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RESPONSE OF CEREBRAL MICROVASCULATURE TO BRAIN INJURY

WILLIAM L. MAXWELL*, ALYSON IRVINE*, J. HUME ADAMS†, DAVID I. GRAHAM† AND THOMAS A. GENNARELLI‡

*Department of Anatomy, University of Glasgow, Glasgow G12 8QQ, U.K.
†University Department of Neuropathology, Southern General Hospital, Glasgow G51 4TF, U.K.
‡Division of Neurosurgery, University of Pennsylvania, Philadelphia, U.S.A.

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SUMMARY

There is increasing evidence that there is a direct response of the cerebral microvasculature to head injury. We have investigated using SEM and TEM the response of microvessels within the white matter of the baboon brain to lateral head acceleration. There is rapid endothelial disruption and swelling of perivascular astrocytes near the sites of petechial haemorrhage. The formation of microvilli in all vessels reaches a peak at 6 h and extends at least 5 mm from the site of haemorrhage. The astrocyte response suggests a partial recovery by 6 h. The endothelial response is most marked in arterioles and venules and is maintained for 6 days after injury. We suggest there is a biphasic cerebrovascular response to brain injury. First there is rapid astrocytic swelling possibly correlated with transient disruption of the blood–brain barrier. This is followed by morphological changes in the endothelium of all vessels which are most marked in arterioles and venules and extend considerable distances throughout the neuropile. This response is discussed in the light of disruption of the blood–brain barrier.

KEY WORDS—Endothelium, endothelial microvilli, perivascular astrocytes, cerebral microvasculature, electron microscopy, baboon, blood–brain barrier.

INTRODUCTION

There is now a considerable body of literature concerning the vascular response to experimental head injury (see ref 1 for a review). It has also been suggested that neural damage may be secondary to vascular damage or obstruction[2] as a result of (a) increased extravascular pressure from swollen tissue, petechial haemorrhage, and extraluminal clots; and (b) intravascular events. The latter may be correlated with changes in the functional status of the blood–brain barrier. The vascular response appears to be a function of the severity of the trauma.[1]

It is well recognized that endothelial microvilli occur in cerebral blood vessels in response to an ischaemic episode, but Dietrich *et al.*[3] stated that quantitative data were not available. Endothelial microvilli also develop after other types of cerebral insult[4,5] together with other endothelial alterations such as the formation of 'balloons' and 'craters'[5] and disruption of the blood–brain barrier.[1] After head injury, these vascular abnormalities appear to be caused by the dramatic post-traumatic hypertensive episode.[6] However, no study has either extended observations on the occurrence of endothelial alterations over a period of greater than 4 h after insult or reported a change in the distribution of endothelial alterations some distance from the site of the lesion. The formation of endothelial microvilli is extremely rapid under conditions of global ischaemia[3] but it seems unlikely that global ischaemia would occur in human head injury; rather there would be continued circulation, at least partial, during the traumatic and post-traumatic episodes.

We decided to investigate endothelial changes within the brain parenchyma using material in which diffuse axonal injury (DAI) had been induced

Addressee for corresondence: W. L. Maxwell, Department of Anatomy, University of Glasgow G12 8QQ, U.K.

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by lateral head acceleration in the anaesthetized baboon.[7] Under these conditions, petechial haemorrhages are particularly common in the central white matter. We have investigated the response of the brain microvasculature within the corpus callosum to determine (a) whether there is a differential response between different sized vessels within the microvasculature, (b) the time course of the development and possible regression of the microvilli after head acceleration with circulation maintained throughout the experimental period, (c) whether there is a response some considerable distance from the site of petechial haemorrhage.

METHOD

Scanning electron microscopy (SEM) was carried out on the microvasculature of the corpus callosum near regions of petechial haemorrhage after lateral head acceleration. Details of the method of induction of head acceleration, while under phencyclidine (1 mg/kg) katamine and nitrous oxide anaesthesia, are given elsewhere.[7] Experimental animals, subjected to lateral head acceleration, were electively killed at 20 min, 1, 4, 6, 12 h, and 6 days after the acceleration by transcardial perfusion with 5 per cent glutaraldehyde in phosphate buffer while under deep anaesthesia. Control animals were killed by perfusion while under deep anaesthesia.

