scanning electron microscopic study of arterial cushions in rats: a novel application of the corrosion replication technique. Anat.Rec., 203,419-428.

Castenholz A, Zölter H, Erhardt H. (1982) Structures imitating myocites and perycites in corrosion casts of terminal blood vessels. A methodical approach to the phenomenon of "plastic strips" in SEM. Mikroskopie (Wien), 39, 95-106.

Coyle P, Jokelainen PT. (1982) Dorsal cerebral arterial collaterals of the rat. Anat.Rec., 203, 397-404.

Dinsdale HB, Robertson DM, Chiang TY, Mukherjee SK. (1971) Hypertensive cerebral microinfarction and cerebrovascular reactivity. Europ.Neurol., 6, 29-33.

Dobson J. (1970) Historical Introduction, in: Anatomical techniques (second edition), Tompsett DH, E.&S. Livingstone, Edinburgh and London, xi - xvi.

Duvernoy HM, Delon S, Vannson JL. (1981) Cortical blood vessels of the human brain. Brain Res.Bull., 7, 519-579.

Gannon BJ. (1978) Vascular casting, in: Principles and techniques of Scanning Electron Microscopy - Biological Applications, Vol.6, Hayat MA (ed.), Van Nostrand Reinhold Co., NY, 170-193.

Gannushkina IV, Galayda TV, Shafranova VP, Rjassina TV. (1977) Further studies of the role of geometry of arterial system of the brain in "breakthrough" of cerebral blood flow autoregulation. Acta Neurol.Scand.(Suppl.64), 56, 392-393.

Garbett PK, Gibbins JR. (1980) A scanning electron microscope study of the microvasculature of the cremaster muscle. Micron, 11, 449-450.

Greensmith JE, Duling BR. (1984) Morphology of the constricted arteriolar wall: physiological implications. Am.J.Physiol., 247, H687-H698.

Hart MN, Sokoll MD, Davis LR, Henriquez E. (1978) Vascular spasm in cat cerebral cortex following ischemia. Stroke, 9, 52-57.

Heros RC, Zervas NT, Varsos V. (1983) Cerebral vasospasm after subarachnoid hemorrhage: an update. Ann.Neurol., 14, 599-608.

Hodde KC, Veltman WAM. (1979) The vascularization of the pineal gland (Epiphysis cerebri) of the rat. Scanning Electron Microsc. 1979;III:369-374.

Hodde KC, Nowell JA. (1980) SEM of microcorrosion

casts. Scanning Electron Microsc.1980;II: 88-106.

Hodde KC, Nordborg C, Johansson BB. (1984) Pial artery casts of normotensive and hypertensive rats. Beitr.Elektronenmikroskop.Direktabb.Oberfl. (BEDO), 17, 209-214.

Holley JA, Fahim MA. (1983) Scanning electron microscopy of mouse muscle microvasculature. Anat.Rec., 205, 109-117.

Johansson BB. (1984) Cerebral vascular bed in hypertension and consequences for the brain. Hypertension, Suppl.III, 6, 81-86.

Johnson PC. (1980) The myogenic response, in: Handbook of Physiology, sect.2,The Cardiovascular System, vol.II, Vascular Smooth Muscle, Bohr DF, Somlyo AP, Sparks Jr HV (eds), Amer.Physiol.Soc., Washington, D.C., 409-442.

Jokelainen PT, Jokelainen GI, Coyle P. (1982) Non-random distribution of rat pial arterial sphincters, in: Cerebral Blood Flow: Effects of Nerves and Neurotransmitters, Heistadt DD, Marcus ML (eds), Elsevier North Holland,Amsterdam, 107-115.

Kardon RH, Kessel RG. (1979) SEM studies on vascular casts of the rat ovary. Scanning Electron Microsc. 1979;III: 743-750.

Kojimahara M, Ooneda G. (1980) Ultrastructural observations on bifurcations in rat cerebral arteries. Virchows Arch.B Cell Path., 34, 21-32.

Kontos HA, Wei EP, Dietrich WD, Navari RM, Povlishock JT, Ghatak NR, Ellis EF, Patterson Jr JL. (1981) Mechanism of cerebral arteriolar abnormalities after acute hypertension. Am.J.Physiol., 240, H511-H527.

