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Disturbance in the Intramural Circulation of the Major Cerebro-pial Arteries After Experimental Subarachnoid Haemorrhage

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Summary

The intramural fluid circulation of the cerebral arterial wall was investigated using horseradish peroxidase (HRP) as a tracer which was injected intravenously or intracisternally in dogs with or without subarachnoid haemorrhage (SAH). In the control dogs, the endothelial barrier function was confirmed for intravenous HRP, whereas the intracisternal HRP passed freely through the interstitial spaces of the adventitia and media to reach the intima within a few minutes. However, on the 5th day after SAH the barrier function of the intima for intravenous HRP was lost. In addition, there was a marked decrease in the amount of HRP reaching the intima when injected intracisternally. The intercellular space appears to be the main route for leakage of HRP into the subendothelial layer from the arterial lumen. Obstruction of the interstitial space in the adventitia by blood elements may be the cause of the disturbed intramural circulation of cerebrospinal fluid. These results suggest that this disturbance in the intramual circulation of the cerebral arterial wall plays a role in the development and/or progression of delayed cerebro-arterial narrowing after SAH.

Keywords: Subarachnoid haemorrhage; horseradish peroxidase; blood-brain barrier; experimental vasospasm.

Introduction

The intramural circulation of cerebral arteries is different from that of systemic arteries. The intima of cerebral arteries shows a barrier function equivalent to the blood-brain barrier of the capillaries in the brain[2, 6, 10]. Furthermore, cerebral arteries have no vasa vasorum[3, 20]. Cerebrospinal fluid (CSF) may nourish the cerebral arterial wall and/or remove noxious agents in the arterial wall in the same way that the vasa vasorum serve the systemic arteries[3, 20].

Vasospasm following subarachnoid haemorrhage (SAH) presents severe clinical problems. It is well known that there is a close association between vasospasm and the amount of blood in the subarachnoid space[4, 11]. The pathogenesis of vasospasm is still poorly understood. A few studies have addressed the metabolic abnormalities in the arterial wall after SAH[14]. In this study, we evaluate the disturbance in intramural circulation of the cerebral arterial wall after experimental SAH and we discuss its role in the pathogenesis of vasospasm.

Materials and Methods

Twenty-four mongrel dogs of both sexes, ranging in weight from 8 to 12 kg were divided into eight groups as shown in Table 1. A subarachnoid haemorrhage (SAH) was produced by two successive injections of 5 ml blood given 48 hours apart. The dogs were anaesthetized with intraperitoneal sodium pentobarbital (35 mg/kg body weight) and then tracheal intubation and artificial ventilation with room air were instituted. Subsequently, under sterile conditions, 5 ml of fresh autologous arterial blood obtained from the femoral artery was injected into the cisterna magna. After the injections the dogs were tilted in a head down position for thirty minutes to facilitate settling of the blood in the basal cisterns by gravity. The second SAH was produced 48 hours after the first in the same manner, and the dogs were sacrificed 48 hours after the second SAH. For morphological examination, the dogs were anaesthetized deeply with an overdose of pentobarbital, and perfused transcardially with 10 *l* of 0.5% glutaraldehyde and 3.5% paraformaldehyde in 0.2 M Millonig's buffer (pH 7.4). Fifteen minutes before perfusion, 20% HRP solution in saline (HRP: 200 mg/kg body weight, Sigma type II: RZ 1.52) was given intravenously (3 control dogs and 3 SAH dogs). Dimorphoramine was injected intravenously in order to prevent HRP-induced hypotension.

