SUMMARY  Topographic distribution of barrier function in normal canine cervico-cephalic arteries was studied using horseradish peroxidase (HRP) and Evans blue as tracers. The carotid sinus of the internal carotid artery (ICA) was conspicuously permeable to HRP when compared to other areas of major cervical arteries. The cavernous portion of the ICA also showed prominent permeation of HRP, especially through the outer surface, which is covered with venous endothelial cells. On the luminal side of the cavernous portion of the ICA, barrier deficiency was noted at angulated segments such as the carotid siphon. Intracranial segments of both ICA and vertebral arteries demonstrated incomplete barrier function of the first to 4 mm from the origin of the intradural segments. These areas were considered to be transitional zones in barrier function between extra- and intracranial arteries. Focal, but definite, barrier disruption was also noted at the distal ends of the ICA and other arterial branching sites of major intracranial arteries. While opening of the interendothelial junctions was considered to be one of the mechanisms causing increased permeability in the cavernous ICA, the mechanisms for the permeation of HRP into the major cerebral arteries could not be confirmed ultrastructurally.

Materials and Methods

Five adult female mongrel dogs, weighing between 5.1 and 6.6 kg, were anesthetized with intravenous sodium pentobarbital (Nembutal, 30 mg/kg body weight). Endotracheal intubation was performed and anesthesia was supplemented with a mixture of 60% N₂O and 40% O₂, administered during ventilation. The femoral artery and vein were catheterized to monitor blood pressure and to administer drugs. A capnometer was used to monitor expiratory CO₂ concentration, which was held between 35 and 40 mmHg. During the experiment, arterial blood pressure, PaCO₂ and pH of each animal were maintained within normal range. Evans blue (2% solution, 2.5 ml/kg body weight) was injected intravenously 30 minutes prior to fixation. Diphenhydramine hydrochloride (5 mg/kg body weight) was used in order to prevent hypotension and the histamine release reaction induced by horseradish peroxidase (HRP) administration. Ten minutes before fixation, HRP (200 mg/kg body weight, Sigma Type II) was injected intravenously and each animal was then sacrificed by perfusion fixation using 2.5% paraformaldehyde — 2% glutaraldehyde — 0.1 M phosphate buffered fixative (pH 7.3) under a pressure of 100–120 mmHg for 15 minutes. The brain and cervical arteries were removed, including cavernous and cervical portions of the internal carotid arteries (ICA), external carotids and common carotid arteries, and the specimens were kept in 0.1 M cacodylate buffered fixative (pH 7.3) for five hours. After washing overnight, the specimens were incubated for determination of peroxidatic activity. The incubated sections were examined with an operating microscope and were prepared for electron microscopy. The sections were post-fixed in osmium tetroxide, stained en bloc with uranyl acetate, dehydrated through graded acetone, and embedded in epoxy resin. One micron sections were cut and appropriate areas were selected for ultrathin sectioning. The sections were examined with Hitachi (H-600) electron microscope.

Results

The results in the present study are illustrated in figure 1.

Macroscopic Findings

While HRP-reaction products in cervico-cephalic arteries were relatively easily detected as brown-colored areas with operating microscopy, it was essential-
Intracranial Arteries

The proximal portion of the intradural internal carotid artery was stained with HRP-reaction products in continuity of the stained area of the cavernous portion of the ICA (fig. 2B, 2C). The vertebral arteries were also stained at the proximal portion, 1 to 4 mm from the point of dural penetration (fig. 2D). Small areas of focal staining were found at arterial bifurcations such as the apex and lateral wall of the distal ends of the internal carotid artery, the origin of the azygous trunk of the anterior cerebral artery, the bifurcation of the anterior spinal arteries and basilar arteries, and other branching sites of the major cerebral arteries (fig. 2B, 2C & 2D). Brain parenchyma was not stained with HRP-reaction products except at the hypophysial infundibulum, median eminence and choroid plexus.

Electron Microscopic Findings

Carotid Sinus

HRP-reaction products were present in the subendothelial space, but the density of staining was relatively low in contrast with that of the macroscopic and light microscopic staining. HRP-labeled vesicles were noted on the abluminal membranes of the endothelium (fig. 3A). We did not detect continuous staining with HRP-reaction products throughout the entire thickness of the interendothelial junctions from the lumen to the subendothelial space.

