Acta Neuropathol (1991) 82: 118 – 126

Acta Neuropathologica
© Springer-Verlag 1991

The relationship between plasma protein extravasation and remote tissue changes after experimental brain infarction*

C. Nordborg[1], T. E. O. Sokrab[2], and B. B. Johansson[2]

[1] Division of Neuropathology, Department of Pathology I, University of Göteborg, S-413 45 Göteborg, Sweden
[2] Department of Neurology, University of Lund, Lund, Sweden

Received October 9, 1990/Revised, accepted March 14, 1991

Summary. Extravasated endogenous serum albumin and fibrinogen were identified immunohistochemically in coronal brain sections from normotensive Wistar Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) after permanent ligation of the right middle cerebral artery. Infarcts were seen in all the SHR but only in 6 out of 14 WKY. Six hours after ligation, extravasated proteins were located primarily within the borders of the infarcts whereas after 24 h and later there was an increasing spread in the white matter. After 7 days, a protein immunoreactivity was seen far outside the infarcted areas, mainly in the white matter and occasionally extending somewhat into the contralateral side. Three weeks after permanent ligation, the immunoreactivity for plasma proteins had a similar extension but was less intense than after 7 days. A gliosis was noted within the protein-positive regions. From 72 h and onwards the immunoreactivity for albumin but not for fibrinogen extended via the white matter into the ipsilateral thalamic nuclei, where marked, mainly cytolytic nerve cell damage and gliosis was found. The close spatial correlation with albumin immunopositivity and the histological features of the thalamic lesions indicate that the propagation of extravasated plasma constituents or degradation products from the infarct may influence the character, timing and extent of remote tissue changes after cerebral infarction.

Key words: Brain infarct – vasogenic edema – thalamus – neuron – degeneration

* Supported by grants from the Swedish Medical Research Council (Project 14X-4968), the Swedish Heart Lung Foundation, King Gustaf V and Queen Victoria Foundation and from the 1987 Foundation for Stroke Research

Reported in part at the XIVth International Joint Conference on Stroke and Cerebral Circulation, San Antonio, Texas, February 9–11, 1989. Published as an abstract in Stroke 20:146 (1989)

Offprint requests to: C. Nordborg (address see above)

During the last few years a number of experimental studies have suggested that extravasated plasma constituents may exert a harmful effect on the brain tissue. Chronic, multifocal plasma extravasation is considered to be a major pathogenetic factor in spontaneously occurring brain lesions in stroke-prone spontaneously hypertensive (SHRSP) and renal hypertensive rats [6–8, 20–22]. The carotid infusion of hyperosmolar solutions causes an extravasation of plasma proteins as well as severe cerebral structural changes [24, 25]. Although the pathogenesis of brain lesions may be complex in the latter model, recent studies in fact indicate that the plasma constituents per se may be noxious. Thus, short-lasting blood-brain barrier (BBB) opening induced by adrenaline infusion, aortic clamping or epileptic seizures may cause neuronal damage with a spatial relationship to the extravasation of plasma proteins [26–28].

In cerebral infarction, extravasated plasma constituents spread outside the infarct border. The present study addressed the question of whether the transport of plasma components and/or degradation products along nerve fibre tracts might add to the cerebral tissue damage by causing remote parenchymal changes. Rats subjected to the occlusion of the middle cerebral artery (MCA) were, therefore, studied at various times after the insult in terms of the possible correlation between the spread of extravasated plasma proteins and parenchymal morphological changes. Spontaneously hypertensive rats (SHR), which are known to develop large cerebral infarcts after MCA ligation, were used, as well as normotensive Wistar Kyoto rats, which develop smaller and less constant infarcts [3, 5, 11].

Material and methods

Four- to five-month-old male SHR (n = 15) and WKY (n = 14) were used. The animals were anesthetized with methohexital (Brietal) 50 mg/kg i.p. Catheters were inserted in the tail artery and one tail vein. After awakening, the animals were given a period of 60 min to recover before the mean arterial blood pressure (MAP)

was measured. The rats were re-anesthetized with methohexital (0.5% solution) 1 mg/kg i.v. with additional doses as needed during the operation. Normothermia was maintained by external heating regulated by rectal temperature. The trachea was exposed via a small midline incision and an endotracheal tube was inserted under direct eye observation. The animals were mechanically ventilated with an oxygen-room air mixture (20% : 80%). Arterial blood samples were drawn for the determination of pH, pO_2, pCO_2, glucose concentration and hematocrit. The operative procedure was essentially the same as that described by Tamura et al. (29). The right MCA was occluded between the lenticulo-striate artery and the rhinocortical branch by means of a square knot using a 10-0 mono-filament nylon thread. The rats thus subjected to permanent MCA ligation were then allowed to live for 6 h (three SHR; two WKY), 24 h (two SHR; two WKY), 72 h (two SHR; two WKY), 1 week (four SHR; four WKY) or 3 weeks (four SHR; four WKY). After the post-operative survival time, all the rats were re-anesthetized and, after an inital flush with physiological saline for about 1 min the brains were fixed by perfusion through the left heart ventricle with a 4% formaldehyde solution in 0.1 M phosphate buffer, at pH 7.4 and 37 °C.

The brains were sectioned in 2.0-mm-thick coronal sections in a brain cutter and were then dehydrated and embedded in paraffin. For immunohistochemistry, 5-μm-thick sections were placed on chrome gelatine-coated slides. Extravasated plasma proteins were demonstrated with anti-rat albumin (1 : 8000, 1 : 16 000) and anti-human fibrinogen (1 : 2000; Dakopatts A/S, DK 2600, Glostrup, Denmark), which has been shown to cross-react with rat fibrinogen on agarose gel immunodiffusion [6]. Glial reaction was demonstrated with antiserum to bovine glial fibrillary acidic protein (1 : 1000, Dakopatts A/S), which cross-reacts with rat GFAP (Dakopatts; product information). Bound antibodies were visualized with a commercially available avidin-biotin-peroxidase complex (Vectastain ABC-kit, Vector Laboratories, Burlingame, Calif.) using diaminobenzidine as a chromogen. Alternative sections for routine light microscopy were stained with hematoxylin-eosin or a combination of 0.1% celestine blue and 1% acid fuchsin.

