Scanning Electron Microscopy

Volume 1986 | Number 4 Article 28

9-5-1986

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Enrico D. F. Motti
Laboratory of Microsurgery of the Institute of Neurosurgery

Hans-Georg Imhof
Laboratory of Microsurgery of the Institute of Neurosurgery

Robert C. Janzer
Institute of Neuropathology

Klaus Marquardt
The University of Zürich

Gazi M. Yasargil
Laboratory of Microsurgery of the Institute of Neurosurgery

SCANNING ELECTRON MICROSCOPY/1986/IV (Pages 1501-1513)
SEM Inc., AMF O'Hare (Chicago), IL 60666-0507 USA

0586-5581/86$1.00+05

THE CAPILLARY BED IN THE CHOROID PLEXUS OF THE LATERAL VENTRICLES: A STUDY OF LUMINAL CASTS

Enrico D.F. Motti*, Hans-Georg Imhof, Robert C. Janzer°, Klaus Marquardt°° and Gazi M. Yasargil

Laboratory of Microsurgery of the Institute of Neurosurgery, °Institute of Neuropathology and °°SEM Laboratory, Dept. of Surgery, of the University of Zürich, Rämistrasse 100, CH-8091 Zürich, Switzerland

(Received for publication March 01, 1986, and in revised form September 05, 1986)

Abstract

Micro-angioarchitecture of the choroid plexus of the lateral ventricles is investigated in microcorrosion casts of animal and human preparations studied with the scanning electron microscope. The capillary bed in the diverse regions of the tissue belongs to one of three patterns: (1)-a network of capillary meshes that envelop the larger arteries and veins predominates in the central segment. (2)-in the villous regions a "leaf-like" organization of sinusoids is found together with (3)-fronds of "glomerular" formations. "Glomeruli" are formed when arterial afferents and venous efferents converge in a quasi hilar structure before branching in arterio-venous loops. Nodular thickenings are observed on glomerular capillaries. The preparations studied (rat, dog, human) are remarkably similar and differ mostly in degree of occurrence of common architectural patterns. Arterio-venous communications are found at the hilus of human glomerular formations.

KEY WORDS: arterio-venous communications, brain, capillary, choroid plexus, corrosion-casting, dog, human, microcirculation, rat, thoroughfare channel

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

Phone No. (0039-2)8376290

Introduction

An object of study since remote times (Dohrmann,1970), the structure and functions of the choroid plexus (CP) have yet to be completely elucidated (Dohrmann,1970; Milhorat,1976; Masuzawa and Sato,1983). The characteristic vascularity to which the CP owes its name has mostly been studied in injected and cleared preparations or by histological techniques, both essentially two-dimensional means of investigation. *In vivo* observation, also a method of study (Putnam and Ask-Upmark,1934; Pogorzelski,1963; Doumoto et al., 1984), cannot usually attain the necessary magnification to discern angioarchitectural detail.

Following the introduction of observation of microcorrosion casts by scanning electron microscopy (SEM) (Nowell et al.,1970; Murakami,1971), the three-dimensional features of CP vascularization have been presented in much greater detail in four recent studies:
in the toad (Lametschwandtner et al.,1978),
in the cat (Miodonski et al.,1979), in the rat (Hodde,1979) and in man (Kessel and Kardon,1979). These reports however did not document the terminal microcirculatory arterio-venous (A-V) path.

In the course of corrosion casting experiments aimed at producing replicas of the brain vasculature for SEM observation (Motti et al.1984; Motti et al.,1986) we obtained excellent casts of the CPs of the lateral ventricles in rat, dog and human preparations which allowed three-dimensional examination of the microvascular segment.

The present study reports on our findings limited to the capillary segment of the CP vascularization wherever it was possible to trace a route from artery through capillaries to vein.

Materials and Methods

Specimens

Four ZBZ-Cara rats (University Hospital of Zurich breed of white rats) of both sexes weighing

an average of 250 g, were used, out of a large series prepared for investigation of microcorrosion casts of the brain vasculature (Motti et al.,1986).

Four mongrel dogs of both sexes weighing between 15 and 20 Kg, and aged 7 months to 1 year, were prepared for corrosion casting after being sacrificed at the completion of pial window observation experiments (Motti et al.,1983).

Three human brains (two males and one female, aged respectively 23, 52 and 67 yrs) were obtained shortly after death precipitated by non-neurological causes. The corrosion casting procedure started within as little as 6 hrs in the youngest subject and within 12 hrs in the others.

Surgical procedure

In the animals we dissected free a large segment of the common carotid artery (CCA) bilaterally using microsurgical technique. The external jugular vein (EJV) was also exposed bilaterally. CCAs were incised and cannulated (with 19 gauge intravenous catheter (Deseret Co.,Sandy,Utah) in rats, standard i.v.-line tubing in dogs). The catheters were prefilled and connected (by means of a three-way junction) to the same bottle of wash-out solution (see below) which was allowed to flow. Both EJVs were divided after completion of the cannulation procedure.

The isolated human brains were floated in a container filled to the brim with isotonic saline and the stumps of the internal carotid arteries and the basilar artery (or the vertebral arteries when preserved) were ligated to disposable Luer locks connected to standard i.v.-line tubing.

Wash-out procedure

The vasculature was then washed with 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 height of the bottle of wash-out solution was adjusted to about 1 meter above the heart of the animal and ca. 100 ml and 500 ml were employed respectively in rats and dogs. 500 ml were also sufficient for the human brains. 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.30ml in rats) of isotonic saline. Effluent was normally clear at the end of perfusion with only minimal streaks of blood.

Resin injection

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:

	RAT	DOG
1) Batson's N.17 component: A(monomer)	4.17	100 ml
B(catalyst)	1	9 ml
C(promoter)	0.08	0.5 ml
(D)Sevriton	4	60 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	100 ml
B(catalyst)	1.5	12 ml
C(promoter)	0.08	0.8 ml
(D)Sevriton	1	48 ml

+ a small quantity of red colored paste provided with the Batson's N.17 kit (Polysciences, Inc.,Warrington PA)

The same amounts employed for *in situ* injection in dogs were used in the human brains (without cephalic vasculature to absorb excess acrylic mixture).

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, using 50 ml syringes (10 ml syringes in rats) and performed applying pulsations (Bradley and Sacks, 1981). The total quantities injected averaged 6 ml for the first compound and 2 ml for the second in rats. In dogs and in human brains all the prepared quantities were injected. The rationale for using the two compounds of different viscosity and the pulsating manual injection was entirely empirical, as preliminary trials produced the best results with the above procedure.