After immersion fixation for at least 7 days, blocks of the corpus callosum, with areas of petechial haemorrhage, were removed and processed for wax embedding. Five μm paraffin sections were cut and examined for longitudinal sections of patent blood vessels. These could be readily seen in a flattened wax section floating on a drop of water and viewed with the microscope condenser racked down to increase contrast. Once a section had been obtained with blood vessels passing in the plane of the section into the area of haemorrhage, the block was dewaxed. It was then placed in molten wax for 5–10 min before

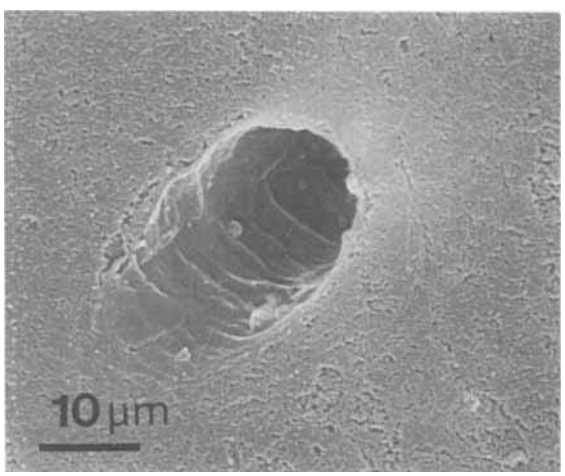

Fig. 1—A scanning electron micrograph of a control intermediate size vessel and the surrounding neuropile. The vessel endothelium is smooth and the neuropile compact

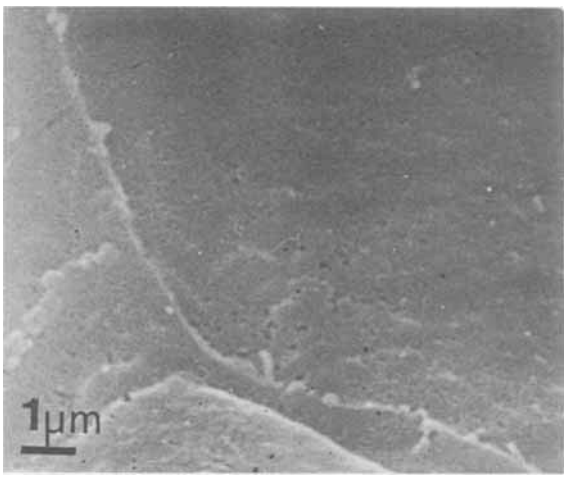

Fig. 2—A scanning electron micrograph of the endothelium of a control arteriole. The boundary line between two endothelial cells runs diagonally across the figure. There are a small number of microvilli on the boundary

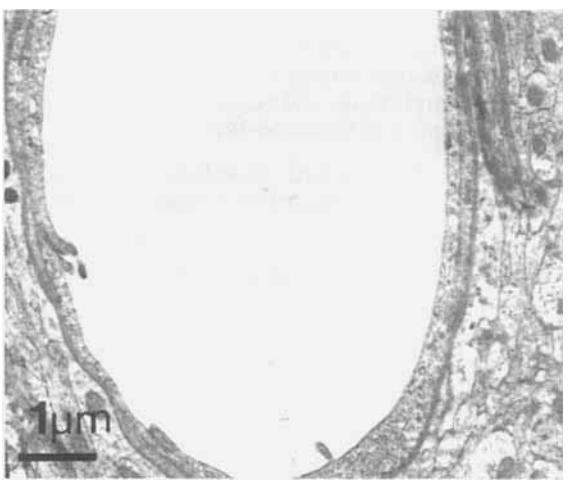

Fig. 3—A transmission electron micrograph of a control capillary showing the small number of microvilli on the endothelial surface

Table I—Mean number of microvilli/25 μm^2 area (and standard error)

	Capillaries	% Change from control	Intermediate vessels	% Change from control	Arterioles and venules	% Change from control
Control	4·875 ± 2·74		11·138 ± 4·82		5·90 ± 1·38	
20 min	8·018 ± 1·99	+64	14·24 ± 5·43	+28	19·059 ± 3·39	+223
1 h	11·228 ± 4·82	+130	16·67 ± 5·25	+50	16·937 ± 5·90	+187
4 h	14·714 ± 1·77	+202	19·30 ± 7·12	+73	20·04 ± 5·21	+240
6 h	18·241 ± 6·05	+274	17·00 ± 4·73	+53	40·11 ± 7·54	+580
12 h	4·05 ± 0·71	−20	14·59 ± 7·94	+31	17·738 ± 7·02	+201
6 days	3·73 ± 1·29	−23	13·37 ± 4·76	+20	23·63 ± 6·52	+300

Counts are the means of four fields per vessel; ten vessels per size category for each experimental group.