Routine histological and immunohistochemical findings in the infarcted hemisphere were compared with those on the contralateral side in the same coronal section. The infarct size was determined with point counting on coronal sections from the infarcted hemisphere. A x1 objective and a x10 eye-piece with a square lattice grid was used. The grid was oriented randomly over the section. The infarct volume was expressed as number of point hits over the infarct in per cent of total number of hemispheric hits.

Results

Body weights, mean arterial pressures (MAP), blood gases, hematocrit and blood glucose levels are given in Table 1.

Table 1. Physiological parameters

	n	Body weight (g)	MAP (mmHg)	PaO_2 (kPa)
WKY	14	313 ± 42	125 ± 25	13.2 ± 3.1
SHR	15	305 ± 38	173 ± 35	13.0 ± 2.4
	PCO_2 (kPa)	pH	Hematocrit (%)	Blood glucose (mmol/l)
WKY	5.7 ± 1.1	7.41 ± 0.06	50.5 ± 2.3	7.4 ± 1.2
SHR	5.8 ± 0.8	7.40 ± 0.04	50.7 ± 2.0	7.5 ± 1.1

Table 2. Infarct volume in percent of ipsilateral hemisphere volume

	6 h	24 h	72 h	1 week	3 weeks
SHR	37	38	13	28	15
	29	40	36	23	14
	37			28	11
				52	21
WKY	22	32	0	15	2
	7	0	0	4	0
				0	0
				0	0

Cortex and white matter

Large cortical infarcts, extending into the lateral caudatoputamen in some animals, were seen in all the SHR (n = 15) subjected to permanent MCA ligation. Only 6 of the 14 WKY developed infarcts, which were smaller on average than those in the SHR (Table 2); three of the infarcts were confined to part of the caudatoputamen. There was no edematous expansion of the infarcts in the rats which survived for 3 weeks after ligation, which explains the smaller relative volume of the lesions in these animals. The plasma protein extravasation was mainly confined to the infarcts 6 h after ligation, whereas perifocal and subpial spread was seen after 24 h. The spread of plasma constituents into the bundles of the internal capsule and the corpus callosum increased during the 1st post-operative week and a faint immunopositivity occasionally extended slightly over the midline into the contralateral portion after 7 days in some SHR as well as in one of the WKY which had developed an infarct. Three weeks after ligation the spread of plasma proteins in the cortex and white matter was similar to that seen after 7 days, but the staining was generally weaker. From 72 h post-operatively and onwards, a positive GFAP staining was seen in the regions which were positive for plasma proteins, i.e., perifocally and subpially in the infarcted hemisphere as well as in the adjacent white matter, bundles of the internal capsule and in the corpus callosum, where it extended somewhat into the contralateral hemisphere in some animals. There was no apparent difference in the spreading pattern of plasma proteins between infarcted SHR and the few WKY, which developed infarcts. A marked attenuation of the peripheral cytoplasm was seen in scattered nerve cells close to the infarct border.

Thalamus

Seventy-two hours after ligation in SHR an immunopositivity for albumin but not for fibrinogen was noted in the most lateral part of the ipsilateral thalamus, with the exception of the reticular nucleus. Since the thalamic immunopositivity was continuous with that in the internal capsule, it appeared to be the result of a spread of extravasated proteins from the infarct. The protein