Fixation and corrosion procedures

The brains were removed after standing for 2 hrs at room temperature. Each rat brain and two dog brains were cut along two coronal planes (respectively through the rostral and caudal portions of the lateral ventricles) and the ventricular cavities bilaterally exposed by removing the overlying cerebral convexity. The resulting blocks and, respectively, the other canine and human whole brains were then placed in a KOH 40% solution to corrode away brain tissue. 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 available between movement of the bar and the lowermost portion of the cast. The bottle was

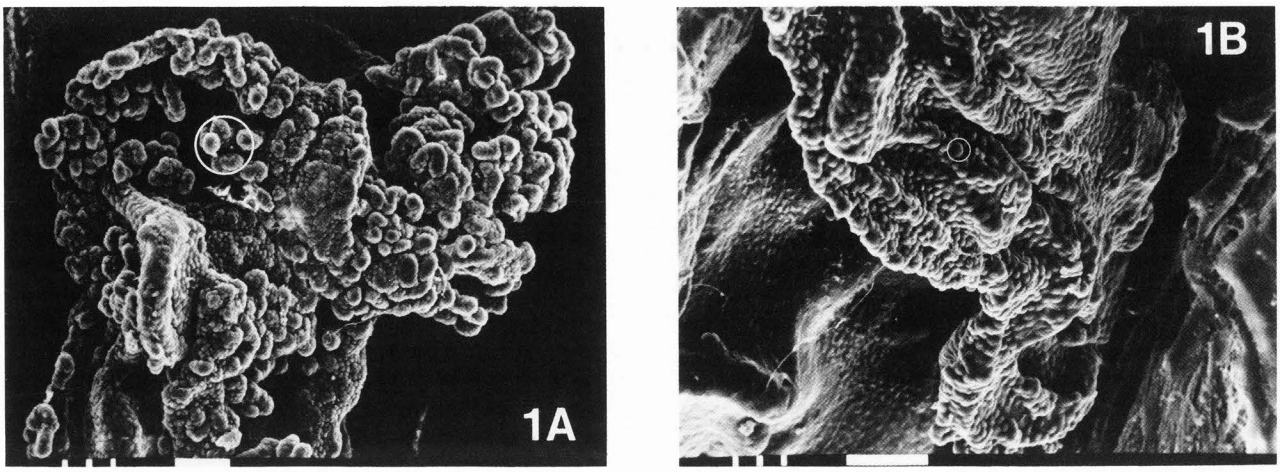


Fig.1. Low magnification views of the surface of the choroid plexus of the lateral ventricle (rat, fixed mat.). (1A) The elaborate infoldings of the villous processes ("fronds") predominate on the free border of the anterior angle. The circle encloses one such efflorescence. Bar=100μm. (1B) Most of the remaining surface ("central segment") displays simple dome protrusions (representing single cells in the cuboidal epithelium) arranged in sheets that rise in folds parallel to the ridges produced by the underlying major vessels. The circle encloses one cellular dome. Bar=100 μm.

then placed onto a magnetic stirrer (operated at low speeds so as to avoid whirls) in order to ensure a continuous delicate stream and tease away the corroded tissue (Motti and Niemeyer,1983). Once complete corrosion of all organic matter was obtained, the casts were washed in numerous successive distilled water baths. The canine and human CPs obtained by whole brain corrosion were carefully dissected free, immersed in distilled water, under the operative microscope. All casts were 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 25kV.

In the specimen in Fig.1 the block with exposed ventricular cavities was fixed in glutaraldehyde 2%, 0.1M cacodylate buffer at pH 7.2, then dehydrated by the critical point method and prepared, as above, for SEM observation.

Results

Practically in all specimens (in this series as in other corrosion casting experiments on the CNS) it was a regular finding to obtain CPs fully perfused by the casting medium (as judged by the observed widespread continuity across the arterial -capillary-venous route) in contrast to the often grossly irregular filling of the cerebro-cortical vessels.

SEM observation of the CP *in situ* shows two types of surfaces: elaborate villous infoldings at the rostral portion (Fig.1-A) and generally at the free edges, that give way to smooth folds over the central segments (Fig.1-B). The two surface types are remarkably similar in the considered species and differ only in degree of spatial representation; villous-fronds being increasingly distributed along the free edges and a wider portion of the central segment (as drawn by Miodonski et al., 1979) as we rise on the phyletic ladder (Hudson and Smith,1952; Millen and Woollam,1953).

Beneath the superficial single-layer cuboidal epithelium, three micro-angioarchitectural patterns can be distinguished:

1- In the smooth central segments a mesh of fine capillaries surrounds all larger vessels evenly (Fig.2-A,B,C,D). The capillaries with diameter ≤10 μm predominate and are mostly linear in morphology, disposed longitudinally to the larger vessels and joined by short bridges. The A-V path in such surfaces is not easily identified and afferents and efferents can be extremely distant one from the other. It is the larger vessels which produce the ridges over which the epithelial folds are formed (Fig.1-B).

2- In the regions of the so-called fronds/ villi the capillary bed raises itself in a kinky pattern of tortuous larger capillaries interspersed with shorter bridges of smaller caliber. Precapillary arterial afferents are here much more frequent, arising almost at regular intervals with a "T" morphology from 20-50 μm arterioles and entering a vein after very short paths 100-400 μm long (Figs.3-A,B; 4-A,B).

We have defined "capillaries" the relatively large vessels (>10 μm) that can be seen to originate from a T-shaped side branch (Motti et al.,