In other experiments, immediately (3 control dogs), 7.5 minutes (3 control dogs) and 15 minutes (3 control dogs and 4 SAH dogs) before perfusion, 20% HRP solution in saline (HRP: 20 mg/kg) was injected into the cisterna magna of the dogs in the head down position. Additionally 3 dogs with no treatment and 2 dogs with SAH but no HRP injection were perfused transcardially like the other dogs. After perfusion the large pial arteries were removed, washed and immersed in 0.1 M phosphate buffer (pH 7.4) for 4 hours. The HRP was visualized by incubation at room temperature for 15 minutes with 0.03% diaminobenzidine tetrahydrochloride and 0.003% hydrogen peroxide in 0.05 M Tris-HCl buffer (pH 7.6). Samples were post-fixed in 1.0% osmium tetroxide in 0.1 M Millonig's buffer at pH 7.4,

Table 1. *Summary of Experimental Group*

Animal group	The number of cases
a) Control	
No treatment	3
Intravenous HRP injection	3
Intracisternal HRP injection	
immediately before fixation	3
7.5 min before fixation	3
15 min before fixation	3
b) SAH	
SAH only	2
Intravenous HRP injection	3
Intracisternal HRP injection	
15 min before fixation	4
Total	24

dehydrated with an ascending series of ethyl alcohol baths, stained en bloc with 2.0% uranyl acetate, and embedded in epon 812. Ultra-thin sections were then cut on a Porter-Blum ultra-microtome equipped with a diamond knife. The sections were stained with lead citrate and examined in a Hitachi H-300 electron microscope. For light microscopic examination, 1 μm thick sections were cut on a Porter-Blum ultra-microtome and stained with toluidine blue.

Results

In all dogs receiving two injections of autologous arterial blood into the cisterna magna, the production of SAH was confirmed by gross inspection at the time of sacrifice on the 5th day. Subarachnoid clot and/or xanthochromic staining of the arachnoid and pial membranes were seen consistently on the ventral surface of the brain stem (Fig. 1). In control dogs without any treatment and in those with SAH but no HRP injection, HRP-reactive products were not observed in the wall of the large pial arteries.

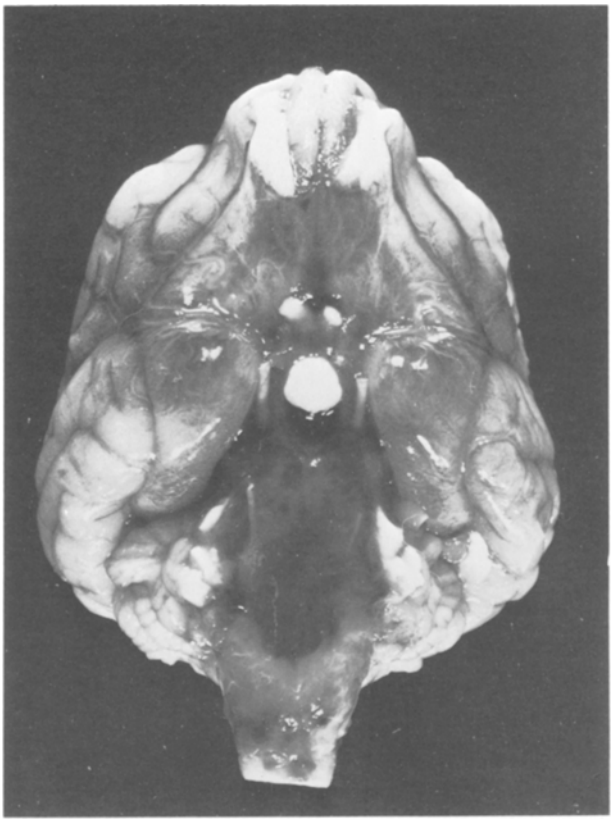


Fig. 1. Ventral aspect of a brain of a dog at the time of sacrifice on the 5th day after the 1st SAH. The subarachnoid clot is seen on the anterior surface of the brain stem

Endothelial Permeability to HRP

Electron microscopic studies in control dogs demonstrated no HRP-reactive products in the subendothelial space of the basilar arteries except within the marginal folds or a small number of the luminal plasmalemmal vesicles (Fig. 2). In dogs with SAH, degenerative changes of the endothelium and corrugation of the elastic lamina were frequently observed. HRP-reactive products were observed in the interendothelial space, subluminal plasmalemmal vesicles and subendothelial spaces of the basilar arteries (Figs. 3 a and b).