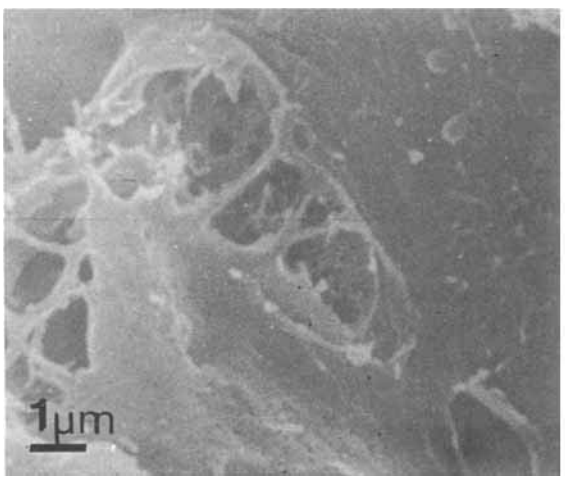

Fig. 4—A scanning electron micrograph of the endothelium of a capillary at 20 min survival near a region of petechial haemorrhage. Large crater-like deficiencies occur in the endothelium

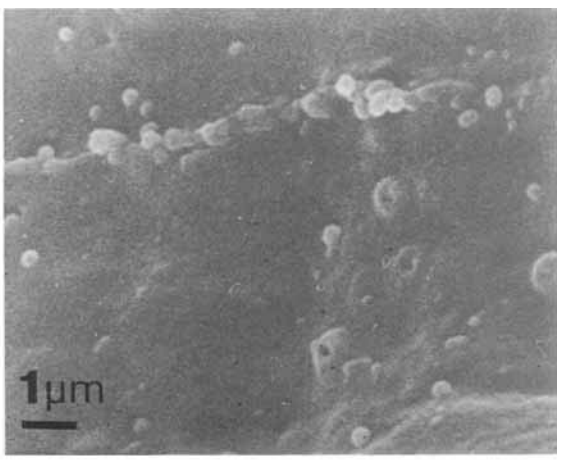

Fig. 5—A scanning electron micrograph of the endothelium of an arteriole at 20 min survival 3·3 mm from a haematoma. Microvilli are numerous along the boundary between the endothelial cells. Over the cell body a number of small balloons or umbilicated blebs are visible

being placed in xylene. The xylene was maintained at 37°C and changed four times over the next 24–48 h, each change using a new, clean, glass bottle, cap, and label to avoid a wax precipitate. The block was then transferred to absolute alcohol in new, clean bottles, followed by a second change before critical point drying. After gold/palladium coating, the specimen was viewed in a Jeol 300T SEM.

Photographs were taken at three magnifications along four perpendicular axes centred over the middle of the site of petechial haemorrhage. A field at × 100 allowed measurement of the distance between the larger vessels and the site of haemorrhage; × 1000 allowed measurement of the mean diameter of the vessel; × 7500 allowed counting of the number of microvilli/blebs/balloons within 25 μm^2 areas on the endothelial surface of the blood vessels. All vessels along each of the four axes were recorded from the centre of the haemorrhage to the edge of the tissue block, a distance of 4–5 mm.

The counts for each survival time were grouped according to vessel size: capillaries with a luminal diameter up to 10 μm, intermediate vessels (10–50 μm), and arterioles and venules (50–300 μm). For each survival period the counts for vessels within these size ranges were pooled, and the mean number of microvilli/25 μm^2 and the standard error calculated. These data were used to plot a table of