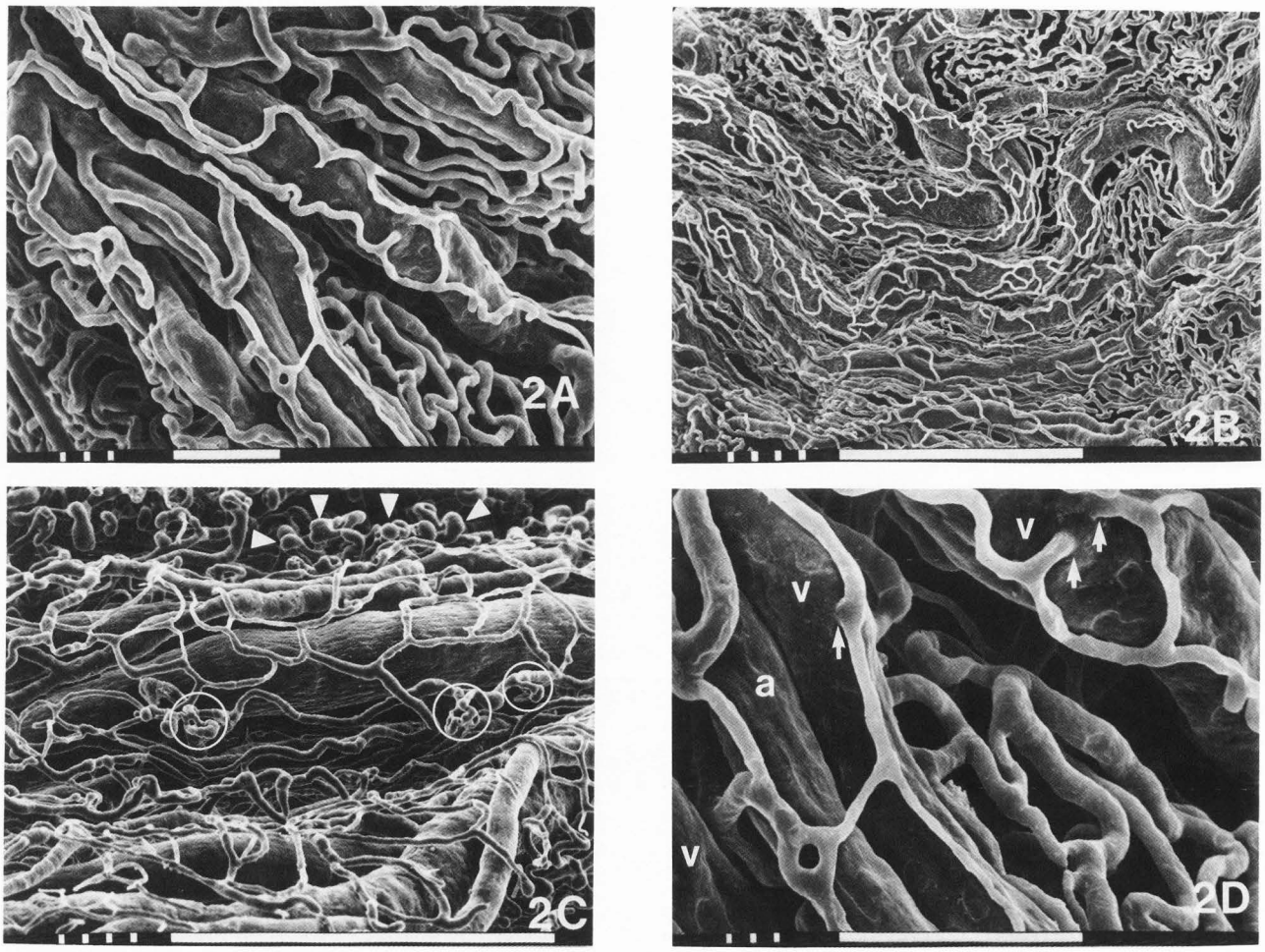


Fig.2. A network of capillary meshes envelops the large vessels of the choroid plexus (CP): (2A) Rat. Bar=100 μm. (2B) Dog. Bar=1 mm. (2C) Human. Bar=100 μm. Small efferents entering a much larger vein are a common occurrence as shown by arrows in (2D): (Enlarged detail of 2A) Nuclear imprints of venous endothelial cells are round-oval and irregularly distributed (v) while imprints of arterial endothelial cells (a) are elongated and parallel to vessel axis. Bar=100 μm. Also visible in (2C): capillaries with "nodular thickenings" (arrowheads) and representatives of "microglomerulus" (circles).

Fig.3-A,B. "Leaf-like formation" in rat CP: a capillary field fed by a "T-form" arterial afferent (T) is drained by many venous efferents in a rather bidimensional distribution. Note the many bridge-capillaries (some indicated by asterisks) and the range of capillary sizes. Bar=100 μm.

1986) of an arteriole and can be followed (sometimes uniting to form a post-capillary venule) to their entrance into a vein. Capillaries also originate from the tapering end of arterioles after they have attained capillary diameter but this is obligatorily a less frequent observation. Some of these capillaries are lying flat to the observer, resembling the description of Maillot and Koritke (1975) and we call them "leaf-like formations" (Figs. 3,4,5).

The leaf-like formations are much more numerous in the human CP where they crowd close together with bubble-like appearance as if out-pouched by accumulation of material in their interior (Fig.5), which is rigorously devoid of vessels.

3- Other capillaries are organized in a three-dimensional structure that stands out with "glomerular" appearance from the background vessels. They are mostly found at the free borders in the regions of the so-called fronds-villi, but may occur anywhere. In these glomerular formations the capillaries are sinusoidally dilated with a diameter of 13-25 μm. Some are very simple, exhausting the A-V route in 400 μm, others are much more elongated. It is also recognizable almost a hilus structure as both vein and artery often come from the same aspect of the vascular network (Figs. 6,7,8,9). On the contrary,

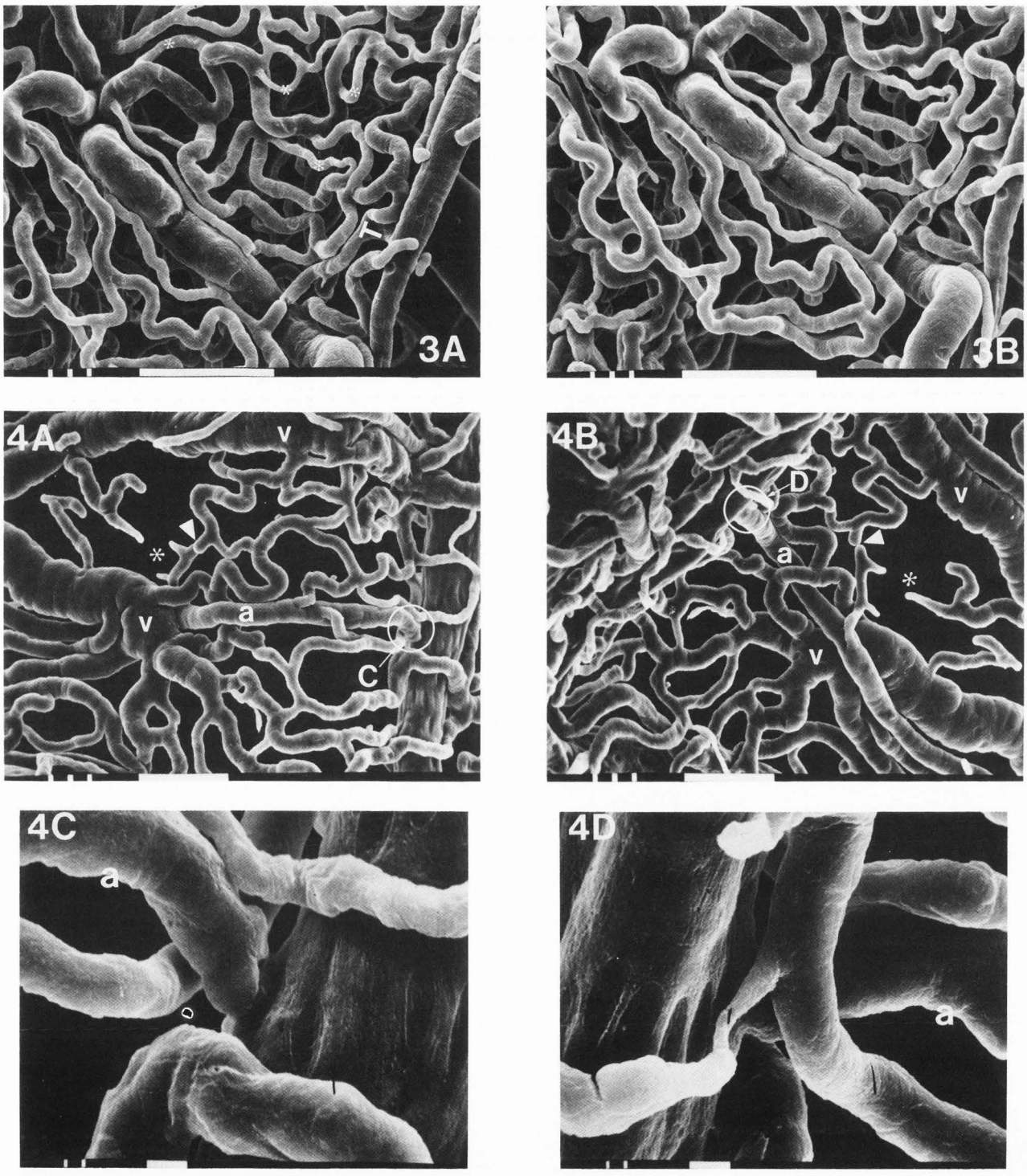


Fig.4. (A,B) "Leaf like" formation in dog CP: artery (a) and veins (v) are seen to contribute to the capillaries from a front and a backside view: an unobscured way of observation that is possible in this grossly bidimensional structure. Note focal filling defects (* and arrowhead) apparently due to a micro-quantity of wash-out solution being trapped between two advancing fronts of the injected resin. Bar=100μm. (C,D) Slightly rotated front and backside views of the area of origin (circle) of the arterial vessel are shown at higher magnification. A constriction is present at the emergence of the side branch (a) from the larger arterial trunk. Bar=10 μm.