HRP Permeation from the Adventitia to the Intima

In control dogs, immediately after HRP injection, HRP-reactive products could be seen on the external surface of the adventitia of the basilar arteries (Fig. 4 a). At 7.5 minutes, HRP-reactive products could be seen in the intercellular space of the innermost portion of the media (Fig. 4 b). At 15 minutes, HRP-reactive products could be seen in the subendothelial space and subluminal plasmalemmal vesicles of the endothelial cells

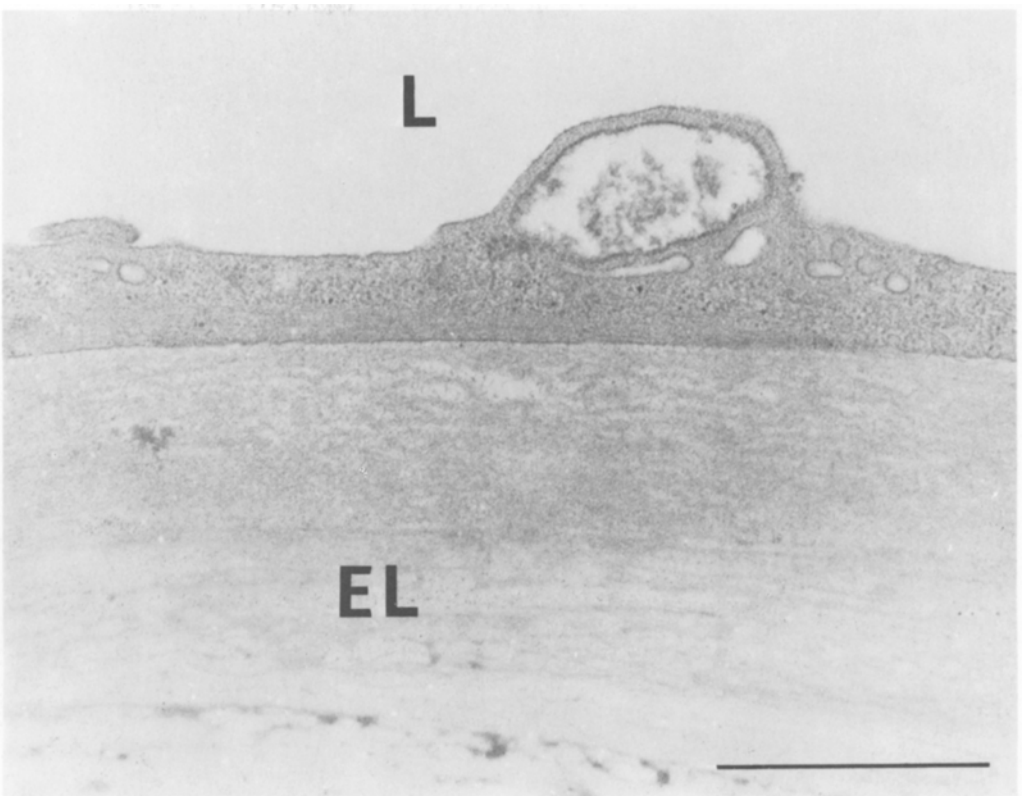


Fig. 2. Electronmicrograph of a basilar artery from a control dog which was intravenously injected with HRP solution 15 minutes before perfusion. HRP-reactive products are seen within the marginal folds, but the subendothelial space is devoid of HRP-reactive products. × 36,000, bar = 1 μm, *L* lumen, *EL* elastic lamina

(Fig. 4c). Light microscopic studies revealed HRP-reactive products in the adventitia and the intima at 15 minutes after cisternal injection of HRP (Fig. 5a). In contrast, in the dogs with SAH, fewer HRP-reactive products were observed in the subendothelial space of the constricted basilar arteries at 15 minutes after cisternal injection than in control dogs (Fig. 5c). Light microscopic studies also revealed fewer HRP-reactive products in the intima of the basilar arteries of dogs with SAH than of those without SAH, although a sufficient quantity of HRP-reactive products was observed in the adventitia (Fig. 5b). This decrease in the amount of HRP in the subendothelial layer of arteries was associated with the severity of the morphological change and the amount of covering clot. In other pial arteries such as the middle cerebral arteries which were covered with less subarachnoid clot, the HRP reached the subendothelial layer as much as in the control dogs.