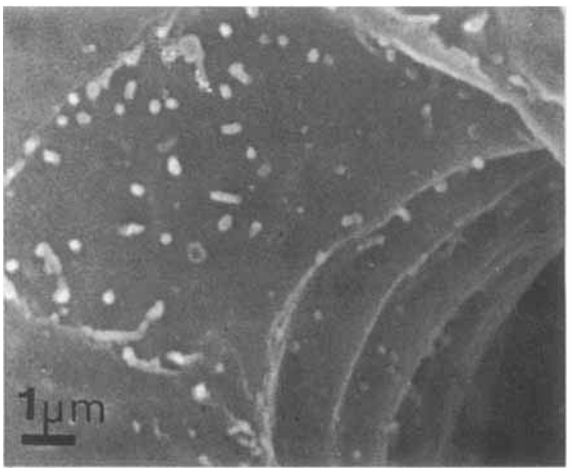

Fig. 6—A scanning electron micrograph of an arteriole at 20 min survival 1·6 mm from a petechia. Microvilli are numerous and occur in all areas of the endothelial surface

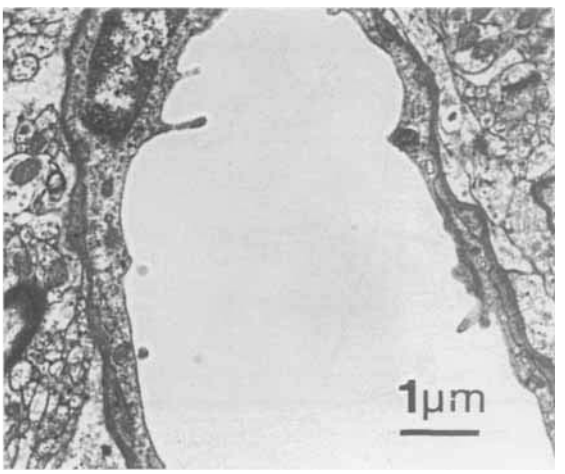

Fig. 8—A transmission electron micrograph of a capillary wall at 20 min survival and 4·2 mm from a petechia. Despite the occurrence of a number of microvilli on the endothelial surface, the related astrocyte foot processes appear normal

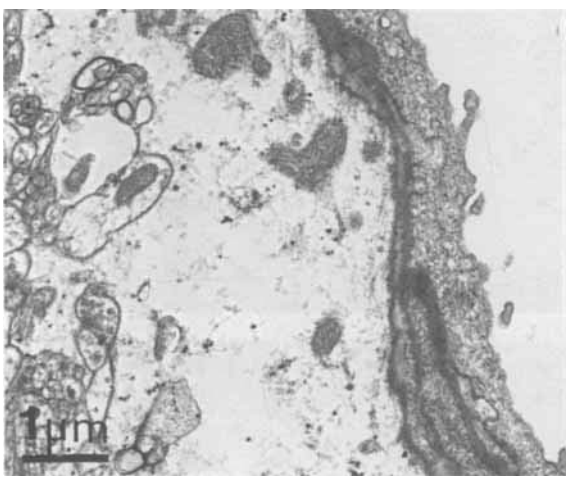

Fig. 7—A transmission electron micrograph of a capillary at 20 min survival and 2·0 mm from a haematoma. The underlying astrocyte foot processes are swollen and contain small groups of glycogen granules and intact mitochondria

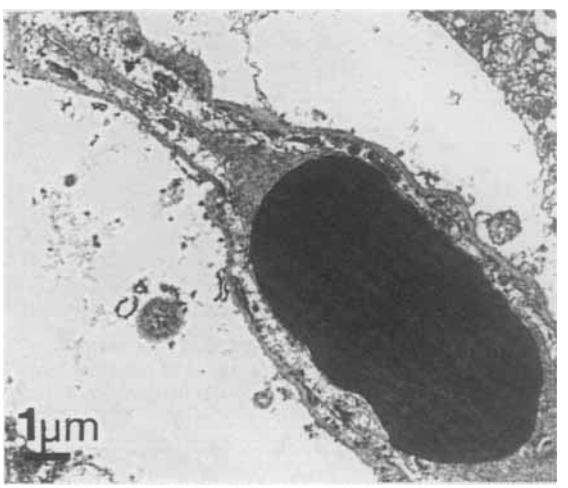

Fig. 9—A transmission electron micrograph of a capillary at 1 h survival within 500 μm of a petechia. There is blood stasis with erythrocytes trapped within the vessel lumen. The vessel is collapsed and surrounded by grossly enlarged astrocyte foot processes containing little recognizable cytoplasmic components

the numbers of microvilli/25 μm^2 in each category of vessel for each survival time. Further statistical analysis was not attempted since results were obtained from only one animal at each survival time.