"leaf-like formations" anastomose more freely with surrounding fields.

Both in "leaf-like" and "glomerular" formations short and small (5-10 μm) bridging capillaries are seen to enter perpendicularly much larger capillaries and veins (Figs.3,6,7,8,9). On large capillaries there are instances of a "threaded surface" as if it were the imprint of an irregularly spiraling ligature on the outside; this is shown mostly on capillaries closer to the arterial feeder (Figs. 7-B, 8-C).

Practically limited to the larger capillaries in glomerular formations is the occurrence of luminal dilatations (defined "bulgings of the lumen" by Putnam and Ask-Upmark (1934), "moniliforme" by Maillot and Koritke (1975) and "nodular thickenings" by Miodonski et al. (1979)) mostly at the bends of convolutions (Figs. 6-B, 7-B, 8-B, 9). On occasion, the dilatations demonstrate also a distinct ovoid area surrounded by a luminal imprint (Fig.8-C) of the type usually associated with cell borders. The nodular thickenings are noticeable in the rat (Fig.6-B), well developed in

Fig.7. Free border of the dog CP. (7A) a large glomerular formation is visible (arrows). Bar=1 mm. (7B) close-up view of the glomerular formation. Large venous efferents (V) drain the glomerular capillaries. Dilatation of the lumen (nodular thickening) is obvious at the bends of capillary loops (circles). A spiraling thread (arrows) imprints most of the capillary replica. (*)mark some bridging capillaries. Bar=100 μm. →

Fig.8. In a small glomerular formation (dog) it is possible to follow the arterial-venous path: (8A) an artery (a) and a vein (v) coming from opposite directions with sparse branchings along their terminal course, curve together into a hilar structure before ramificating extensively. Bar=100 μm. (8B)(Enlarged detail of 8A) Hilar area (h). The artery (a) gives off "T-forms" to feed the tortuous capillaries that show nodular thickenings (circles). Bar=100 μm. (8C) "T-forms" are marked by (T). Irregularly threaded constrictions (empty arrows) are more common on the post-arteriolar capillaries. Ovoid imprints are indicated by circles. (*)marks a bridging capillary. Bar=100 μm.

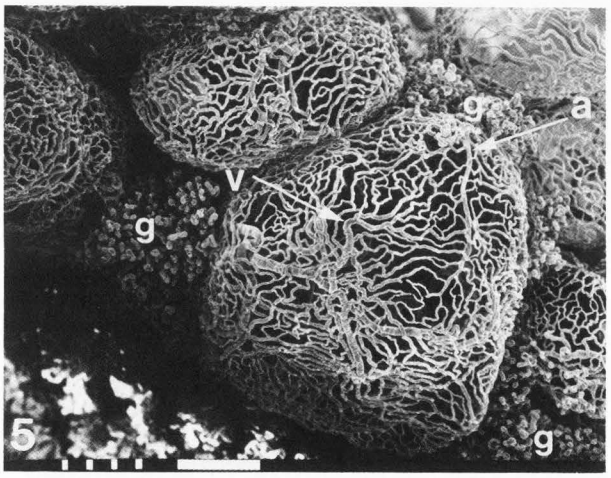
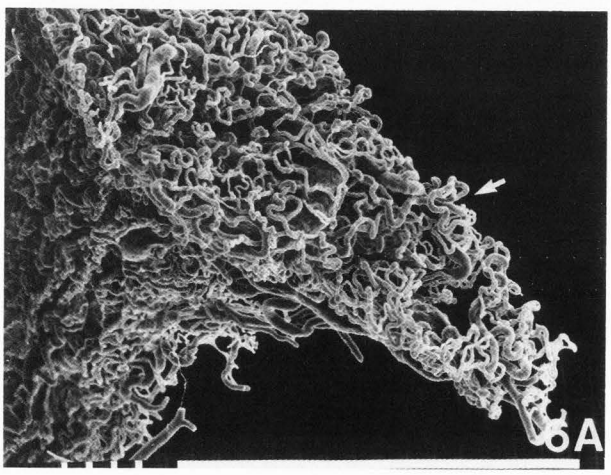
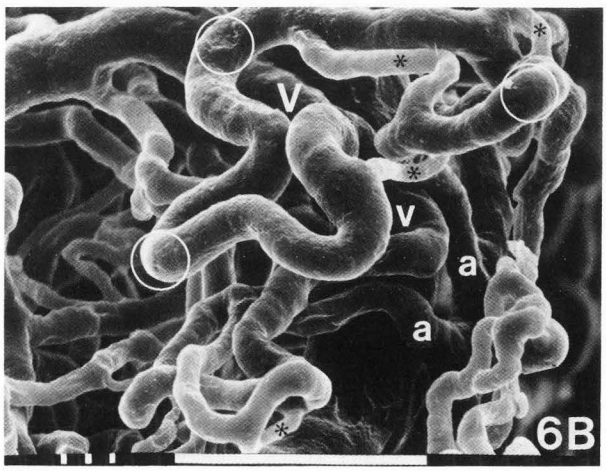
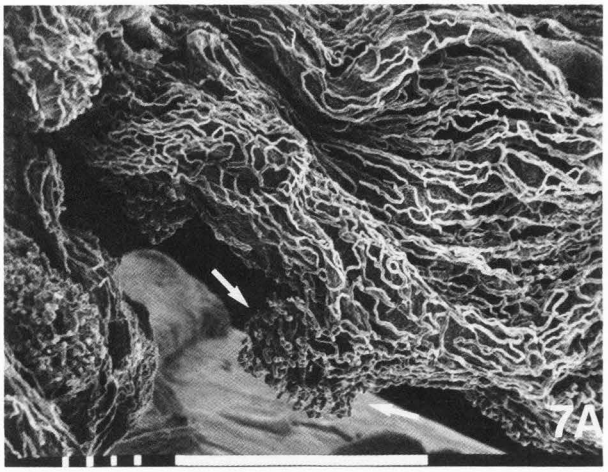


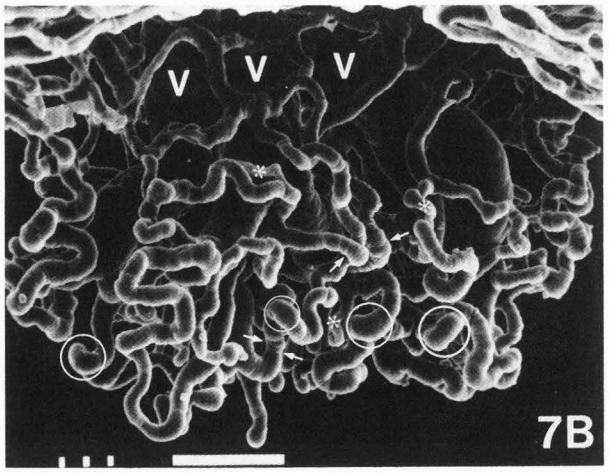
Fig.5. The "leaf-like" structure assumes a "bubble" morphology in the human CP with the distended outpouchings crowding close to one another. (a) artery and (v) vein. Clusters of convoluted capillaries with "nodular thickenings" (glomerular formation, g) crop up in the resulting clefts. Bar= 1 mm.