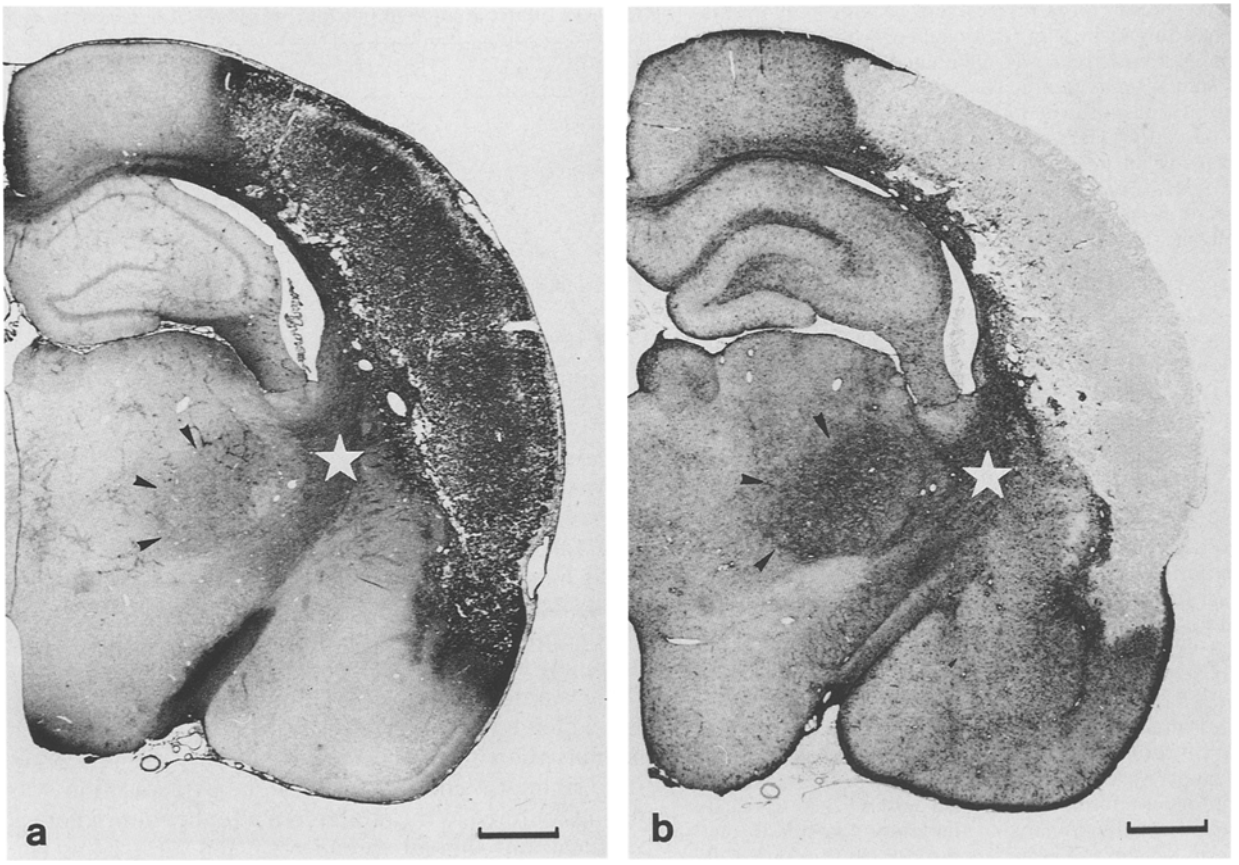

Fig. 1 a, b. The right hemisphere of a spontaneously hypertensive rat (SHR) subjected to 7 days of permanent middle cerebral artery (MCA) ligation. **a** The immunopositivity for albumin has spread from the infarct into the white matter and also extends from the internal capsule *(star)* into the ventral thalamic nuclei *(arrowheads)*. **b** A marked gliosis is seen around the infarct, in the white matter *(star)* and in the ventral, thalamic nuclei *(arrowheads)* **a** Anti-albumin + hematoxylin; **b** anti-GFAP + hematoxylin. Bars **a, b** = 1000 μm

deposition was more prominent after 7 days (Fig. 1). The immunopositivity, which had a finely granular component (Fig. 2), extended into the medial geniculate body in all the SHR and into the lateral geniculate body in sections where this nucleus could be localized. In contrast to the white matter, there was no decrease but instead an increase in immunopositivity with time. After three weeks, the lateral part of the thalamic lesion displayed a coarse granular immunopositivity for albumin, whereas the staining in its medial parts was finely granular (Fig. 3). The tissue appeared normal in routine stains 72 h after ligation, but in the SHR which were allowed to survive for 7 days there was a very close spatial correlation between neuronal changes and the albumin deposition, which was found in the neuropil. The majority of the neuronal perikarya were albumin negative and appeared to be "cytolytic" with an attenuated peripheral cytoplasm and nuclei which were somewhat reduced in volume. Other nerve cells were condensed with strongly acidophilic, albumin-positive cytoplasm and fragmented nuclei. A moderate gliosis was found in the same area (Fig. 2).

More advanced thalamic lesions were encountered in the SHR which were killed 3 weeks after ligation. The lateral part of the lesions appeared to be spongiotic with dilated microvessels. The volume and number of neurons was reduced but neither "cytolytic" nor acidophilic cells were found. There was a very heavy gliosis with numerous large astrocytes some of which were strongly albumin-positive. The medial part of the lesion had an appearance similar to that 7 days after ligation with numerous "cytolytic" nerve cells and a milder gliosis. However, there were no strongly acidophilic, shrunken neurons (Fig. 3, 4).

Two infarcted WKY, which were allowed to survive for 7 days after ligation, displayed a thalamic immunopositivity for albumin, which also extended into the lateral and medial geniculate bodies in one of the rats. There was a moderate gliosis within albumin-positive areas. Scattered nerve cells were densely acidophilic but no cytolytic neurons were encountered. The only infarcted WKY which was kept alive for 3 weeks after ligation had a spongiotic, gliotic thalamic lesion and there was also some gliosis in the medial geniculate body. Neither extravasated protein, neuronal degeneration nor gliosis was encountered in the contralateral thalamus in any of the SHR or infarcted WKY included in the present study and thalamic lesions were not seen on the ligated or contralateral side in any of the non-infarcted WKY.