Lametschwandtner A, Lametschwandtner-Albrecht U. (1983) Scanning electron microscopy of corrosion casts of the cerebral vessels. Advantages and limitations, in: The Cerebral Veins - An experimental and clinical update, Auer LM, Loew F (eds), Springer-Verlag, Wien, NY, 93-97.

Legait E. (1946) Bourrelets valvulaires et bourrelets sphinctériens au niveau des artères cérébrales chez les Vertébrés. Arch.Biol., 57, 447-458.

Meiselman HJ, Cokelet GR. (1975) Fabrication of hollow vascular replicas using a gallium injection technique. Microvasc.Res., 90, 182-189.

Meyer JS, Waltz AG, Gotoh F. (1960) Pathogenesis of cerebral vasospasm in hypertensive encephalopathy. I- Effects of acute increases in intraluminal blood pressure on pial blood flow. Neurol., 10, 735-744.

Meyer JS, Rogers RL, Mortel KF. (1985) Prospective analysis of long term control of mild hypertension on cerebral blood flow. Stroke, 16, 985-990.

Motti EDF, Imhof H-G, Xian-li Chu. (1983) Calvarial window - description of a one-stage procedure for acute and chronic implants. Surg.Neurol., 19, 80-85.

Motti EDF, Imhof H-G, Marquardt K. (1984) Cerebral microcirculation - Scanning Electron Microscope (SEM) study of luminal casts. Int.J.Microcirc. Clin.Exp. (Abstract), 3, 552 (583).

Motti EDF, Imhof H-G, Yasargil MG. (1986a) The terminal vascular bed in the superficial cortex of the rat - A SEM study of corrosion casts. J.Neurosurg., 65, 834-847.

Nakai K, Imai H, Kamei I, Itakura T, Komari N, Kimura H, Nagai T, Maeda T. (1981) Microangioarchitecture of rat parietal cortex with special reference to vascular "sphincters". Scanning electron microscopic and dark field microscopic study. Stroke, 12, 653-659.

Nopanitaya W, Aghajanian JG, Gray LD. (1979) An improved plastic mixture for corrosion casting of the gastrointestinal microvascular system. Scanning Electron Microsc. 1979;III: 751-755.

Phelps PC, Luft JH. (1969) Electron microscopical study of relaxation and constriction in frog arterioles. Am.J.Anat., 125, 399-428.

Potter RF, Groom AC. (1983) Capillary diameter and geometry in cardiac and skeletal muscle studied by means of corrosion casts. Microvasc.Res., 25, 68-84.

Rhodin JAG. (1967) The ultrastructure of mammalian arterioles and precapillary sphincters. J.Ultrastructure Res., 18, 181-223.

Rhodin JAG. (1973) Dimensions and geometry of the microcirculation: introductory remarks. Microvasc.Res., 5, 313-315.

Robertson DM, Dinsdale HB, Hayashi T, Tu J. (1970) Cerebral lesions in adrenal regeneration hypertension. Am.J.Pathol., 59, 115-131.

Rodda R, Denny-Brown D. (1966a) The cerebral arterioles in experimental hypertension. I- The nature of arteriolar constriction and its effects on the collateral circulation. Am.J.Pathol., 49, 53-76.

Rodda R, Denny-Brown D. (1966b) The cerebral arterioles in experimental hypertension. II- The development of arteriolonecrosis. Am.J.Pathol., 49, 365-381.

Rosenbauer KA, Kegel BH. (1978) Rasterelektronenmikroskopische Technik. Präparationverfahren in Medizin und Biologie. G.Thieme, Stuttgart, 137-138.

Schmidt EE, MacDonald IC, Groom AC. (1983) Luminal morphology of small arterial vessels in the contracted spleen, studied by scanning electron microscopy of microcorrosion casts. Cell Tissue Res. 228, 33-41.

Sobin SS. (1966) Vascular injection methods, in: Methods in Medical Research Vol.11, Rushmer RF(ed), Year Book Medical Publishers, Chicago, IL., 233-238.

Strandgaard S, Jones JV, MacKenzie ET, Graham DI, Farrar JK. (1976) The sausage-string pattern in the pial vessels in acute angiotensine-induced hypertension - vasospasm or vasodilation?. Acta Med.Scand., (Suppl.602), 9-12.

Vinall PE, Simeone FA. (1982) In vitro myogenic autoregulation in cerebral blood vessels, in: Cerebral Blood Flow: Effects of Nerves and Neurotransmitters, Heistad DD, Marcus ML (eds), Elsevier North Holland, Amsterdam, 57-64.