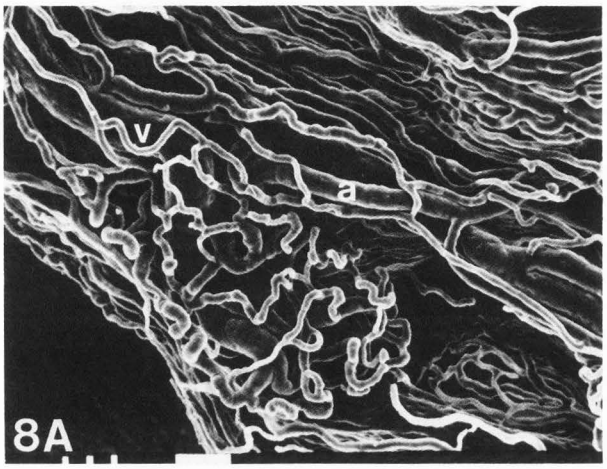
Fig.6. Free border of the anterior angle of the rat CP. (6A) A glomerular formation is visible (arrow). Bar= 1 mm. (6B) In the rotated close-up view two arterial feeders (a) are seen entering the capillary loops that re-unite in a single venous collector (V). The vessels marked by lowercase letters come together into a quasi-hilar arrangement. The large capillary shows slight focal dilatations (circles). (*)mark bridging capillaries. Bar=100 μm.

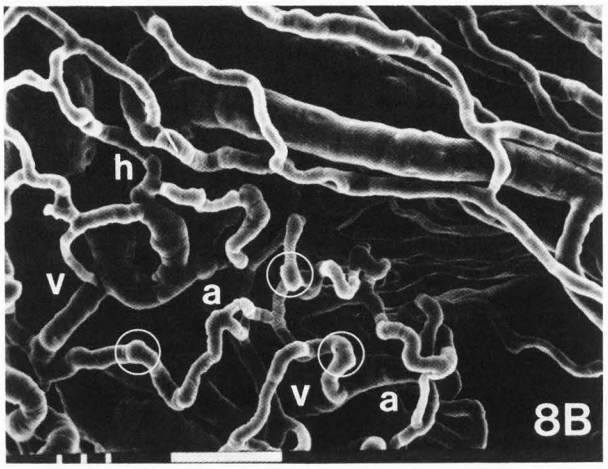


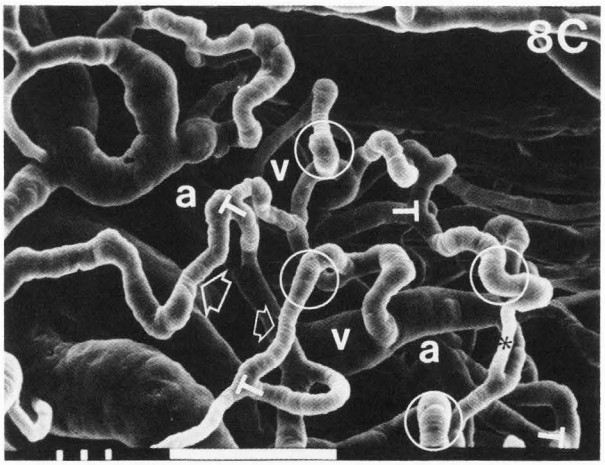












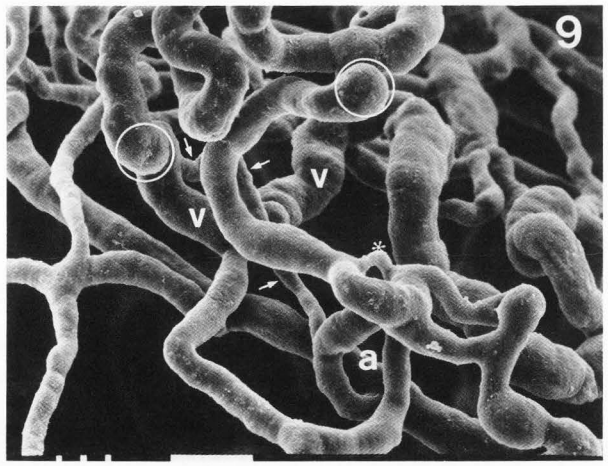


Fig.9. A small glomerular formation (man) shows afferent and efferent vessels turning upwards together from the CP surface and allows with its simpler structure to follow the arterial-venous (a)(v) path. A short communication (arrows) bypasses the convolutions suggesting an operant device (e.g. thoroughfare channel). Dilatation of the lumen (nodular thickening) is obvious at the bends of capillary loops (circles). The asterisk marks a bridging capillary. Bar=100 μm.

the dog (Figs. 7-B, 8-B,C) and more pronounced in man (Figs. 2-C, 5, 9) where they are also associated with saclike cavities applied to the lumen (Fig.10).

In the human "glomeruli" a direct A-V connection was found at the "hilus" in repeated instances (given the complex structure of most glomerular formations even exhaustive search may fail to document them in each glomerulus) suggesting the presence of an operant device (thoroughfare channel) capable to shunt flow from the sinusoidal convolutions (Figs. 9, 10).

In the human preparations the capillary convolutions (mostly on the venous side of the circulation) took a rich variety of forms presenting capillary micro-rings, invaginations of sinusoids and also a structure which we call "venous microglomerulus" 'Figs. 2-C, 11); the functional relation of the latter to the fully developed "glomeruli" or to the rest of CP is a matter for speculation.

In our corroded preparations of the human CP no unusual vascular feature could be detected in association with "glomus chorioideus"; this observation was however hampered by a definite difficulty in obtaining complete corrosion of this formation.

Discussion

Authors have mostly addressed the macroscopic distribution of the major vessel trunks in the context of the CPs although their striking vascular structure as *plexus* aroused interest already in remote times. All past descriptions in different species made reference to the A-V path: documentation of the capillary anatomy is however sparse. This undoubtedly is due to the intrinsic limitations in the techniques employed.

Putnam and Ask-Upmark (1934) made *in vivo* microscopical observations on the cat CP and noted a network of richly anastomosing capillaries with few vessels of intermediate size interposed between them and the large arteries and veins. They also mentioned that the "unusually little difference in colour between arterial and venous blood" might be due either to the large "unusually coarse" capillaries or to limited gaseous exchange in the extensive network. This was however doubted by later observers (Millen and Woollam, 1953) who do not think the former authors could even see the capillaries and attribute the observation to blood flowing from arterioles to venules through A-V "connexions". Pogorzelski (1963) studied A-V anastomoses in guinea pigs and rabbits, *in vivo* and histologically, with the aim to investigate the reaction to drugs of the smooth muscle in their walls. Only Millen and Woollam (1953) and Maillot and Koritke (1975) documented however the A-V route in man.

