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The Blood-Brain Barrier to Protein Tracers in Focal Cerebral Ischemia and Infarction Caused by Occlusion of the Middle Cerebral Artery

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Summary. A study was made on the blood-brain barrier (BBB) to protein tracers in focal cerebral ischemia and infarction caused by permanent or temporary occlusion of the middle cerebral artery (MCA) in the rhesus monkey.

Permanent occlusion of the MCA for 1—48 hrs only exceptionally caused extravasation of intravenously injected Evans blue, but temporary occlusion for 4 hrs followed by recirculation for 2 hrs frequently caused exudation of the tracer, particularly in the grey matter of the MCA territory. Reduction of the collateral supply during MCA occlusion of less than 4 hrs did not cause extravasation of the tracer.

If the temporary MCA occlusion caused no or only microscopical brain necrosis, no extravasation of the dye could be detected, irrespective of the survival time of the animal. Monkeys with medium-sized subcortical infarcts and survival times between 3 days and 3 weeks showed exudation of Evans blue in 50% of the cases, whereas almost all animals with large cortical and subcortical infarcts showed abnormal blue staining in parts of the lesions. All animals examined 23 days or later after the MCA occlusion did not show any changes of the BBB. Extravasation of the tracer during the first 3 weeks after MCA occlusion is therefore related to the size of the resulting brain necrosis, but restitution of the BBB occurs thereafter irrespective of infarct size.

Zusammenfassung. Die Bluthirnschranke (BHS) für Proteintracer bei focaler cerebraler Ischämie und bei Hirninfarkten infolge dauerndem oder passagem Verschluß der A. cerebri media (ACM) wurde beim Rhesusaffen untersucht.

Dauernder Verschluß der ACM für 1—48 Std verursachte nur ausnahmsweise einen Austritt von i.v. injiziertem Evansblau, während vorübergehende Klemmung von 4 Std Dauer gefolgt von Wiederdurchblutung durch 2 Std häufig einen Austritt der Markierungssubstanz, vor allem in den grauen Anteilen des Versorgungsgebietes der ACM bewirkte. Drosselung der Kollateralversorgung während Verschlusses der ACM für weniger als 4 Std verursachte keinen Austritt des Tracers.

Wenn der passagere Verschluß der ACM keine oder nur eine histologisch faßbare Hirngewebsnekrose verursachte, so war unabhängig von der Überlebenszeit des Tieres kein Farbstoffaustritt nachweisbar. Affen mit mittelgroßen subcorticalen Infarkten und Überlebenszeiten zwischen 3 Tagen und 3 Wochen zeigten Evansblauaustritte in 50% der Fälle, während fast alle Tiere mit großen corticalen und subcorticalen Infarkten eine abnorme Blaufärbung von Teilen der Läsionen aufwiesen. Keines der Tiere, die 23 Tage oder länger nach Verschluß der ACM untersucht wurden, zeigte Störungen der BHS. Der Austritt des Tracers während der ersten 3 Wochen nach dem Verschluß der ACM steht daher in Beziehung zur Größe der resultierenden Hirngewebsnekrose, doch ist die darauffolgende Restitution der BHS-Funktion unabhängig von der Größe des Infarkts.

Key-Words: Blood-Brain Barrier — Cerebral Ischemia — Cerebral Infarction.

Introduction

It is well known that both global and focal cerebral ischemia may be complicated by brain edema (White *et al.*; Edström and Essex; Spector). This complication is particularly hazardous in cases of cerebral infarction in which the resulting swelling may constitute a "space occupying lesion" which can produce secondary vascular lesions.

Based on experimental evidence, two major types of brain edemas can be recognized, i.e. vasogenic and cytotoxic (Klatzo). In the former the essential event is injury to cerebral vessels which leads to increased vascular permeability and escape of serum constituents, including proteins, into the surrounding brain parenchyma. In cytotoxic edema, the essential event is a direct toxic action on various cellular elements. Such toxicity leads to swelling of the involved elements whereas vascular permeability itself may remain undisturbed.

Little is known about the relative importance of vasogenic and cytotoxic factors in the production of edema related to cerebral infarction. Transient global cerebral ischemia produced by arterial clamping for several hours causes brain swelling without exudation of circulating protein tracers (Broman, 1949; Olsson and Hossmann), but Denny-Brown and Meyer and Meyer observed an early subpial extravasation of trypan blue after clamping of the MCA in the monkey. Broman (1944) has also noticed a perifocal staining in one human case of encephalomalacia tested post mortem with trypan blue. Microembolism is also associated with an early increased cerebrovascular permeability (Broman, 1940).

Restitution of normal vascular permeability after cerebral infarction has not been studied in detail. Some relevant data are available from studies of other forms of brain injuries. A restitution of blood vessel permeability to trypan blue occurs within a few weeks after traumatic (Macklin and Macklin) or thermal injury (Broman *et al.*; Lee and Olszewski).

The present report is concerned with the cerebrovascular permeability to protein tracers in focal ischemia and infarction produced by occlusion of the middle cerebral artery. Particular attention has been directed toward (1) the time course of permeability changes and (2) the localization of such changes within the cerebrum. This study is also part of a series of investigations designed to determine the maximal brain tolerance to temporary occlusion of this artery in the rhesus monkey and to elucidate various pathogenetic aspects of ischemic brain injury (Crowell *et al.*; Crowell and Olsson, 1970a, b).