Walter GF, Auer LM, Sayama I. (1983) Morphological analysis of contractile elements in pial and intraparenchymal veins after in vivo perfusion fixation, in: The Cerebral Veins - An experimental and clinical update, Auer LM, Loew F (eds), Springer-Verlag, Wien, NY, 57-63.

Waltz AG, Sundt JM. (1967) The microvasculature and microcirculation of the cerebral cortex after arterial occlusion. Brain, 90, 681-696.

Werber AH,Heistad DD. (1984) Effects of chronic hypertension and sympathetic nerves on the cerebral microvasculature of stroke-prone spontaneously hypertensive rats. Circulat.Res., 55, 286-294.

Wilkins RH. (1975) Intracranial vascular spasm in head injuries, in: Handbook of Clinical Neurology, Vinken PJ, Bruyn GV (eds), Vol.23, Injuries to the Brain and Skull. North Holland, Amsterdam,163-197.

Discussion with Reviewers

S.W.Carmichael: The length of the constriction enveloped by the putative smooth-muscle cells in Fig.5 is about 43 μm. The constricted length in Fig.1 (for example) is much less. Please explain these differences in the length of the constricted area along the cerebral vessels.
Authors: Assume the circular constriction shown in Fig.1 is produced by the "noose" formed by a single "minimal constrictive unit" consisting of either two or more SMCs ringing the vessel lumen. The event that we are witnessing would be dynamically the same in Fig.1 and Fig.5 (and respectively in all other instances similar to Fig.1 and Fig.5) and the difference between them would then consist only in the number of contiguous "ring-units" being recruited in the spastic event.

A.Lametschwandtner: In Fig.5 you show very convincingly the coincidence of vasoconstriction and the presence of "SMC-remnants" at this particular region. In other figures, particularly in Figs.1, 4,6,8,9 and 11, you demonstrate localized circular constrictions without any "SMC-remnants" at the cast. Do you consider these constrictions as being caused by smooth muscle cell(s) and if so, how do you explain the obvious lack of "SMC-remnants" here?
Authors: Corrosion was carried out in our specimens with the intention to remove all tissue. SMCs are not supposed to withstand the corrosion process. Nonetheless patches of uncorroded tissue coating the casts are common and something similar to SMCs has been noted on casted vessels in many studies, mostly in the brain (Castenholz et al., 1982, text ref.). At the site in Fig.5 (and also partly in Fig.6) two seldom occurring phenomena are concomitant: the SMCs that resisted corrosion are apparently those whose contraction produced the circular narrowing (that elsewhere is displayed "bare" on the cast). It is then not the lack of SMC remnants that we should try to explain, but rather their occasional persistence in order to obtain eventually their regular preservation (Dermietzel et al., 1979).

F.N.Low: In the liver certain endothelial cells are so placed that they are able to block or effectively inhibit) portal circulation into a sinusoid and accommodate hepatic artery flow, apparently as a means of aerating the tissue surrounding the sinusoid. In your preparations is there any indication that endothelial cells participate actively in arterial constriction?
Authors: We have no information on the traces left by "independent" dynamic behavior of each vessel wall constituent (e.g., endothelial vs. SM cells). No portion of the vessel wall is regularly preserved by standard corrosion: on occasion we observe SMCs, endothelial nuclei, internal elastic lamina still adhering to the cast. A vessel wall component adhering to the site of a purported dynamic event is an even more rare occurrence. In the rat cortical circulation we noted that a "rough" surface occurred regularly in the immediate precapillary section (Motti et al., 1986a, text ref.) which is commonly thought to be only sparsely equipped with SMCs: it could then be construed as a sign of endothelial reactivity.

A.Lametschwandtner: You name structures which hitherto have been termed "plastic strips" "smooth muscle cell remnants". Have you done a specific investigation on the nature of these structures enabling you to consider these "SMC-remnants" as actually still consisting of muscle

tissue having resisted the crude corrosion procedure because it has been fixed for a long time in formalin or as consisting of resin?

Authors: No, we have not. The investigation that you outline (by X-ray microanalysis possibly following addition of a marker to the casting compound (Gannon, 1978, text ref.)) could be the most direct way to demonstrate the nature of the "plastic strips". We noted SMCs remnants also in casts of brains which were not fixed in formalin.