The *in vivo* impression of intense vascular engorgement produced by the CP is parallelled by the outcome of the acrylic injection: strikingly well filled CPs were obtained in almost all cases (also among preliminary series of animals (unpublished) in which very poor injection of the cerebral cortex was obtained). Capillary size and micro-architecture may play a role in the observed ease of filling of the CPs, as well as the macroscopic vascularization to the CP, with its richly anastomosing arteries at the base of the brain and the large distensible veins. The latter consideration and the circumstances of acrylic injection (un-physiologic pressure gradients, simultaneous filling and distension of all segments, viscosity) remind us many factors contribute to render unsafe absolute measurements based on casts (Lametschwandtner and Lametschwandtner-Albrecht, 1983).

The levels of organization of the terminal vascularization in the CP that we describe (capillary mesh, leaf-like formations, glomeruli) could be identified at least in part with anatomical features described by other authors (e.g. the "ultimate villi" in Findlay (1899), the "central capillary of villus" in Dohrmann and Bucy (1970)). Univocal interpretation is however difficult due to the heterogeneous terminology.

Our investigation finds one common element (the typical T-form offshoots of terminal arterioles) with the prevailing cerebrovascular organization and confirms the singularity of most microvascular features of the CP, as noted by most authors:

- network capillaries
- large capillaries
- focal capillary dilatations

All these features (both the three organizational levels and the typical capillary morphology) are missing in cerebrocortical microvascularization (Motti et al.,1986) and are remarkably similar in the three species described. The similarity between species at the microvascularization level mimics the ultrastructural similarity (Davis et al., 1973). Short A-V communications at the origin of glomerular formations without the intervention of a capillary field have been found only in the human CP, as Millen and Woollam (1953) observed in their comparative study of rabbit and human CPs. Their function as operant devices in the guise of thoroughfare channels awaits confirmation.

Different morphological organization commonly subserves different functions and while the CP is thought to be a centre of CSF production, identification of specialized activities is presently very difficult considering that (although a higher

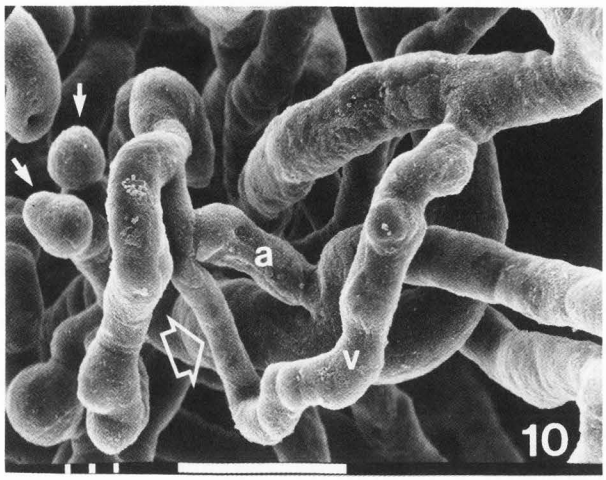


Fig.10. Instance of arterio-venous (a)(v) communication (empty arrow) at the origin (hilus) of a human glomerular structure. Pronounced "nodular" saclike cavities are visible (white arrows) at the top of a capillary loop. Bar=100 μm.

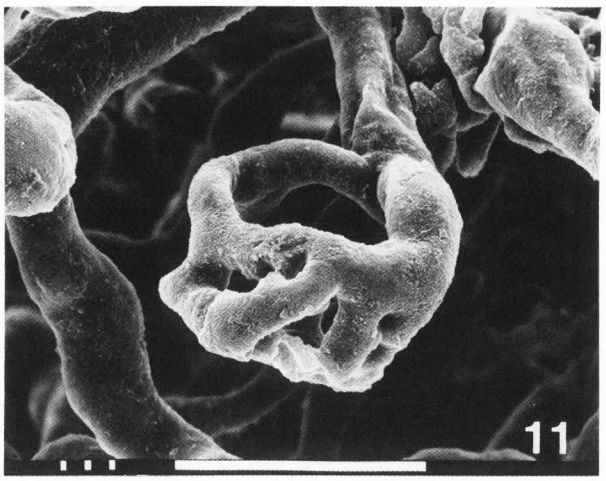


Fig.11. Instance of a peculiar structure ("microglomerulus") which is sparsely distributed over venous branches of the choroid plexus of the lateral ventricle in man. Bar=100 μm.

alkaline phosphatase activity is localized in the endothelium of capillaries) no metabolic difference has been noted between choroid plexuses in all locations (Masuzawa and Sato, 1983).

Acknowledgments

The authors would like to thank Dr.Stephanos Geroulanos for his collaboration and support in the Laboratory of Scanning Electron Microscopy of the Dept. of Surgery and also both Cecile Thalman and Rosemarie Frick for their technical assistance in the Laboratory of Microsurgery. Dr.P.Schianchi was instrumental in arranging the procurement of the human specimens.

E.Motti worked on this project while on leave from the Institute of Neurosurgery of the University of Milano, Italy.

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

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

Davis DA,Lloyd Jr.BJ, Milhorat TH (1973) A comparative ultrastructural study of the choroid plexuses of the immature pig. Anat.Rec.,176, 443-454.

Dohrmann GJ (1970) The choroid plexus: a historical review. Brain Res.,18, 197-218.

Dohrmann GJ, Bucy PC (1970) Human choroid plexus: a light and electron microscope study. J.Neurosurg., 33, 506-516.

Doumoto Y, Toya S, Lisaka Y,Kawase T, Sato S, Niyahara Y (1984) In vivo microcirculation of the choroid plexus, in: Progress in Microcirculation Research (2) - Courtice FC, Garlick DG, Perry MA (Eds.), Committee in Postgraduate Med.Education, Kensington, 210-215.

Findlay JW (1899) The choroid plexuses of the lateral ventricles of the brain, their histology, normal and pathological, (in relation specially to insanity). Brain, 22, 161-202.

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

Hodde KC (1979) The vascularization of the choroid plexus of the lateral ventricle of the rat. Beitr.Elektronenmikroskop.Direktabb.Oberfl.(BEDO), 12, 395-400.

Hudson AJ, Smith CG (1952) The vascular pattern of the choroid plexus of the lateral ventricle. Anat.Rec., 112, 345-346.

Kessel RG, Kardon RH (1979) Tissues and Organs.

A text-atlas of scanning electron microscopy. Freeman & Co., San Francisco, CA, 82-83.

Lametschwandtner A, Albrecht U, Adam H (1978) The vascularization of the anuran brain - The choroid plexus of the fourth ventricle - A scanning electron microscopical study of vascular corrosion casts. Acta Zool.(Stockh.), 59, 229-237.

Lametschwandtner A, Lametschwandtner-Albrecht U (1983) Scanning electron microscopy of corrosion casts of 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.

Maillot C, Koritke JG (1975) Les reseaux capillaires de la toile choroidienne rhomboencephalique chez l'homme. Arch.Anat.Histo.Embr.norm. et exp., 58, 167-176.

Masuzawa T, Sato F (1983) The enzyme histochemistry of the choroid plexus. Brain, 106, 55-99.