After these analyses the tissues were carefully removed from the SEM stub and transferred to acetone to dissolve the silver paint used to attach the specimen to the SEM stub. The block was then cut into 2 mm cubes and transferred to propylene oxide before embedding in resin. Semi-thin and thin sections were cut from areas close to the gold-coated region of the block, and at right-angles to the gold-coated surface. The thin sections were stained with uranyl acetate and lead citrate before viewing in a Jeol 100S TEM. Thin sections allowed analysis of the integrity of the endothelium and astrocyte foot

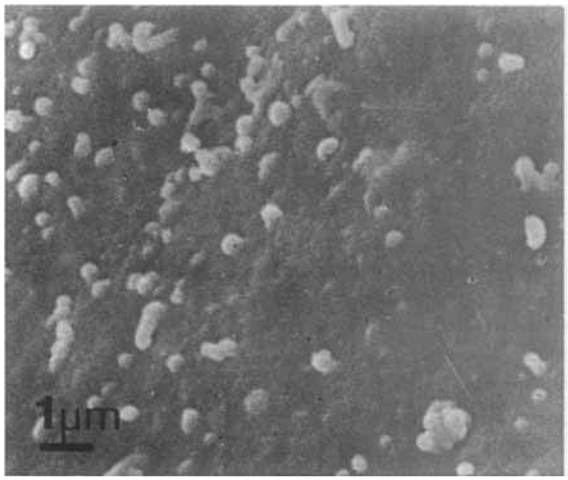

Fig. 10—A scanning electron micrograph of a capillary 1·5 mm from a petechia at 6 h survival. The endothelial surface is covered by numerous microvilli

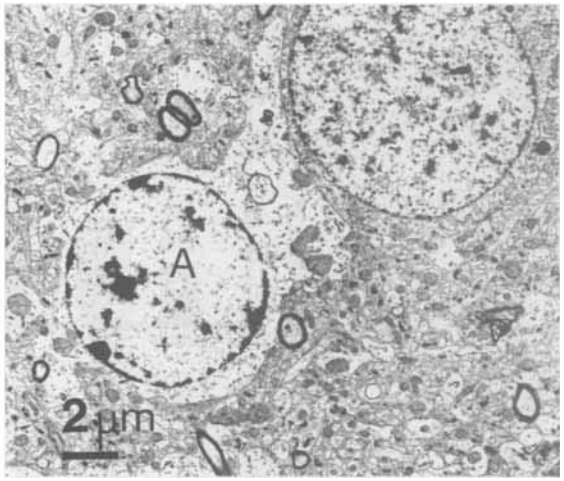

Fig. 12—A transmission electron micrograph of the neuropile at 6 h survival 3·5 mm from the petechia. The neuropile is intact but contains swollen astrocytes (A)

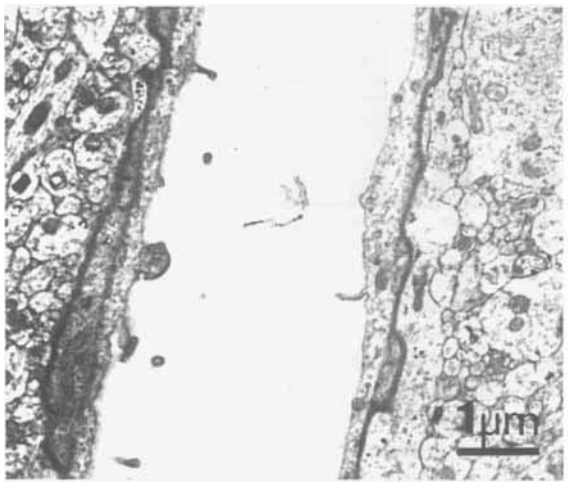

Fig. 11—A transmission electron micrograph of a capillary at 6 h survival 900 μm from a haematoma. Numerous microvilli occur on the endothelial surface, but the astrocyte foot processes are much less swollen than at 1 h