A.J.Miodonski: What was the rationale to use Hypnorm as an anesthetic instead of sodium pentobarbital which is applied mostly, especially for mammals? Fentanyl citrate exerts rather strong depressive action on the breathing center like morphine does. Fluanisone, on the other side, is a drug which is augmenting the action of hypotensive agents (e.g. Papavarin, used in your washing solution), besides if injected i.v., even accidentally during i.m. administration, it can cause a circulatory collapse and thus exert strong inconvenient and unpredictable action especially on the brain circulation status.

Authors: The anesthesia procedure we describe was based on the practice of experimental surgery extending over many years in our laboratory. The goal to have freely breathing rats remain still for the hours needed to complete microsurgical procedures avoiding as much as possible the need for follow-up administration (with good control of secretions and with quick recovery) was more easily obtained with Hypnorm as compared with other drugs (including sodium pentobarbital). In this novel series of experiments we tried to avoid introducing too many innovations in the surgical set-up. Breathing difficulties and circulatory collapse would become evident in the course of surgical preparation: anyway before the injection of the casting compound. The agonal stage was precipitated by sudden interruption of blood flow to the brain at the time the washing solution (and papaverin) entered the cephalic circulation (overwhelming flow in the vertebral arteries). No safe inference can be made on the cerebral circulation and drug interactions at the agonal stage. Any synergism of drugs and circumstances causing a vascular bed as dilated as possible (in order to obtain better perfused specimens in the smallest ramifications) is anyway desirable as our aim was the investigation of the three-dimensional "architecture" of microvessels.

A.J.Miodonski: There is (in my opinion) a discrepancy between the rather large amount of the washing fluid used (ca.130 ml) against the very small quantity of injected corrosion casting compound (ca.8 ml). Do you think that the washing procedure, even done with dextran-saline solution, could produce a slightly progressive edema? Do you think, based on your experience, that such a small amount of casting compound (only 8 ml) is sufficient for replacing a washing fluid contained within the brain vasculature?

Authors: Wash-out perfusion was continued as long as the reflux became clear (ca.5-10 min). On the contrary injection of the casting compound was limited in time (3-5 min) by progressing polymerization. Both quantities injected (of washing solution and of casting compound) exceed many times the small intracranial volume of the rat, which of course is larger than the intravascular volume of the brain perfused *in situ*. It is also evident how the amounts of injected solutions must be relatively greater than the volume to be filled: mixing effects, distention of the whole vascular bed, losses etc. account for this necessity. The discrepancy between the large amount of wash-out solution vs. the small amounts of resin needed to substitute contents of the brain vasculature can, in our opinion, be explained by considering the different solubilities and viscosities of the fluids concerned. Wash-out solutions dilute blood, casting compounds displace both. Some authors omit altogether a "preperfusion" and inject directly the casting compound (Morrison and Van Buskirk, 1984). We do consider edemigenic the wash-out solutions: isotonic saline much more than the dextran solution that we employed after the first unsatisfactory trials with isotonic saline (Motti et al.,1986b).

Among different possible rating scales we have decided that for our purposes the quality of a specimen can best be assessed in the course of SEM observation by the frequency with which it is possible to follow consistently the arterial-capillary-venous path in different cerebral areas, denoting complete filling of the capillary bed. Some of our best specimens in this regard (Motti et al., 1986a, text ref.)have been obtained with the same reported amounts. As we noted, the large venous sinuses are often insufficiently filled by the amount of resin employed and also the large bridging veins show signs of emulsion of resin with the washing fluids (Fig.7).

A.J.Miodonski: What was the rationale to apply cannulation only to both CCAs? Such approach will cut off, let us say, "more physiological" conditions (by exploiting all afferent vessels, i.e. both internal carotides and both a.a.vertebrales with at the same time controlled artificial respiration for keeping appropriate oxygenation of blood after thoracotomy) for attenuation of eventual over-pressure of fluids used when they are administered via pars ascendens of the aorta

(with closing of the aorta descendens - p.thoracalis as well as both axillares) utilizing a cannula as large as possible, otherwise too much pressure has to be put on the syringe. (See also paper by A.Castenholz, 1983). The uneven distribution of pressure in your model was demonstrated in the test which you performed.