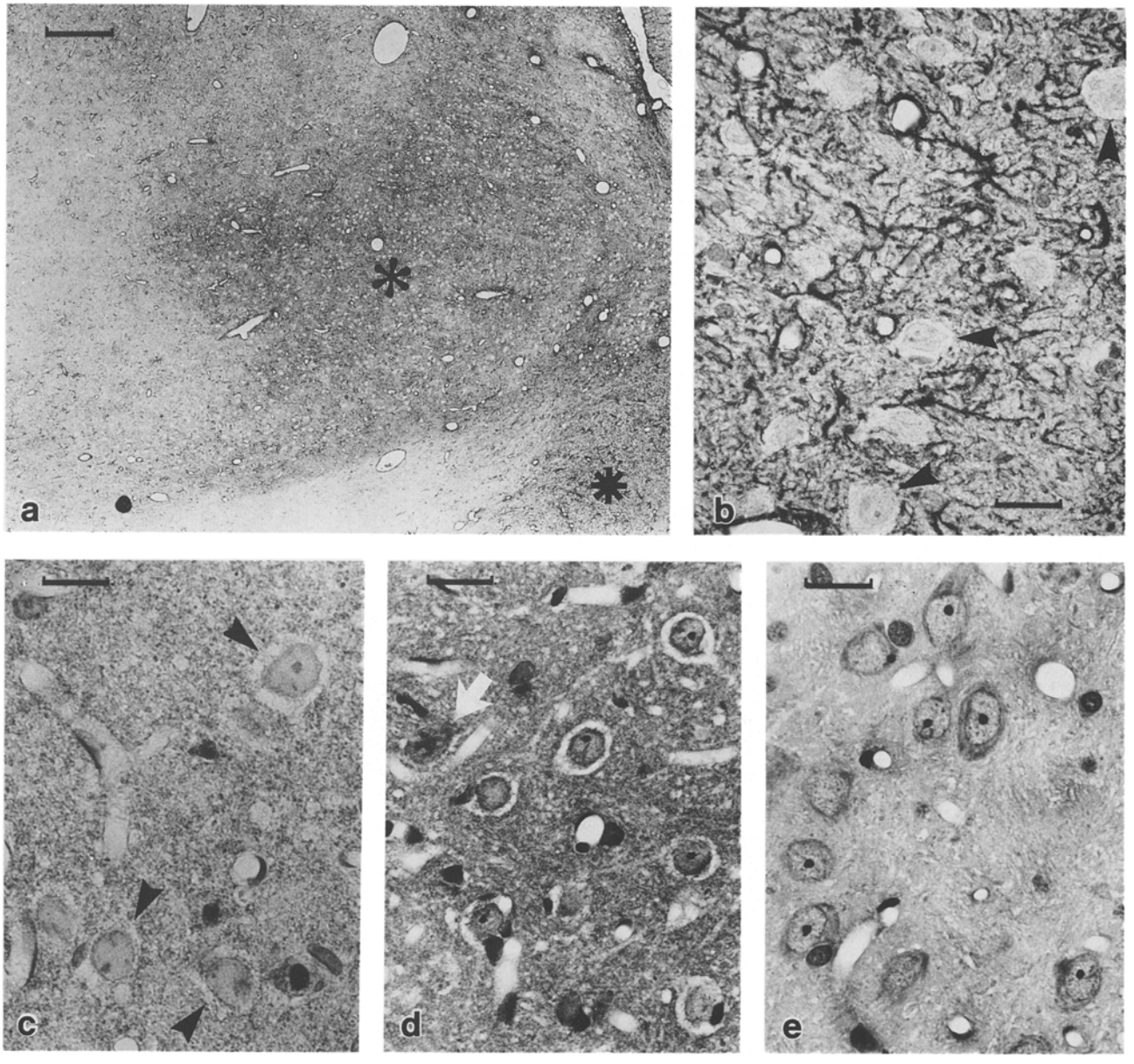

Fig. 2 a–e. Thalamic lesion in the infarcted hemisphere of an SHR after 7 days of permanent, unilateral MCA ligation. **a** Gliosis in the ventral nuclei *(six-pronged asterisk)* and in the internal capsule *(asterisk)*. **b** The gliosis was moderate with slender astrocytes among the cytolytic nerve cells (*arrowheads*). **c** There was a finely granular immunopositivity for albumin in the neuropil and in scattered shrunken nerve cells, but the cytolytic nerve cells appeared to be negative *(arrowheads)*. **d** Marked attenuation of the peripheral cytoplasm and a somewhat reduced nuclear size in cytolytic nerve cells. Scattered nerve cells appeared to be shrunken and acidophilic, often with a fragmented nucleus *(arrow)*. The neuronal changes were strictly confined to albumin-positive parenchyma. Note the slightly spongiotic neuropil. **e** Contralateral thalamus with denser but less acidophilic neuropil. **a, b** Anti-GFAP + hematoxylin; **c** anti-albumin + hematoxylin; **d, e** acid fuchsin + cresyl violet. Bars **a** = 330; **b, d, e** = 24; **c** = 16 μm

Substantia nigra and locus coeruleus

A gliosis was found in the ipsilateral substantia nigra, mainly its reticular part, in rats with a caudoputamen lesion (Fig. 5). Neuronal cytolysis was seen in this nucleus 7 days after ligation in one SHR, which had an extensive caudatoputamen infarction (Fig. 5). In this rat alone there was an immunopositivity for albumin in the nigral neuropil, which was spatially well correlated with the cytolytic neuronal change.

There were no cytolytic nerve cells in the locus coeruleus in any of the animals at 7 days or 3 weeks after ligation. A moderate gliosis was encountered in the ipsilateral nucleus in one SHR 7 days after ligation (Fig. 6).

Discussion

The immunohistochemical findings in the present study as well as previous observations clearly demonstrate that