The subendothelial space of the adventitial surface of the cavernous portion of the internal carotid artery contained HRP-reaction products. A small number of HRP-labeled micropinocytotic vesicles were found at the abluminal membrane of the endothelial cells, and reaction products were present at the interendothelial junctions. The subendothelial space of the arterial lumen contained varying densities of HRP-reaction products, the highest being just beneath the interendothelial junctions (fig. 3B). Some intercellular junctions were stained throughout the entire thickness of the vessel wall from the arterial lumen to the subendothelial space (Figure 3C). A large number of the HRP-labeled vesicles were noted at the junctional area and the abluminal membrane of the endothelial cells (fig. 3B, 3C). There were very few HRP-labeled vesicles on the luminal surface or within the cytoplasm of the endothelium.

Intracranial Major Arteries

In the intracranial arteries, except in the focally stained areas, HRP-reaction products were not found in the subendothelial space. There were no HRP-labeled micropinocytotic vesicles attached to the abluminal membrane nor were there any HRP-labeled vesicles on the luminal surface of the endothelial cells (fig. 3D). HRP-reaction products were detected in the subendothelial spaces of the focally stained areas. The staining was usually so low in density, however, that even under electron microscopy reaction products were detectable only as HRP-labeled vesicles attached to the relatively dense abluminal membrane of the en-
dothelial cells (fig. 3E). Among the stained areas in the intracranial arteries, the distal portion of the internal carotid artery showed HRP-reaction products in the endothelial spaces. In these areas, abluminal HRP-labeled vesicles were relatively increased in number (fig. 3F). However, luminal HRP-labeled vesicles were not detected. Despite the presence of HRP-reaction products in the subendothelial spaces, opening of the interendothelial junctions was not found in the intracranial arteries, probably due to the small amount of HRP permeation into the macroscopically stained area.

Discussion
Though it is generally assumed that major cerebral arteries have barrier function similar to the parenchym-
nal microvessels of the brain\textsuperscript{1-3} and that systemic arteries do not possess definite barrier function\textsuperscript{2,3,6} the present study has revealed that there were topographic differences in barrier function between the various parts of the cervico-cephalic arterial system.

Among the cervical arteries, the carotid sinus showed a well demarcated stained area, corresponding to its anatomical localization. It may be speculated that the specific absence of barrier function at the carotid sinus is related to the presence of baroreceptors or

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**FIGURE 3.** A: Endothelial cells at the carotid sinus. HRP-reaction products were present in the subendothelial space and the abluminal pinocytotic vesicles (arrows). × 30000. B: Luminal site of the cavernous portion of the ICA. HRP-reaction products are noted in the subendothelial space and in a large number of pinocytotic vesicles. EL: Elastic lamina. × 10000. C: Luminal site of the cavernous portion of the ICA. HRP-reaction products are seen in the interendothelial junction (arrow). × 15000. D: Unstained segment of the basilar artery. There are neither abluminal nor luminal HRP-labeled vesicles in the endothelium. × 12000. E: Bifurcation site of the basilar artery. Very low density HRP-reaction products are present in the subendothelial space. One vesicle contains a relatively dense concentration of HRP-reaction products (arrow). × 20000. F: Distal portion of the ICA (C1). HRP-reaction products are seen in the subendothelium and micropinocytotic vesicles of the endothelium (arrows). EL: Elastic lamina, L: Lumen, E: endothelium. × 16000.
vasoregulatory function at this area, and perhaps also
to the anatomical characteristics of the vascular wall
components. The cavernous portion of the internal
carotid artery was characterized by diffuse staining
with HRP-reaction products through the adventitial
side. The endothelial cells covering the outer surface
do not possess definite barrier function. In contrast, the
endothelial cells covering the arterial lumen showed focal barrier deficiency, especially at the angulated
segments. It is likely that the deficiency of barrier
function at the carotid sinus might be physiological,
since the carotid sinus showed clear demarcation of the
stained area with homogeneous staining which was
overlapped by relatively dense staining at the ostia of
the branches. On the other hand, in arterial segments
showing barrier disruption on the luminal side of the
cavernous and distal portion of the internal carotid
artery and vertebro-basilar systems staining was defi-
nitely localized at the angulated area and arterial bifur-
cation. The barrier disruption might be attributed to
hemodynamic stresses or other pathological processes
related to atherogenesis and aneurysm formation.