Authors: As your description makes evident, the search for "more physiological" conditions increases surgical complexity. We believe in swift preparation of the animal and quick completion of the whole procedure. The vertebral arteries in the rat have only a small fraction of the CCA diameter. Our surgical approach has insured good filling of the cerebral vasculature allowing investigation of the terminal capillary beds (Motti et al.,1986a text ref., 1986b). Apparently that was not attained in published studies of the rat brain (Miodonski et al.,1976; Nakai et al.,1981 text ref.; Yoshida and Ikuta, 1984) that employed the aorta cannulation approach. We agree to the opinion one should cannulate as close as possible to the target organ (Lametschwandtner et al.,1984). Admittedly the "both CCAs" approach has the inherent defects of being likely inadequate for posterior cerebral circulation studies and of being liable to produce uneven distribution of injection pressures also in the anterior circulation. The latter can be controlled however, and had we not altered the distribution of pressures by a chance change in our (uncomplex) experimental setting, possibly we would neither have noted nor understood the phenomena object of this report.

A.J.Miodonski: Did you try to "regulate" the outflow of a washing solution as well as the casting compound from the exposed venae jugulares? According to my experience such a"regulation" i.e. slowing-down of both fluid and resin outflow from the right atrium (with the vena cava inf. closed) gives better filling of brain vessels. This approach "mimics" somehow the "capacitance properties" of veins with the diameter ca.0.5-2.0 mm (but also small arteries within range of the same caliber). (See also paper by Coyle and Jokelainen (1982, text ref.).

Authors: Restricting venous outflow is an interesting variable to introduce in the carotid / jugular experimental preparation. Any maneuver that engorges the venous system would seem inappropriate for normal injection studies, since venous distention has been implicated in ischemia and arterial spasm (Denny-Brown and Horenstein, 1956; Hart et al., 1978 text ref.). In some cases we delayed the opening of both venae jugulares. We noted no dramatic difference in results, however we did not systematically compare specimens for this variable.

A.J.Miodonski: Do you think that the time of 2 h was long enough for nearly full polymerization of the casting compound, the more that it was done at room temperature only. According to Nopanitaya's procedure, which you adopted, injected specimens were left *in situ* ca.15 to 30 min, then removed and immersed for 1-2 h in hot (80°C!!) water bath (Nopanitaya et al.,1978, 1979 text ref.). What was a rationale for subsequent fixing of the injected brains in 4% formalin? I cannot find any comments about this step of the procedure. If the time of 2 h, during which the injected brains were left standing *in situ* at room temperature, was not sufficient for developing adequate polymerization of the corrosion compound, one can presume that, after placing "polymerized" brains in 4% formalin, further progression of "already existing" vasoconstriction will take place due to the shrinkage of tissues caused by this agent.

Authors: The end-point of polymerization in a cast is difficult to determine (Lametschwandtner et al., 1984). We also used longer "stand-by" times for the casted resin to polymerize. No difference in sturdiness or surface quality has been noted between specimens left standing longer than 2 h and the 2 h only. We avoided the hot water bath as in preliminary series the brain tended to swell massively in it, producing numerous deformations and diffuse breaks in the casted vasculature. The casting medium must definitely be liquid at the time a dynamic event occurs in order to be imprinted by it (as discussed in the text). In our opinion, if 2 h were a time interval insufficient for adequate polymerization of our casting compound, we would then obtain somewhat rubbery casts, not brittle as well cured ones, but nonetheless very much harder than casting medium in its "impressionable" liquid state.

The most common problem in our experimental series of casts of the brain vasculature has been inadequate or very slow corrosion. The best perfused specimens, with apparently complete capillary bed, have been exceedingly difficult to free from cerebrovascular and meningeal tissues. If corrosion was not satisfactory within a few weeks, further treatment (for as long as 6 months to one year) would not affect sensibly the partially corroded "resistant" specimens (mummification). We then decided to store some good-looking specimens pending acquirement of a better corrosion procedure and also of the means and time to follow the concurrent corrosion of many specimens. We resorted to storage in formalin 4% which apparently did no damage to the surface of the casts and actually appeared to quicken the pace of corrosion once it was finally decided 3 months to 1 year later. We attempted to reduce the known shrinkage of tissue produced by formalin by employing it at the

low 4% concentration, although 20% formalin, even injected intravascularly at the time of sacrifice, was not reported to produce either vessel shrinkage or vasospasm (Peerless et al., 1981).