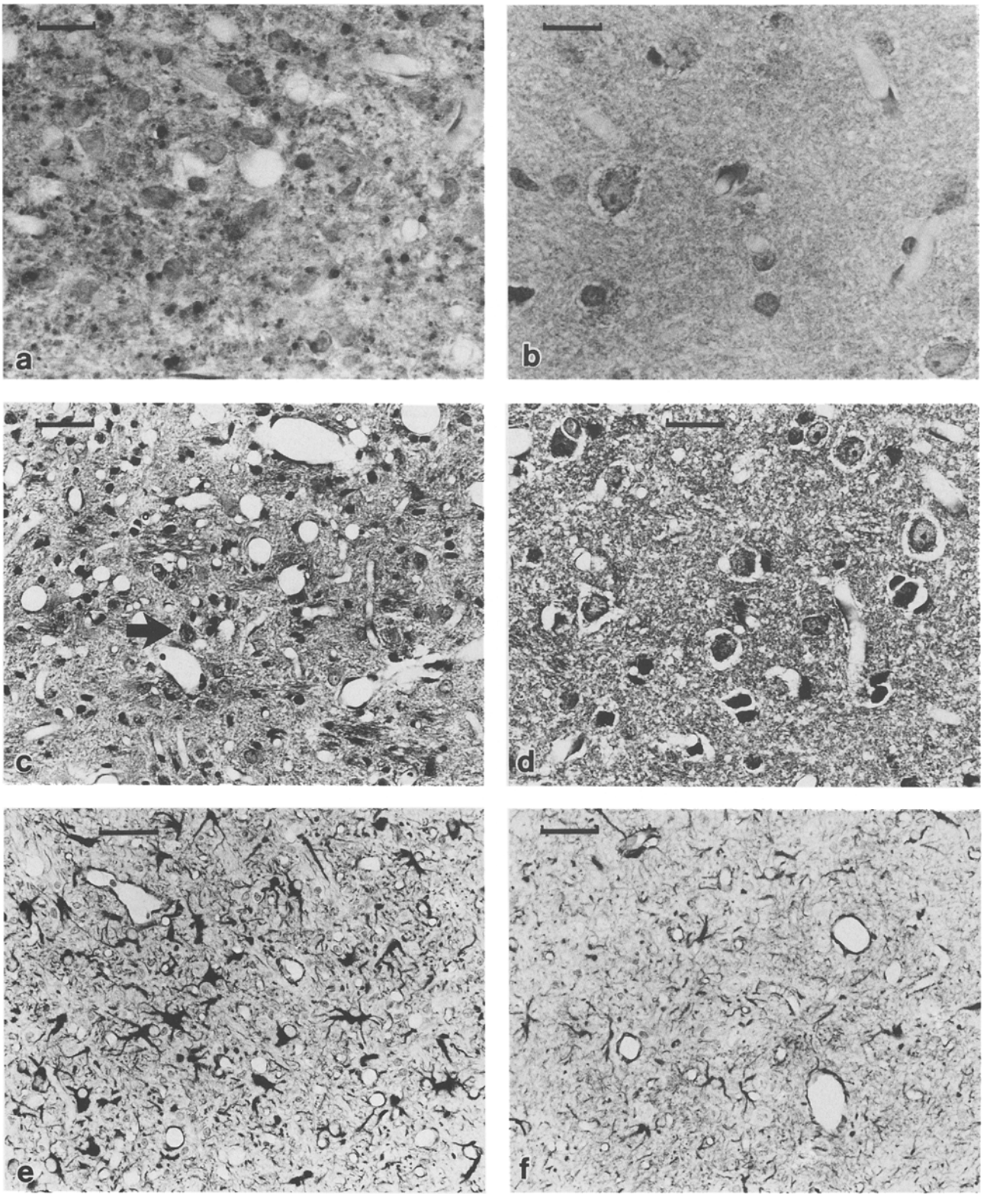

Fig. 3a–f. Thalamic lesion in the infarcted hemisphere of an SHR after 3 weeks of permanent, unilateral MCA ligation. **a** In the lateral part of the lesion there was a coarse granular immunopositivity for albumin in the neuropil as well as some positivity in astrocytes and neurons. **b** In the medial part of the lesion the immunopositivity for albumin was finely dispersed in the neuropil. **c** The lateral part of the lesion was markedly spongiotic. The number of neurons *(arrow)* was reduced, but there was no attenuation of the peripheral neuronal cytoplasm. **d** A marked attenuation of the peripheral cytoplasm was seen in numerous nerve cells in the medial part of the lesion and the neuropil was acidophilic and slightly spongiotic. **e** A heavy gliosis with numerous large astrocytes was seen in the lateral part of the lesion. **f** The gliosis was moderate in the medial lesion with slender astrocytes. **a, b** Anti-albumin + hematoxylin; **c, d** acid fuchsin + cresyl violet; **e, f** anti-GFAP + hematoxylin. Bars **a, b** = 16; **c** = 48; **d** = 24; **e, f** = 95 μm

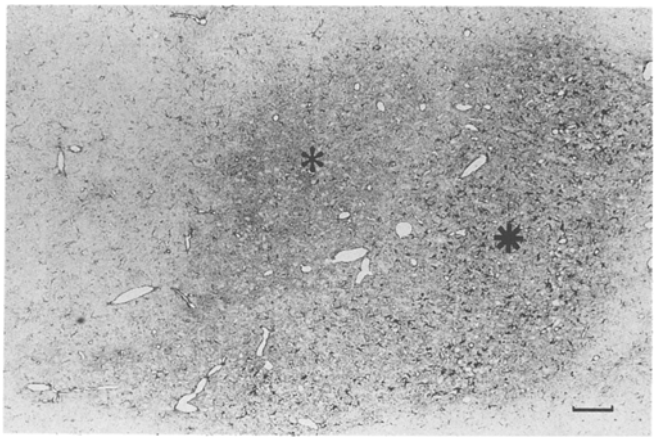

Fig. 4. Thalamus of the infarcted hemisphere in an SHR 3 weeks after permanent MCA ligation. The gliosis is more advanced in the lateral part of the lesion *(asterisk)* than in the medial part *(six-pronged asterisk)*. Anti-GFAP + hematoxylin. Bar = 167 μm

extravasated plasma proteins spread more readily in white than in gray matter [2, 13, 14]. The relationship between GFAP and plasma protein immunoreactivity indicates that the accumulation of plasma constituents and/or degradation products from the infarct may be harmful to the brain tissue and cause astrocytic reaction, although the degeneration of nerve fiber tracts from the infarct and retrograde neuronal degeneration evidently also cause gliosis [12]. The latter was illustrated by an apparent GFAP reaction despite negative protein stainings in the substantia nigra and the locus coeruleus.

It is unlikely that the lesions in the thalamic nuclei are ischemic. The arterial supply to thalamus in normotensive rats is not affected in the present experimental model [30]. Furthermore, the thalamic blood flow on the right, ligated side is not decreased compared to the left, non-ligated side in SHR studied in our laboratory (Zhang, Grabowski and Johansson, in preparation). Moreover, the noticed discrepancy between a strong positivity for albumin and a negative staining for