Proximal segments 1 to 4 mm from the origin of the
intradural segments of both internal carotid artery and
vertebral arteries showed increased permeability.
These areas are probably transitional zones of endothe-

tial barrier function between extra- and intracerebral
arteries. Bevan8 investigated precise topographic dif-

ferences in pharmacological vascular properties be-

tween major cerebral and systemic arteries of rabbits.
He reported that the transition site in the carotid artery
was just proximal to the carotid canal while the tran-
sition site in the vertebral artery occurred just rostral to
the emergence of that artery from the foramen of the
lateral process of the atlas. There was no evidence for a
gradual transition of characteristics but rather there
appeared to be an abrupt change.8 It is interesting that
at the site of transition there are differences between
the pharmacological responses of medial smooth mus-

cle and barrier function in the endothelium.

There are a few reports concerning barrier function
or increased permeability in the major cerebral arter-

yes. 9, 8 Sadoshima et al, 9 reported that deposition of
fibrinogen-fibrin as a result of increased permeability
was seen within intimal pads at the bifurcation of hu-

man major cerebral arteries even in neonates, and the
amount of deposition increased with age. Topographic
distribution of the arterial segments permeable to HRP
in the dog were compared with those showing athero-
sclerotic changes in human.9

Increased permeation of the plasma components is
one of the most important causative factors in athero-
genesis.11 Conspicuous, and characteristic staining
with HRP at the carotid sinus indicates that the endo-

thelium at the carotid sinus is extraordinarily perme-
able to plasma proteins. Since atherosclerotic changes
are generally most frequently seen in the region of the
carotid sinus among the cervico-cephalic arteries,
increased permeation seen in the present study indicates
that barrier deficiency could be closely related to athero-
genesis. Focal barrier-disrupted areas in the major
cerebral arteries corresponded to those sites which
were most directly influenced by hemodynamic stress.
These focal increases in the permeability at the branch-

ing sites may be related to the development of cerebral
saccular aneurysms.

In the present study, we could not delineate all of
the mechanisms for the HRP permeation through the endo-
thelial cells, because the amount of permeated HRP
was usually too small to detect ultrastructurally despite
the macroscopic presence of the stained areas. We
could not find continuous staining of interendothelial
junctions at the carotid sinus, despite macroscopic and
light microscopic dense staining. It is possible that
HRP in the intercellular opening was completely
washed out during perfusion fixation. Except for the
carotid sinus and cavernous portion of the internal carot-
id artery, the staining with Evans blue in the cervico-
cephalic arteries was too low in density to detect under
an operating microscope, so Evans blue dye may be
inappropriate as a tracer for investigating barrier func-
tion in the cervico-cephalic arterial system of normal
animals.

Recognition of the physiological absence of barrier
function in the major cerebral arteries is essential for
evaluation of barrier changes or disruption in these
vessels under various experimental conditions. The
physiological barrier deficiency in the cerebral arteries
may influence not only the arterial response to some
vasoactive substances, but also the vascular reaction
following subarachnoid hemorrhage, systemic hyper-
tension, and atherosclerosis.

References

1. Reese TS, Kamovsky MJ: Fine structural localization of a blood-

brain barrier to exogenous peroxidase. J Cell Biol 34: 207–217,

1969

2. Kurozumi T: Electron microscopic study on permeability of the

aorta and basilar artery of the rabbit — with special reference to the

changes of permeability by hypercholesteremia. Exp Mol Pathol

23: 1–11, 1975


atherosclerosis in the cholesterol-fed rabbit. Atherosclerosis

30: 137–145, 1978

4. Zervas NT, Liszczak TM, Maybert MR, Black PM: Cerebrospinal

fluid may nourish cerebral vessels through pathways in the adventi-
tia that may be analogous to systemic vasa vasorum. J Neurosurg

56: 475–481, 1982

5. Kamovsky MJ: The ultrastructural basis of capillary permeability


mechanism for increased endothelial permeability in experimental


7. Rees PM: Electron microscopical observations on the architecture

of the carotid arterial walls with reference to the sinus portion. J

Anat 103: 35–47, 1968

8. Bevan JA: Sites of transition between functional systemic and
cerebral arteries of rabbits occur at embryological junctional sites.
Science 204: 635–637, 1979


in the development of cerebral atherosclerosis. Atherosclerosis

34: 93–103, 1982


M, Nara Y, Yamori Y: Permeability of intracranial extracranial


11. Jorgensen L, Packham MA, Rowell HC, Mustard JF: Deposition of

formed elements of blood on the intima and signs of intimal

injury in the aorta of rabbit, pig, and man. Lab Invest 27: 341–350,

1972
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M Yamashita, N F Kassell, T Sasaki, S Fujiwara, M Zuccarello and A Spallone

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