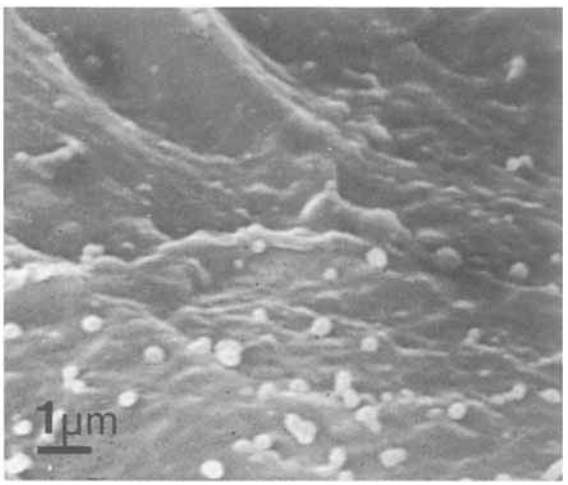

Fig. 13—A scanning electron micrograph of an arteriole at 6 days. There are still numerous microvilli on the endothelial surface

processes of vessels closely related to those previously examined in the SEM.

RESULTS

Control animals

Only a few microvilli were seen by SEM on the luminal surfaces of microvessels of all sizes (Figs 1 and 2), most of them along the border between endothelial cells. Counts (with standard errors) for control vessels in the three size categories are given in Table I. The mean number of microvilli/25 μm^2 was $7{\cdot}304 \pm 2{\cdot}98$ (Figs 14–16). TEM analysis showed an occasional microvillus (Fig. 3) in a blood vessel within normal brain parenchyma. The endothelial cells and related astrocyte foot processes were structurally normal.

Experimental animals

Within 20 min of acceleration of the head, major vascular disruption occurred immediately adjacent

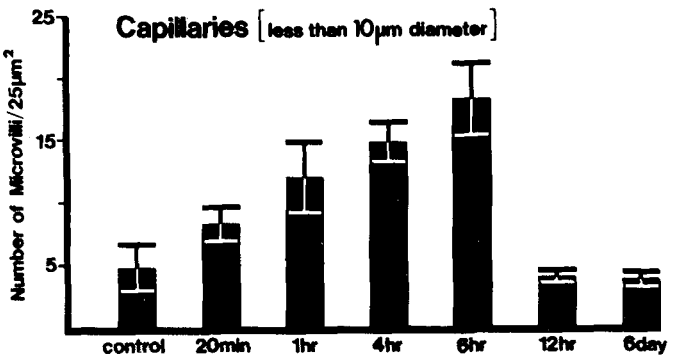

Fig. 14

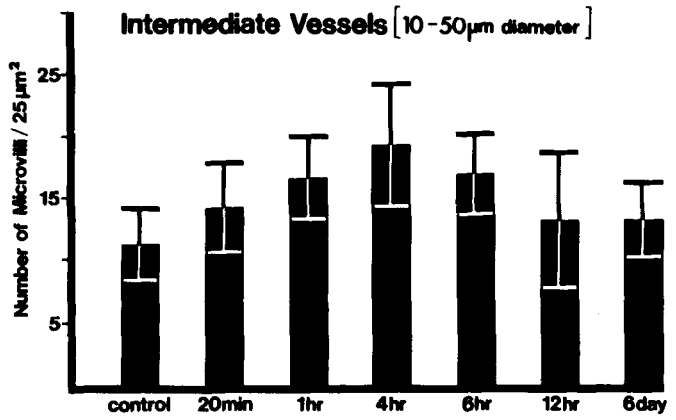

Fig. 15

to the area of petechial haemorrhage. SEM showed areas of disruption of endothelial cells with the production of craters (Fig. 4). Some distance (more than 2·5 mm) from the haemorrhage there were numerous cytoplasmic balloons and blebs, some of them umbilicated (Fig. 5). These observations support the suggestion of Wei *et al.*[6] that cytoplasmic balloons form first and then rupture to give rise to craters, the large craters occurring at the border between endothelial cells resulting from the coalescence of smaller ruptured balloons.

Counts of microvilli showed an increase in all categories of vessel (Table I; Figs 14–16) compared with the controls, but the increase was most marked in the arterioles and venules (Fig. 16). Microvilli were not only more numerous but were also now scattered more uniformly over the surface (Fig. 6), rather than being largely restricted to the cell boundary. With TEM there was marked swelling of the perivascular foot processes of astrocytes associated with reactive vessels (Fig. 7). However, this response did not occur uniformly throughout the tissue; in areas distant (more than 2·5 mm) from the haemorrhage petechia, astrocyte foot processes had a normal ultrastructure (Fig. 8) although microvilli were present on the endothelium.