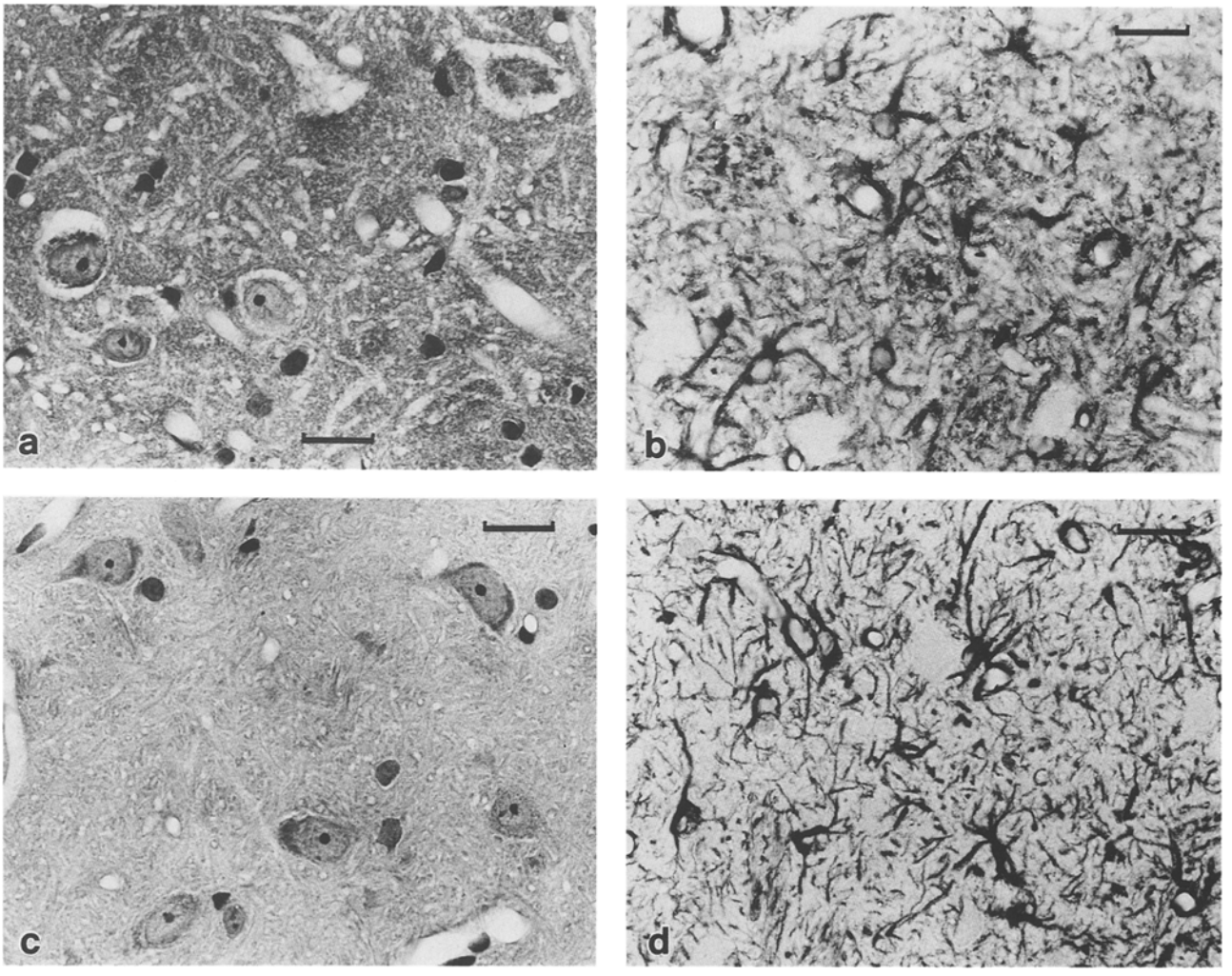

Fig. 5a–d. The ipsilateral substantia nigra in animals with an infarction extending into the caudatoputamen 7 days after MCA ligation. **a** In the animal with an immunopositivity for albumin in the nigral neuropil, the nerve cells were cytolytic with attenuated peripheral cytoplasm. Slight spongiosis of the neuropil. **b** A moderate gliosis was seen in the same region. **c** In animals with albumin-negative substantia nigra, the neuropil was dense and less acidophilic. There was no cytolysis but a moderate gliosis **(d). a, c** Acid fuchsin + cresyl violet; **b, d** anti-GFAP + hematoxylin. Bars **a–d** = 24 μm

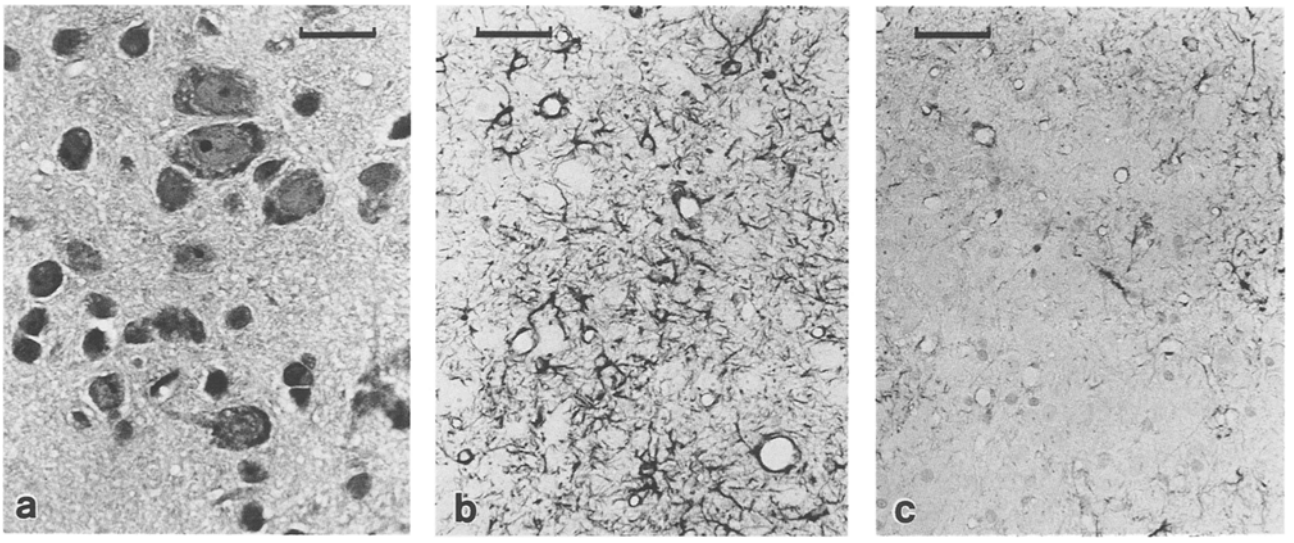

Fig. 6. **a** The ipsilateral locus coeruleus in an SHR with a brain infarct 7 days after MCA ligation. The neuropil is dense and there is no cytolytic neuronal change. **b** The gliosis is moderate. **c** Contralateral nucleus without obvious gliosis. **a** Acid fuchsin + cresyl violet; **b, c** anti-GFAP + hematoxylin. Bars **a** = 24; **b, c** = 48 μm