If, in spite of all evidence to the contrary, "already existing" vasoconstricted casted vessels were to shrink further during subsequent exposure to formalin, they would supposedly do so in similar degree as surrounding tissues. Only absolute measurements (diameters, lengths, etc.) would then be affected in the overall specimen, as in common histological technique. Morrison and Van Buskirk (1984) stored enucleated casted eyes of monkeys in 10% formalin, before corrosion, without untoward effects.

F.N.Low: At the risk of further complicating an already complex situation I call attention to the presence of arterial valves in cerebral arteries of the rat (Rosen, 1967). These would certainly affect luminal casts and were interpreted to play a part in maintaining constant intracerebral blood flow. Can you comment on this?

Authors: Morphological studies on the "intimal thickenings" at branching sites are abundant. Arterial "sphincters" have also been recognized by the corrosion casting method in several studies (Hodde et al., 1977; Nakai et al. 1981 text ref.; Casellas et al., 1982 text ref.). In our own observations of the cerebral vasculature we encounter them *in vivo* (employing the operative microscope in the practice of microsurgery and the Ultropak microscope (Wild-Leitz AG, Zürich) in calvarial window experiments (Motti et al., 1983, text ref.)) as well as in corrosion casted specimens. Only corrosion casts, however, enable us to detect a measure of systematic distribution to the extent they regularly occur in cortical precapillary vessels (Motti et al., 1986a, text ref.). Jokelainen et al. (1982, text ref.) found a systematic distribution of sphincters in pial vessels by a $BaSO_4$ injection technique. In the present study sphincters appear to have been affected by constriction in some instances (Figs.6,8). However in most occurrences it could not be assessed whether they actively constricted (and to which extent) or whether they were occasionally made more conspicuous by the respective alterations in the walls of both the vessels they belonged to. This is no secondary topic. As blood flows from a main vessel into a branch a substantial pressure drop in the branch is expected. Vawter et al. (1974) have calculated that a local narrowing accentuates such pressure drop. Very slight variation of the width of constriction would cause dramatic changes in the pressure drop, to the point that occlusion of the sphincter is not required to render flow negligible in a branch.

Additional References

Castenholz A.(1983) Visualization of periendothelial cells on arterioles and capillaries by scanning electron microscopy of ultrasound treated and Plastoid® injected brains in rats. Scanning Electron Microsc. 1983;I: 161-170.

Denny-Brown D, Horenstein S, Fang HCH. (1956) Cerebral infarction produced by venous distention. J.Neuropathol.Exp.Neuropathol., 15, 146-180.

Dermietzel R, Eichner R, Schönenberg I. (1979) The cerebral microvasculature: a reinvestigation by scanning electron microscopy. Microvasc.Res., 17, S 91, (Abstract)

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

Lametschwandtner A, Lametschwandtner U, Weiger T. (1984) Scanning electron microscopy of vascular corrosion casts - technique and applications. Scanning Electron Microsc.1984;II: 663-695.

Miodonski A, Hodde KC, Bakker C.(1976) Rasterelektronenmikroskopie von Plastik-Korrosions-Präparaten: Morphologische Unterschiede zwischen Arterien und Venen. BEDO, 9, 435-442.

Morrison JC, Van Buskirk EM. (1984) Sequential microdissection and scanning electron microscopy of ciliary microvascular castings. Scanning Electron Microsc. 1984;II: 857-865.

Motti EDF, Imhof H-G, Janzer RC, Marquardt K, Yasargil GM. (1986b) The capillary bed in the choroid plexus: a study of luminal casts. Scanning Electron Microsc. 1986;IV: 1501-1513.

Nopanitaya W, Grisham JW, Aghajanian JG,Carson JL. (1978) Intrahepatic microcirculation: SEM study of the terminal distribution of the hepatic artery. Scanning Electron Microsc. 1978;II: 837-842.

Peerless SJ, Ishikawa R, Hunter IG, Peerless MJ. (1981) Protective effect of Fluosol-DA in acute cerebral ischemia. Stroke, 12, 558-563.

Rosen WC. (1967) The morphology of valves in cerebral arteries of the rat. Anat.Rec.,157, 481-488.

Vawter D, Fung YC, Zweifach BW.(1974) Distribution of blood flow and pressure from a microvessel into a branch. Microvasc.Res., 8, 44-52.

Yoshida Y, Ikuta F.(1984) Three-dimensional architecture of cerebral microvessels with a scanning electron microscope: a cerebrovascular casting method for fetal and adult rats. J.Cereb.Blood Flow Metab., 4, 290-296.