By 1 h, the numbers of microvilli in capillaries and intermediate vessels had increased (Figs 14 and 15), but their numbers were still lower than in arterioles and venules. SEM and TEM suggest than there is stasis of blood with erythrocytes trapped within collapsed vessels, confirming other findings.[2] The astrocyte foot processes related to these vessels were disrupted, with loss of cytoplasmic organelles (Fig. 9). In other areas of the tissue containing unreactive vessels the astrocyte foot processes were intact.

Over the next 5 h there was a 62 per cent increase in microvilli in capillaries, a smaller increase in

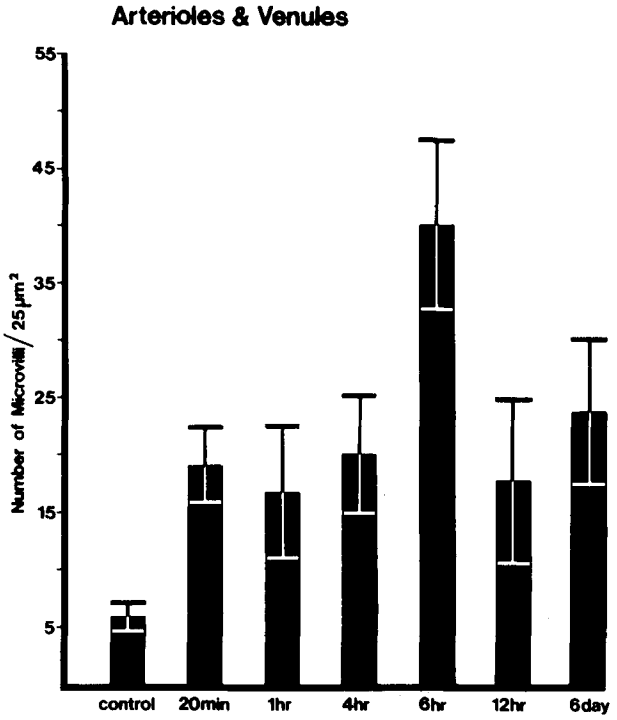

Fig. 16.

Fig. 14–16—Histograms for counts of microvilli/25 μm^2 area (with SE) in the three size categories of cerebral microvessel at the different survival times

intermediate vessels, but a most dramatic 137 per cent increase in arterioles and venules (Table I). Morphological changes were less dramatic than in the first hour but microvilli were still uniformly scattered in large numbers (Figs 10 and 11). Microvilli were present in all vessels up to 5 mm from the centre of a petechia. The response of the perivascular foot processes was now much less marked, although occasional vessels with swollen foot processes were still found. The neuropile had a normal ultrastructure except for the presence of swollen, electron lucent astrocytes (Fig. 12).

By 12 h, the numbers of microvilli in capillaries were reduced almost to control levels (Fig. 14). There was a slight reduction in intermediate vessels (Fig. 15), but in arterioles and venules the numbers remained high (Fig. 16). This differential was maintained even 6 days after injury, at which time the microvilli in arterioles and venules were still four times more numerous than in control animals (Figs. 13 and 16). The numbers in capillaries and intermediate vessels were reduced to control levels within 12 h of injury.

DISCUSSION

Several morphological changes occur in the cerebral microvasculature after a variety of cerebral insults.[1–5] However, no investigation has been extended to a longer post-traumatic interval than 4 h. It is also possible that the rate of microvascular response differs with the type of insult. Ischaemic attacks, global[3] or transient,[8] will obviously induce greater neuronal and glial damage than insults were the cerebral circulation is maintained throughout the experimental period. However, comparison of the endothelial response to global ischaemia in the rat[3] with our own material indicates that the numbers of microvilli occurring are very similar at 20 min survival (mean number 11·937 for ref. 3, 13·77 for our data). With recirculation for 10 min after a 30-min ischaemic period, Dietrich *et al.*[3] reported a 50 per cent reduction in the numbers of microvilli followed by a secondary increase after 1 h of reperfusion, and a further reduction up to 4 h after insult.

In the present experiments, in which cerebral circulation continued during and after the insult, the

time scale of the endothelial changes was different. Although numbers of microvilli were comparable at 20 min, there was a progressive increase in all vessels examined up to 6 h survival. The response was most marked in arterioles and venules, less so in capillaries, and least in vessels of 10–50 μm internal diameter. In the smaller vessels, there was then a loss of microvilli and a return to control values by 12 h. In arterioles and venules, the numbers of microvilli remained high until at least 6 days after injury.