fibrinogen makes a local barrier lesion secondary to parenchymal damage less likely, as it would be expected to cause leakage of both proteins [6, 27]. Rather, the continuous immunopositivity, which could be followed from the internal capsule into lateral parts of the thalamus, indicates that the conspicuous albumin deposition in the thalamic nuclei is the result of a direct spread of edema from the infarct. Tracts from the hemisperic cortex converge at the thalamus and the drainage of edema fluid from large cortical areas might, thus, cause a concentration of plasma constituents in its nuclei. To what extent the strong positivity for albumin after 3 weeks is due to a protracted or recurrent plasma leakage from the infarct remains to be investigated; a biphasic BBB opening has been observed following temporary MCA occlusion [15] but has to our knowledge not been described in permanent occlusion. The long-standing immunopositivity may also indicate that the clearance of plasma constituents is not as effective in the thalamus as it is elsewhere in the brain tissue. It is, in accordance with the discussion above, not likely due to a local protracted or recurrent leakage, since a BBB lesion leading to such a marked accumulation of albumin would also be expected to leak fibrinogen. Likewise, the finely granular albumin positivity, which was seen after 3 weeks medial to the coarsely granular lesion in the lateral thalamus, is most likely due to a propagation of extravasated substances in medial direction.

Macromolecules may be transported intra-axonally from a focal vasogenic edema to remote parts of the brain, even into the contralateral hemisphere [31]. The extent to which the macromolecular transport is intra-axonal in the present model cannot be revealed using light microscopy. An ultrastructural-immunohistochemical analysis is being conducted with a view to elucidating this question. The fine granularity of the albumin stain in the white matter and thalamus may indicate an intraneuronal route [19, 23, 31]. However, the presence of shrunken, albumin-positive thalamic neurons probably reflects the uptake of extracellular protein by dying nerve cells and is no proof of retrograde axonal transport [1, 31]. Moreover, the cytolytic nerve cell change does not appear to be the result of a retrograde axonal transport of noxious substances, since all such neurons were albumin negative.

The ipsilateral thalamic degeneration which is seen after cortical destruction in experimental animals and in stroke patients with thalamic syndromes is ascribed to retrograde neuronal degeneration and the loss of cortico-fugal afferent input, but the pathogenetic mechanisms are not known in detail [10, 17, 18]. The present study revealed a firm spatial correlation between immunopositivity for albumin and thalamic neuronal degeneration. The cytolytic nerve cell change encountered in the thalamus was also found to a limited extent in the edematous border zone of the infarct and it has previously been described in spontaneous edematous lesions in SHRSP [6, 7]. Although the pathogenesis of this cell change is not known, it has been suggested that it is excitotoxic [7]. The present findings, thus, strongly suggest that noxious substances, which are transported along nerve fiber tracts from the infarct, influence the timing, extent and character of the thalamic neuronal degeneration. Accordingly, there were no cytolytic or acidophilic nerve cells nor albumin positivity but only some gliosis in the ipsilateral locus coeruleus, which had also lost its cortical connections. Moreover, in animals with a caudatoputamen lesion, cytolysis was found in the substantia nigra only when the albumin immunopositivity extended into this nucleus.

The fact that cytolytic thalamic neurons were not seen 1 week after ligation in those WKY which developed infarcts may indicate that the propagation of extravasated substances is more rapid in SHR than in WKY. In any condition which affects the BBB, the spread of extracellular substances is highly related to

blood pressure [14]. The present observation does not rule out the pathogenetic role of noxious substances transported from the infarct. On the contrary, the lack of cytolytic neurons in infarcted WKY indicates that a loss of thalamo-cortical projection and cortico-fugal afferent input could not be the only cause of the cytolytic thalamic lesions which were encountered in SHR 1 week after MCA ligation.

The somal response to axon disruption varies greatly between different neuronal populations in the central nervous system [4, 16]. Cortical ablation, therefore, causes extensive thalamic neuronal degeneration, which resembles that in edematous lesions of SHRSP ultrastructurally [18]. On the other hand, axon disruption causes little neuronal response in some other nerve cell populations [9]. The present combined routine histological and immunohistochemical findings indicate that the propagation of noxious substances with the vasogenic edema bulk flow from a tract lesion to the corresponding neuronal perikarya may be one of the modifying factors leading to these discrepancies. It might also serve as one explanation of the differing somal response and improved survival after axonotomy in spinal and cranial motor neurons compared with neurons located entirely within the central nervous system.

In conclusion, a mapping of extravasated plasma proteins after induced brain infarction in rats showed an extension of albumin positivity into the ipsilateral thalamus and, occasionally, into the ipsilateral substantia nigra. Cytolytic nerve cell damage was found in the two latter locations. A close spatial correlation between the cytolytic neuronal change and albumin immunopositivity indicates that the spread of extravasated plasma constituents or degradation products after brain infarction may influence the character, extent and timing of remote, secondary brain lesions.

Acknowledgements. The skilled assistance of Anna-Lena Olsson, Madeleine Jakobsson, Karin Jansner and Hans-Olof Ivarsson is gratefully acknowledged.