Milhorat TH (1976) Structure and function of the choroid plexus and other sites of cerebrospinal fluid formation. Int.Rev.Cytol., 47, 225-288.

Millen JW, Woollam DHM (1953) Vascular patterns in the choroid plexus. J.Anatomy(London),87, 114-123.

Miodonski A, Poborowaska J, Friedhuber de Grubenthal A (1979) SEM study of the choroid plexus of the lateral ventricle in the cat. Anat.Embriol., 155,323-331.

Motti EDF, Niemeyer G (1983) Rasterelektronenmikroskopie an Gefässausgüssen des Auges. Klin.Mbl.Augenheilk., 182, 518-521.

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 (1986) The terminal vascular bed in the superficial cortex of the rat - A SEM study of corrosion casts. J.Neurosurg., 65, 834-847.

Murakami T (1971) Application of the scanning electron microscope to the study of the fine distribution of the blood vessels. Arch.Histol.Japan, 32, 445-454.

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.

Nowell JA, Pangborn J, Tyler WS (1970) SEM of the avian lung. Scanning Electron Microsc. 1970: 249-256.

Pogorzelski JK (1963) Studies on the structure of arteriovenous anastomoses in the choroid plexuses of the cerebral lateral ventricles (Polish). Folia Morphologica (Warszawa), 22, 1-6.

Putnam TJ, Ask-Upmark E (1934) The cerebral circulation. XXIX - Microscopic observations on the living choroid plexus and ependyma of the cat. Arch.Neurol.Psychiat., 32, 72-80.

Discussion with Reviewers

A.Lametschwandtner: Have you also done wash-out of brain vasculature in your specimens using physiological saline only? Does the wash-out solution you have used actually lead to significantly better vascular casts if compared to wash-out with physiological saline or Tyrode's solution only?
Authors: We tried isotonic saline alone as a wash-out solution for the cortical circulation in rats. Outcome has been unsatisfactory as most casted brains showed extravasations and/or grossly incomplete filling. Similar defects in the CPs were apparent inspecting the ventricular cavities in a few specimens of this group. In Motti et al. (1987) we suggest that both defects ("excess of resin" and "lack of resin") may be due to vasospasm caused by vasodistention during resin injection and speculate that this mechanism may have been called into play as early as the wash-out stage in the most defective specimens. Isotonic saline (employed as the first wash-out solution in the same quantities we report for the dextran solution) may compound the problem, at least in the thick felt of cortical microvessels, by its low viscosity and also by eventual changes in the endothelial wall produced by osmotic action. We also obtained unsatisfactory results using the sequence of buffered fixative solutions described by Jokelainen et al. (1980).

K.C.Hodde: What were the viscosities of your different plastic mixtures?
Authors: We did not measure viscosities. We assumed more Sevriton meant less viscous and less Sevriton meant more viscous and employed a series of mixtures with relatively more or less Sevriton as compared to the original description of Nopanitaya et al.(1979, text ref.).

A.Lametschwandtner: You mention that usage of two compounds of different viscosity and of the pulsating manual injection was entirely empirical. Did you also use just one compound and continuous manual injection? What were the differences in the outcome of casting studies?

Authors: We found the compound originally described by Nopanitaya et al.(1979, text ref.) had many nice qualities but did not fill reliably the cortical microvessels. We then experimented with a range of different relative concentrations of components before obtaining what became standard mixture-1 and standard mixture-2. The diverse mixtures were always injected alone. Note our two compounds vary in Sevriton concentration but also in type of added pigment. Results can be summarized as follows:
Mixtures type-1 (Sevriton excess) tended to produce:
1)satisfactory injection of capillaries
2)frequent extravasations (with maximal Sevriton concentrations)
3)weak casts that easily collapsed
Mixtures type-2 (Sevriton restricted) tended to produce:
1)poor or no injection of capillaries
2)rare or no extravasations
3)self-sustaining casts.
We adopted the sequential injection of the two mixtures on the following rationale: the "thin-weak" resin, that fills the whole organ down to the microvascularization, is displaced by the "thick-strong" resin in the relatively larger ramifications only and acquires stability by being appended to the latter as a web. Pigments were employed mostly as "metal netting in reinforced concrete": the colored particles make the casts sturdier. The regular pigments provided with Batson's kit contain particles too large to enter the smaller ramifications and their use was accordingly restricted to mixture-2 only. Continuous injection appeared as well to have a role in the production of extravasations and incomplete filling. Continuous injection may possibly make it easier to set in motion the reactive sequence of vasospasm produced by vasodistention that we suggested (Motti et al., 1986).

A.Lametschwandtner: You very impressively document that in the smooth central segments of the CP a mesh of fine capillaries invests all larger vessels evenly (Fig.2; especially Fig.2-A and 2-D). Hodde (1979, text ref.) in his study (rat) termed these capillaries "garland capillaries" and reports them to sheath arteries only. Would you please comment on this matter. Studying the CP of the lateral ventricle of the rabbit by the corrosion casting technique we have found these capillaries also sheating arteries as well as veins (Weiger et al., 1986).
Authors: Your finding confirms that complete absence (Miodonski et al.,1979, text ref.) as well as only partial presence of "garland capillaries" (Hodde, 1979, text ref.) likely represent incomplete injection.

A.Lametschwandtner: Have you any suggestion upon the function(s) the capillaries sheating arteries and veins could have? Are there any fine structural studies done on these vessels revealing the nature of the capillary endothelium?
Authors: We entertained a number of hypotheses; among others a heat exchange arrangement and a sensor device coupling intravascular and intracranial (CSF) pressures. We have however no information on these hypothetical functions. It could be safely stated that the mesh of fine capillaries represents the stromal vasculature underlying the simple cuboidal epithelium of the central segment.

Unusual "fenestrations" have been described by diverse authors (cited in Peters et al., 1976) in the "villi" as belonging to the endothelial wall of large capillaries only. Regarding these we do not know whether the fenestrations (thinning of the endothelial cell) can cause any change at all on the surface of the casts and, were it so, if it is their impressions that we are occasionally seeing (e.g., the ovoid imprints noted on dilated capillaries, Fig.8-C).

K.C.Hodde: You describe and mention direct A-V connections, A-V anastomoses and A-V communications. Are these the same structural entity and would you call these real arterio-venous anastomoses? Do you have any histology of these?
Authors: The terminology for A-V communications is that of the authors cited. We prefer the purely descriptive terms of "direct A-V communication/connection". The term "A-V anastomosis" is suitable (for the structure described in the "glomerular formation") only as long as applicable to an individual *microvascular* connection between ultimate afferent and efferent vessels to a capillary bed. We have no histology of the wall of these A-V communications.

A.Lametschwandtner: You describe A-V anastomoses at the "hilar" region of "glomeruli" of the human CP suggesting them to function as operant device (thoroughfare channel). In a study on the turtle CP of the lateral ventricle, Weiger (1986) also found these A-V anastomoses in the choroidal villi.
Authors: This corroborative finding that you and your co-worker report in a chelonian is very exciting. The demonstration of A-V connections at the "hilar" region of the glomeruli has been very time-consuming in the mammalian species studied. Complete all-round observation could not be performed in the majority of glomeruli due to the particular location or complex arborization of the individual glomerulus. We are unable to say whether A-V "anastomoses" occur sporadically in glomeruli or represent a regular feature of all or most

glomeruli, that is however recognized only in those selected for their visibility and relatively simple structure.