The functional significance of these endothelial changes is obscure, but it is important to note that, after lateral head acceleration which induces DAI, major pathological changes occur in the microvasculature, in addition to neural damage. After contusion,[2] microvascular obstruction develops within 1 h but is more extensive at 3 h. We have extended these studies to show that the peak of microvascular response is not achieved until 6 h after injury. Since we did not expose the dura in order, say, to induce contusion, it can be argued that the microvascular changes occurring within the brain parenchyma may represent more accurately both the degree and the time scale of the cerebrovascular response to brain injury that might occur commonly in man. Our results also demonstrate that since the peak response does not occur until 6 h after the injury, early application of an appropriate treatment might reduce the progression of brain damage.

After high-level fluid percussion injury,[9] there is an acute increase in cerebral blood flow (CBF) but within 30 min CBF returns to preinjury values, while the cerebral perfusion pressure (CPP) is significantly decreased. Thus, cerebrovascular resistance is decreased after injury. It has been suggested[9] that long-term change (greater than 60 min survival) in vascular resistance occurs in pial arteries 200–400 μm in diameter. However, it has also been suggested[3] that increased microvillus formation may be implicated in post-ischaemic hypoperfusion. In the baboon, there is an abrupt elevation in systemic arterial pressure but cerebral perfusion pressure is less than 60 mm Hg for no longer than 2 min.[10,11] Thus, despite the cerebral perfusion pressure being within normal values for the greater part of the survival period, the greatest response is not obtained until 6 h after injury. This might suggest that the formation of microvilli is not directly related to short-term changes in cerebral perfusion but rather reflects changes in metabolic activity of the endothelial cells or the related neuropile.

After experimental high velocity penetrating head injury,[12] swelling occurs in perivascular astrocyte foot processes in vessels of all sizes both within and without the wound, within 30 min of injury. This astrocytic response occurs despite the absence of widespread severe neuronal and oligodendrocytic abnormality. We can confirm the rapid occurrence of swelling of astrocyte foot processes within 20 min of head acceleration in the neuropile adjacent to the area of petechial haemorrhage. More distant from the petechia astrocyte ultrastructure is normal. The astrocyte foot processes are grossly disrupted by 1 h but the response is much reduced by 6 h and at longer survivals the astrocyte ultrastructure is normal. This suggests that the reaction of the astrocyte foot processes is more rapid than the endothelial changes of reactive vessels, and that astrocyte recovery from the insult is well established by 6 h when the microvillous response is at its peak.

There is now a great deal of evidence for transient opening of the blood–brain barrier after brain injury[1,13] and it has been suggested that the endothelial cell membrane itself could be directly altered by trauma, so allowing diffusion of materials normally segregated by the intact blood–brain barrier of the cerebrovascular endothelium.[14] Other work[4,15] reports peroxidase leakage and raised levels of polyamines, reflecting disruption of the blood–brain barrier, within 2–3 min of fluid percussion or cryogenic brain injury. It has also been suggested that the polyamines[4] mediate stimulation of calcium fluxes raising free cytosolic Ca^{2+} in endothelial cells which triggers the acute activation of vesicular and carrier-mediated membrane transport, resulting in breakdown of the blood–brain barrier. Cryo-injury[4] also induces a five-fold increase in the relative volume of HRP vesicles and microvilli. Other work[14] has, however, questioned whether transendothelial vesicular transport truly reflects disruption of the blood–brain barrier after trauma. Rather the endothelial cell membrane is directly altered, allowing passage of tracers directly through the endothelial cell to reach the perivascular area. Perivascular astrocyte foot processes would be initially exposed to this altered chemical mileau and we suggest that the rapid swelling of astrocyte foot processes is a reflection of responses to disruption of the blood–brain barrier. The fact that the astrocyte swelling is rapid and has begun to recover by the time the microvillous response reaches its peak lends further support to this argument. Also, we were unable to demonstrate increased vesicular activity within reactive vessels.

Thus, we suggest two different responses in brain injury. First, a rapid, relatively localized astrocytic swelling where the blood–brain barrier is disrupted. Second, a more widespread and slower endothelial response resulting in the formation of microvilli on the luminal surface of endothelial cells. This response is most marked in the larger parenchymal vessels and persists for at least 6 days after injury.

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