References

1. Brightmann MW, Klatzo I, Olsson Y, Reese TS (1970) The blood-brain barrier to proteins under normal and pathological conditions. J Neurol Sci 10:215–239
2. Chui E, Wilmes F, Sotelo JE, Horie R, Fujiwari K, Suzuki R, Klatzo I (1981) Immunocytochemical studies on extravasation of serum proteins in cerebrovascular disorders. In: Cervós-Navarro J, Fritschka E (eds) Cerebral microcirculation and metabolism. Raven Press, New York; pp 121–127
3. Coyle P (1986) Different susceptibilities to cerebral infarction in spontaneously hypertensive (SHR) and normotensive Sprague-Dawley rats. Stroke 17:520–528
4. Cragg BG (1970) What is the signal for chromatolysis? Brain Res 23:1–21
5. Duverger D, MacKenzie ET (1988) The quantification of cerebral infarction following focal ischemia in the rat: influence of strain, arterial pressure, blood glucose concentration and age. J Cereb Blood Flow Metab 8:449–461
6. Fredriksson K, Auer RN, Kalimo H, Nordborg C, Olsson Y, Johansson BB (1985) Cerebrovascular lesions in stroke-prone spontaneously hypertensive rats. Acta Neuropathol (Berl) 68:284–294
7. Fredriksson K, Kalimo H, Nordborg C, Johansson BB, Olsson Y (1988) Nerve cell injury in the brain of stroke-prone spontaneously hypertensive rats. Acta Neuropathol 76:227–237
8. Fredriksson K, Kalimo H, Nordborg C, Olsson Y, Johansson BB (1988) Cyst formation and glial response in the brain lesions of stroke-prone spontaneously hypertensive rats. Acta Neuropathol 76:441–450
9. Fry FJ, Cowan WM (1972) A study of retrograde cell degeneration in the lateral mamillary nucleus of the cat, with special reference to the role of axonal branching in the preservation of the cell. J Comp Neurol 144:1–24
10. Fujie W, Kirino T, Tomukai N, Iwasawa T, Tamura A (1990) Progressive shrinkage of the thalamus following middle cerebral artery occlusion in rats. Stroke 21:1485–1488
11. Grabowski M, Nordborg C, Brundin P, Johansson BB (1988) Middle cerebral artery occlusion in the hypertensive and normotensive rat: a study of histopathology and behaviour. J Hypertens 6:405–411
12. Iizuka H, Sakatani K, Young W (1989) Corticofugal axonal degeneration in rats after middle cerebral artery occlusion. Stroke 20:1396–1402
13. Kalimo H, Fredriksson K, Nordborg C, Auer RN, Olsson Y, Johansson BB (1986) The spread of brain oedema in hypertensive brain injury. Med Biol 64:133–137
14. Klatzo I, Wisniewski H, Steinwall O, Streicher E (1967) Dynamics of cold injury edema. In: Klatzo I, Seitelberger F (eds) Brain edema. Springer-Verlag, Berlin Heidelberg New York, pp. 554–563
15. Kuroiwa T, Ting P, Martinez H, Klatzo I (1985) The biphasic opening of the blood-brain barrier to proteins following temporary middle cerebral artery occlusion. Acta Neuropathol (Berl) 68:122–129
16. Lieberman AR (1971) The axon reaction: a review of the principal features of perikaryal responses to axon injury. Int Rev Neurobiol 14:49–124
17. Martin JJ (1969) Thalamic syndromes. Handb Clin Neurol 2:469–496
18. Matthews MA (1973) Death of the central neuron: an electron microscopic study of thalamic retrograde degeneration following cortical ablation. J Neurocytol 2:265–288
19. Mesulam MM (1982) Tracing neuronal connections with horseradish peroxidase. Ibro handbook series: methods in neurosciences. Wiley and Sons, New York, pp 3–135
20. Nag S (1984) Cerebral changes in chronic hypertension: combined permeability and immunohistochemical studies. Acta Neuropathol (Berl) 62:178–184
21. Ogata J, Fujishima M, Tamaki K, Nakatomi Y, Ishitsuka T, Omae T (1980) Stroke-prone spontaneously hypertensive rats as an experimental model of malignant hypertension. 1. A light- and electron-microscopic study of the brain. Acta Neuropathol (Berl) 51:179–184
22. Ogata J, Fujishima M, Tamaki K, Nakatomi Y, Ishitsuka T, Omae T (1981) Vascular changes underlying cerebral lesions in stroke-prone spontaneously hypertensive rats. A serial section study. Acta Neuropathol (Berl) 54:183–188
23. Robertson RT (1977) Bidirectional movement of horseradish peroxidase and the demonstration of reciprocal thalamocortical connections. Brain Res 129:538–544
24. Salahuddin TS, Kalimo H, Johansson BB, Olsson Y (1988) Observations on exsudation of fibronectin, fibrinogen and albumin in the brain after carotid infusion of hyperosmolar solutions. Acta Neuropathol 76:1–10
25. Salahuddin TS, Johansson BB, Kalimo H, Olsson Y (1988) Structural changes in the rat brain after carotid infusions of hyperosmolar solutions. An electron microscopic study. Acta Neuropathol 77:5–13
26. Sokrab T-EO, Johansson BB, Tengvar C, Kalimo H, Olsson Y (1988) Adrenaline-induced hypertension: morphological consequences of the blood-brain barrier disturbance. Acta Neurol Scand 77:387–396

27. Sokrab T-EO, Johansson BB, Kalimo H, Olsson Y (1988) A transient hypertensive opening of the blood-brain barrier can lead to brain damage. Extravasation of serum proteins and cellular changes in rats subjected to aortic compression. Acta Neuropathol (Berl) 75:557–565
28. Sokrab T-EO, Kalimo H, Johansson BB (1990) Parenchymal changes related to plasma protein extravasation in experimental seizures. Epilepsia 31:1–8
29. Tamura A, Graham DI, McCulloch J, Teasdale GM (1981) Focal cerebral ischemia in the rat. 1. Description of technique and early neuropathological consequences following middle cerebral artery occlusion. J Cereb Blood Flow Metab 1:53–60
30. Tamura A, Graham DI, McCulloch J, Teasdale GM (1981) Focal cerebral ischemia in the rat. 2. Regional cerebral blood flow determined by [^{14}C]iodoantipyrine autoradiography following middle cerebral artery occlusion. J Cereb Blood Flow Metab 1:61–69
31. Tengvar C (1986) Extensive intraneuronal spread of horseradish peroxidase from a focus of vasogenic edema into remote areas of the central nervous system. Acta Neuropathol (Berl) 71:177–189