K.C.Hodde: Could you expand on your ideas on what the functional implications might be of the fact that there seem to be several consistently different patterns in the capillary and sinusoidal parts in this vessel bed?
Authors: Many functions have been attributed to the CP. Foremost have been a secretory and a resorptive activity not unlike the manner the composition of urine is determined. We think the demonstration of consistent patterns of angioarchitectural organization may help to understand the "localized" functions in the CP that are currently being identified (Herbert et al., 1986).

Reviewer IV: I find two points bothering me: 1) the inadequacy of any reliable criteria for distinguishing arteries from veins by the appearance of luminal casts and 2) the failure of the injection medium to fill the capillary network completely (Figs.3,4,7,8,9,10). The latter, incomplete perfusion, is a severe technical drawback to the study. A number of the "sac like cavities applied to the lumen" (Fig.10) could be a failure of the perfusate to fill a branch vessel.
Authors: 1) We think the appearance of nuclear impressions is only one fact to take into account in order to reach unequivocal distinction between arteries and veins. In a good cast the vascular tree can be followed from the major to the smaller arterial branches down into capillaries and out into venules leading to larger veins. In this process the quality of the cast will be appraised and the extent of variation of endothelial impressions - as well as the characteristic ramification patterns - will be apparent. The diverse orders of vessels will show some common, some intermediate and some distinctive aspects that ought to be integrated with the occasionally incongruous appearance of ovoid and round nuclear impressions. It has not been uncommon to notice round nuclear imprints on assuredly arterial branches in our cerebral casts. (See the artery (a) in Fig.4 in this report and also the arterial branch in Fig.3 in Motti et al., 1987). How nuclear impressions are formed is still matter for speculation: the recognition of heterogeneity of endothelial impressions with decreasing vessel size led us to search always for the "parent vessel" to whom our supposedly arterial or venous cast was tributary.

2) Cerebral capillary districts show the occasional tendency to fill incompletely, if at all, with the casting material. The completeness of injection in this study is apparently very satisfactory since evidence of both "glomerular" and "garland" capillaries (the latter surrounding both arteries and veins) is lacking, as already mentioned, in previous reports of corrosion casting studies of the CP (text refs.: Miodonski et al., 1979; Hodde, 1979). Figs. 8,9,10 show no incompletely filled capillaries. In Figs. 3,7 there are a few partly filled mesh-work ("garland") capillaries. In the legend to Fig.4 an explanation is advanced for the localized short gaps visible in the reproduction of capillaries; it is also implied that minor gaps (possibly pockets of trapped wash-out solution) are likely to mar most preparations in the capillary-size districts. The stumps of defective (partly injected) capillaries in Figs.3,4 taper abruptly to a smooth hemispherical end. Poor specimens with defective vessels of all sizes commonly show tapering and abrupt termination of vessels. However the stumps of partly filled vessels never flare out in the "bubble" of a sac-like cavity. "Sac-like cavities" are a puzzling uncommon feature. "Blind diverticula" of closely similar appearance have been noted by Jasinski and Miodonski (1981) in the palatine capillaries of Rana esculenta.

A.J.Miodonski: Are you able to present suggestions about the role played, eventually, by the "nodular thickenings" which are present on the larger capillaries in glomerular formations. Please take under consideration following facts: the choroid plexus of the lateral ventricle can be an extra source for oxygen and nourishing substances - what is rather well pronounced in lower vertebrates e.g. tailed amphibians but also during ontogenetical development of the brain in higher vertebrates - for the neural tissue of the hemisphere: secondly, practically the same, even larger in type, nodular thickenings are present on the capillary vessels supplying the mucose membrane of the oral cavity bottom of tailed amphibians which is participating additionally in respiratory functions. Generally one can presume that in such "nodular swelling" or "thickening" which is corresponding with shallow recesses of the capillary lumen, the blood flow will be really slowed down for better extraction of oxygen as well as nourishing substances.
Authors: We have nothing to add to your interesting functional comments. We note that you suggest that "nodular thickenings" (meaning focal enlargements of luminal diameter) belong into one category of devices with "blind diverticula" (meaning "saclike cavities" applied to the lumen) (Jasinski and Miodonski, 1981). In the present study the "nodular thickenings" in the CP of the lateral ventricles show a trend to become more pronounced with increasing phyletic level.

A.Lametschwandtner: Concerning the human brains

you have casted, has there been any special treatment of the patients before death which might have resulted in changes of blood clotting leading to a better/worse result of wash-out of the brain vasculature?
Authors: One patient received subcutaneous Ca-heparin (0.2 ml b.i.d.) for two weeks prior to death. We did not note significant changes in this compared to other cases.

J.J.Taylor: What is the significance or main conclusion of this work and what possible clinical application might it have?
Authors: Morphology is the mother of physiology and we think we have demonstrated in our comparative study that the CP has microcirculatory units of consistent architecture which supposedly subserve different functions. It will be a challenging task for all those busy in the field to probe the functional units of this organ beside the tissue as a whole. Furthermore, what we have presented is the supposedly "normal" angioarchitecture in "normal" CPs and such eminently vascular organ lends itself to investigation by the corrosion casting method in pathological conditions as well. Recognition of deviation from normality requires of necessity the most accurate knowledge of the normal pattern itself. Investigations of this type are underway in experimental hydrocephalus(K. Hodde - personal communication to E.D.F.M).

Additional References

Herbert J, Wilcox JN, Pham KTC, Fremeau RT, Zeviani M, Dwork A, Soprano DR, Makover A,Goodman DS, Zimmerman EA, Roberts JL, Schon EA (1986) Transthyretin - A choroid plexus-specific transport protein in human brain. Neurology, 36,900-911.

Jasinski A, Miodonski A (1981) Vascular arrangement in the oral mucosa of rana esculenta. Scanning electron microscopy of corrosion casts. Arch.Histol.Jap., 44, 215-221.

Jokelainen PT, Webb DR, Yush ED (1980) A combined electron microscopic-microangiographic methodology for cerebrovascular studies. J. Ultrastruct. Res., 71, 116-125.

Motti EDF, Imhof H-G, Martinez Garza J, Yasargil GM (1987) Vasospastic phenomena on the luminal replica of rat brain vessels. Scanning Electron Microsc. 1987; I: in press.

Peters A, Palay SL, Webster H deF (1976) The fine structure of the nervous system, W.B.Saunders Co., Philadelphia, 288.

Weiger T, Lametschwandtner A, Hodde KC, Adam H (1986) The angioarchitecture of the choroid plexus of the lateral ventricle of the rabbit. A scanning electron microscopic study of vascular corrosion casts. Brain Res., 378, 285-296.

Weiger TM.(1986) Die Angioarchitektur der Telencephalen Plexus Choriodidei bei Acipenser ruthenus - Amys crypta elegans und Orcytolagus cuniculus Forma Domestica - eine Rasterelektronmikroskopische Untersuchung mit besonderer Beruchsichtigung der Methodik der Gefässausguss Präparation. Thesis, University of Salzburg, Austria, pp.111.