Discussion

The two-haemorrhage canine model of SAH produces vasospasm which resembles that in patients[7, 18]. In this study, corrugation of elastic lamina and damaged endothelial cells were frequently seen on the 5th day after SAH as previously reported[7, 16, 17]. These morphological changes appear to be closely associated with infiltration of blood elements into the arterial wall as previously reported[1, 7].

When HRP was injected intravenously, it infiltrated through the intercellular space into the subendothelial layer of the constricted basilar artery, which confirms the findings of Sasaki, *et al.*[14]. This leakage into the subendothelial space was most prominently seen in the valley of the constricted arterial lumen. The spread of the intercellular space may be a result of the corrugation of the elastic lamina associated with prolonged severe constriction of the artery[5]. Thus, the leakage of HRP may merely be the results of vasoconstriction. However, it is also probable that the disruption of the intimal barrier function allows the penetration of vasoactive substance from the blood into the smooth muscle layer, which then exacerbates the vasoconstriction.

In the control dogs, HRP which was injected intracisternally traversed rapidly through the adventitia and media of the arterial wall, reaching the subendothelial layer within a short time. It appeared to accumulate in the subendothelial layer, and some was further incorporated within endothelial vesicles. It is

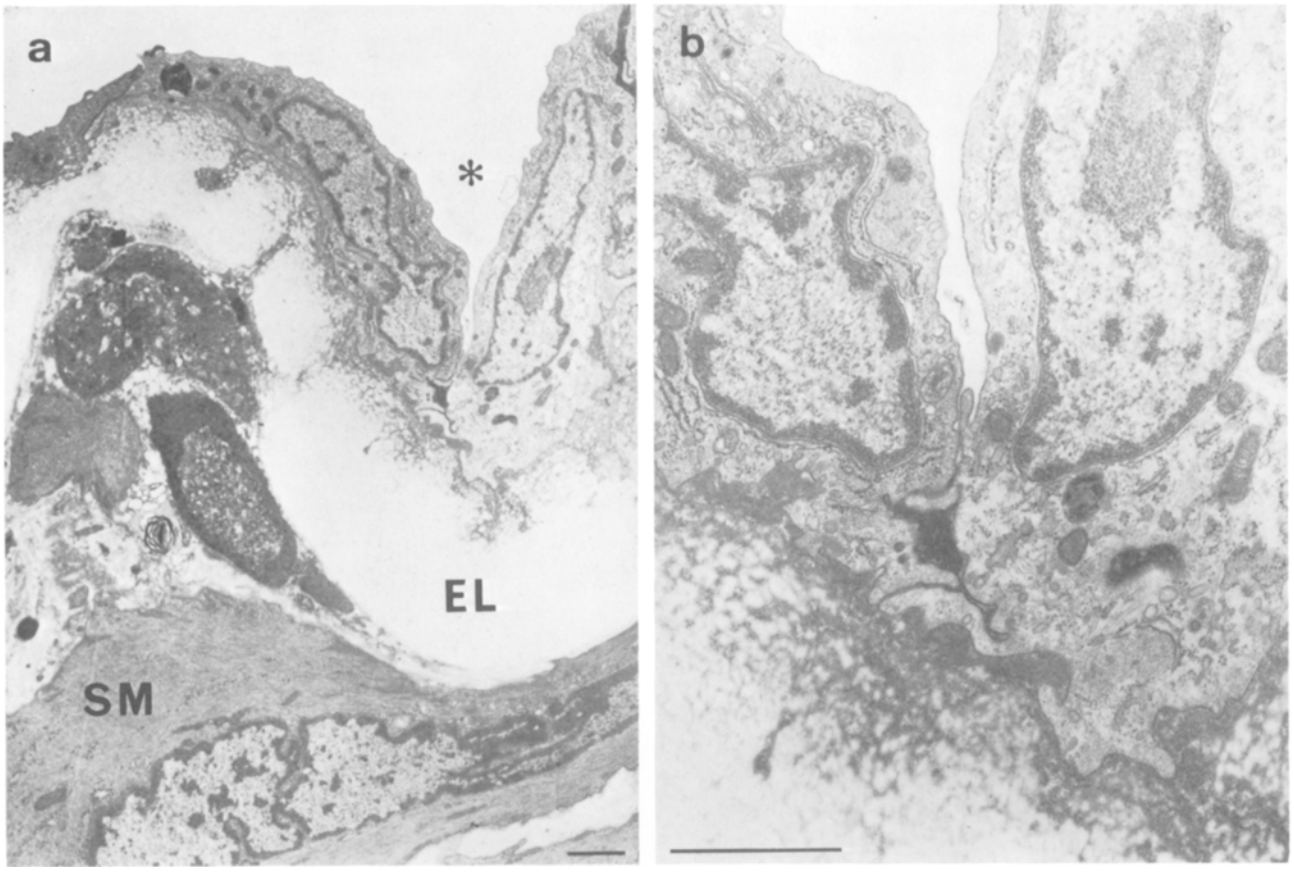


Fig. 3. Electronmicrographs of a basilar artery from a dog injected intravenously with HRP on the 5th day after SAH. a) Corrugation of the elastic lamina and necrotic changes of the smooth muscle cells are seen. HRP-reactive products are seen in the subendothelial space. × 9,000, bar = 1 μm. b) In the valley (*) of the corrugated lumen, an intercellular space is filled with HRP-reactive products. × 27,000, bar = 1 μm, *EL* elastic lamina, *SM* smooth muscle cell

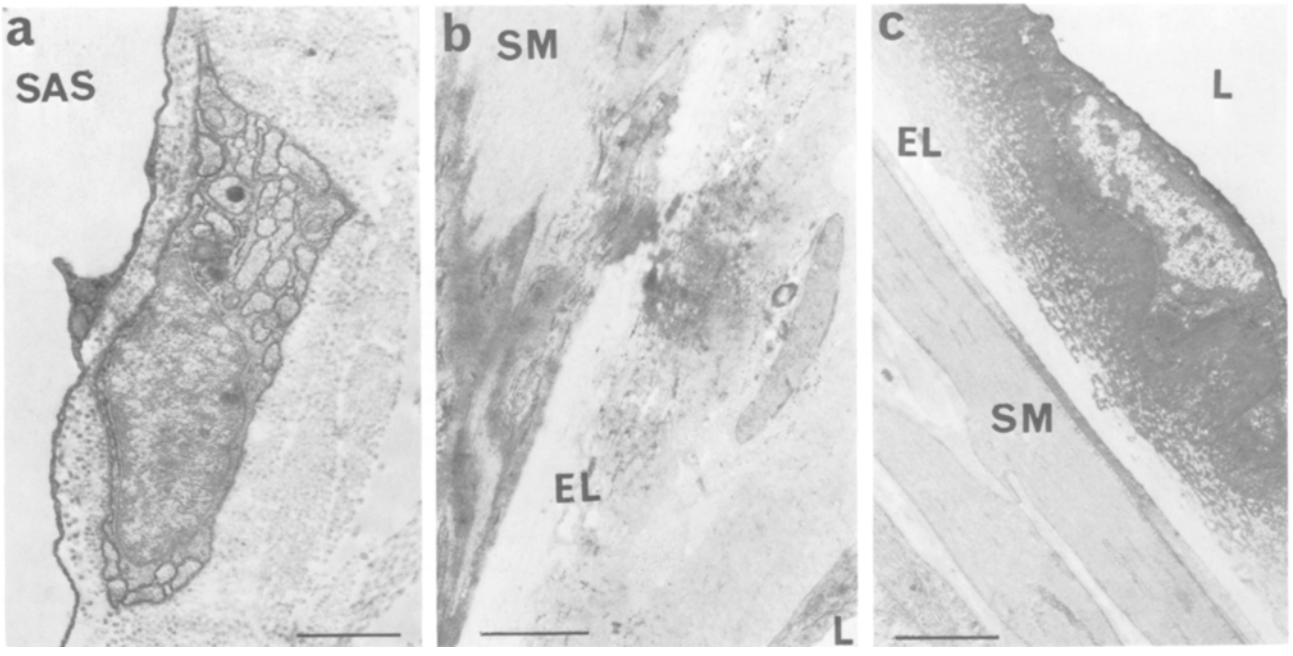


Fig. 4. Electronmicrographs of basilar arteries from control dogs which were intracisternally injected by HRP solution, immediately (a), 7.5 (b), or 15 minutes (c) before perfusion. a) HRP-reactive products are seen along the outermost surface of the adventitia. × 18,000, bar = 1 μm. b) HRP-reactive products are seen in the intercellular space of the innermost portion of the media. × 18,000, bar = 1 μm. c) Both the subendothelial space, and the plasmalemmal vesicles of the endothelial cells are shown containing HRP-reactive products. × 18,000, bar = 1 μm, *SAS* subarachnoid space, *EL* elastic lamina, *SM* smooth muscle cell, *L* lumen

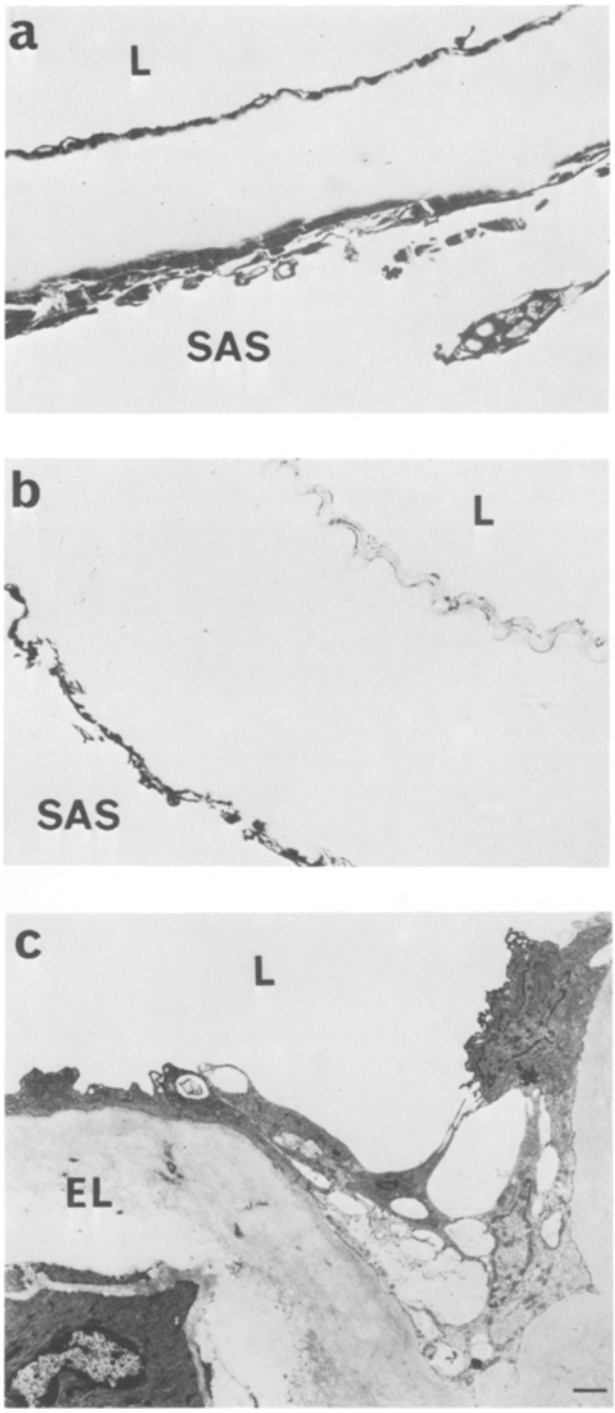
apparent from these results that a direct pathway of CSF circulation exists from the subarachnoid space to the subendothelial layer[20], and endothelial retrograde transport function[19] is present in normal cerebral arterial walls. On the 5th day after SAH, however, a smaller amount of HRP reached the subendothelial layer of basilar arteries 15 minutes after intracisternal injection of HRP. The extent of this decrease in the amount of HRP reaching the subendothelial layer was correlated with the thickness of the clot covering the external surface of the artery, and with the severity of the morphological changes of the arterial wall. These findings strongly suggest that the intramural CSF circulation is disturbed in the arterial wall when it has been surrounded by a subarachnoid haematoma. There are two possible mechanisms for this phenomenon:

1. The actual blood elements within the adventitia may interrupt the CSF pathway to the intima.

2. The opening into the subarachnoid space of the adventitial channel[20] may be blocked by morphological changes such as an increase in the tortuous processes of the fibroblasts and corrugation of the adventitial cells.

The interruption of the CSF pathway may play a role in the progression of the constrictive vasculopathy by introducing abnormalities in the metabolic environment around and within the arterial wall. In fact, it has been suggested that the CSF nourishes the cerebral arterial wall[20]. If so, by the interruption of the CSF pathway within the arterial wall, the smooth muscle cells may develop a state of malnutrition causing organic changes such as vacuole formation or necrosis. It is also probable that poor clearance of the CSF within the arterial wall would induce retention of vasoactive materials, such as erythrocyte breakdown products[9].

It remains to be elucidated whether the disturbance in intramural CSF circulation per se results in prolonged arterial constriction. Although the exact relationship between the intramural circulation and the pathological changes of the vessel wall is not yet clear in cerebral arteries, it has been well studied in systemic arteries whose intramural circulation is supplied by the vasa vasorum. Embolization of the arterial vasa vasorum was reported to lead to intimal thickening and medial necrosis in the canine abdominal aorta[8]. Sakurai *et al.*[12] reported that the disturbance of the venous vasa vasorum is responsible for the accumulation of plasma constituents within the arterial wall and resultant atheroma formation. Recently, we recognized by repeated angiography that the disturbance in the intramural circulation of arterial walls by removal of the



Figs. 5 a and b. Photomicrographs of the basilar arteries from a control dog without SAH (a) and a dog with SAH (b), both injected intracisternally by HRP solution 15 minutes before perfusion. HRP-reactive products are seen in the adventitia and the intima (a). HRP-reactive products are seen in the adventitia, but less than are seen in the intima (b). ×470, toluidine blue staining. *L* lumen, *SAS* subarachnoid space. c) Electronmicrograph of the same artery as (b). Corrugation of the elastic lamina and vaculation of endothelial cells are seen in the basilar artery. The subendothelial space is devoid of HRP-reactive products. × 5,6000, bar = 1 μm, *L* lumen, *EL* elastic lamina

adventitia including the vasa vasorum induces sustained vasoconstriction in carotid arteries (unpublished data).

Disturbances in intramural circulation, particularly the interruption of CSF drainage, may play a role in the progression of constrictive vasculopathy, *i.e.*, "vasospasm" following SAH. The removal of blood from the subarachnoid space in an operation performed early after haemorrhage has been reported by some authors to be beneficial to prevent delayed ischaemic neurological deficits[13, 15]. It is probable that the beneficial effect of this prophylactic haematoma removal results not only from the removal of vasoconstrictive substances but also from the re-establishment of normal CSF circulation within the arterial wall.

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