Material and Methods

Focal cerebral ischemia and infarctions were produced in 101 adult rhesus monkeys (macaca mulatta) by temporary or permanent occlusion of the MCA. Monkeys were anesthetized with phencyclidine hydrochloride and sodium pentobarbital. A microsurgical, retroorbital approach to the MCA origin (Sundt and Waltz) was employed in order to minimize surgical damage to the brain. After exposure of the vessel, the MCA was divided or permanently occluded with a silver clip or a removable Scoville aneurysm clip. In other monkeys, the vessel was temporarily occluded by a Scoville clip for 2, 4, 6—8, or 24 hrs.

The effect of reducing the collateral blood supply following occlusion of the MCA was studied in one group of monkeys. Systemic mean arterial blood pressure was lowered (by hemorrhage) to about 50 mm Hg for 1—4 hrs. The occlusive clip was removed from the MCA at the conclusion of such experiments. Blood pressure was monitored with a femoral catheter and a Statham strain gauge.

Animals were allowed to survive for various periods of time following occlusion of the MCA (Tables 1—4). 1 to 3 hrs before sacrifice, animals received 10—20 ml of a 2% solution of Evans blue intravenously. Some animals had received a similar injection 12—24 hrs earlier. The descending aorta was then cross clamped, the right atrial appendage was opened, and trans-cardiac perfusion was carried out with 2—3 l of 4% paraformaldehyde solution in Millonig's phosphate buffer (pH 7.0).

After perfusion, brains were removed and additionally fixed by immersion in buffered paraformaldehyde solution. Brains from animals which died during the post-operative period were fixed by immersion in the same fixative for 2—3 weeks. Brains were cut in coronal slices 3—5 mm thick and inspected for Evans blue extravasation under a dissecting microscope.

To ascertain more precisely the distribution of the Evans blue fluorescence microscopy was carried out in some blocks of brain tissue. Frozen sections 10 microns thick were mounted in 50% glycerin in water and viewed under a fluorescence microscope equipped with a dark-field condensor and an Osram HBO high pressure mercury lamp. This technique provides sensitive localization of extravasated serum protein since intravenously administered Evans blue is bound mainly to serum albumin (Rawson; Allen and Orahovatz), and the dye-protein complex emits a secondary red fluorescence (Steinwall and Klatzo; Hamberger and Hamberger).

For most animals tissue blocks were also immersed in a mixture of absolute alcohol, glacial acetic acid and formaldehyde, and then embedded in paraffin (Guth and Watson). Sections 10 microns thick were stained with cresyl violet or luxol fast blue-PAS. For histochemical localization of glycogen, sections were stained according to the cold Schiff's method after incubation in 5% Dimedon. Other sections were stained with cresyl violet or with gold chloride, and Wilder's or Bielschowsky's methods were used as reticulum stains.

Some preliminary data were also obtained with the electron microscope with horseradish peroxidase being utilized as a protein tracer. Three monkeys with permanent occlusion of the MCA and 7, 28 and 46 days survival were injected intravenously with 1 g of peroxidase in saline (Type II, Sigma Chemical Co., St. Louis, Mo). These animals were pretreated with the histamine antagonist, pyrilamine maleate, since peroxidase in some species can act as a histamine liberator (Cotran and Karnovsky). After 30 min delay, the animals were perfused through the heart with a cacodylate buffered mixture of glutaraldehyde and paraformaldehyde (Karnovsky). For demonstration of peroxidase activity, samples from the infarcts and from the surrounding brain parenchyma were incubated in a medium containing 3-3′-diaminobenzidine tetrahydrochloride and hydrogen peroxide (Graham and Karnovsky). The tissue was postfixed in osmium tetroxide, dehydrated in methanol and embedded in Araldite. Thin sections were stained on grids with lead citrate (Venable and Coggeshall).

Infarct size was estimated by macroscopical and microscopical inspection and each infarct was graded according to a four point scale:

Grade	Findings
0	No changes typical of infarction.
1	A few microscopical foci of necrosis not exceeding 3 mm in diameter
2	Medium-sized infarct usually confined to the basal ganglia and internal capsule. Exceptionally extends to a small area of the brain surface.
3	Large infarct. Occupies basal ganglia, internal and external capsules, claustrum, insula and extends broadly to the brain surface.

The infarcts were also classified as ischemic or hemorrhagic. The presence or absence of hemorrhagic infarction was determined from the macroscopical appearance since microscopical hemorrhages can be found in most infarcts (Hain *et al.*; Faris *et al.*; Jörgensen and Torvik). Concomitant brain swelling was recognized only when there was an easily visible shift of midline structures to the unoperated side.

Results

Early Changes

The leptomeninges were consistently blue-stained in the area of operation, i.e., the ventral surfaces of the frontal lobe and temporal pole (Fig. 1). In a few

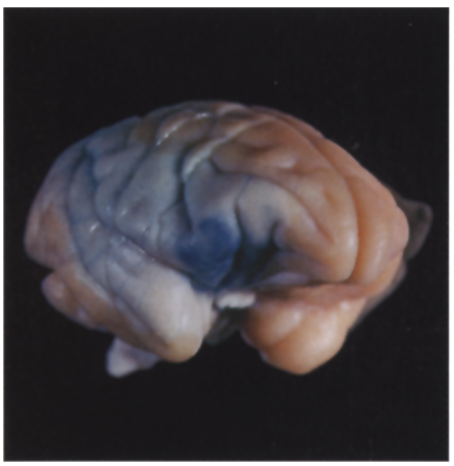


Fig. 1

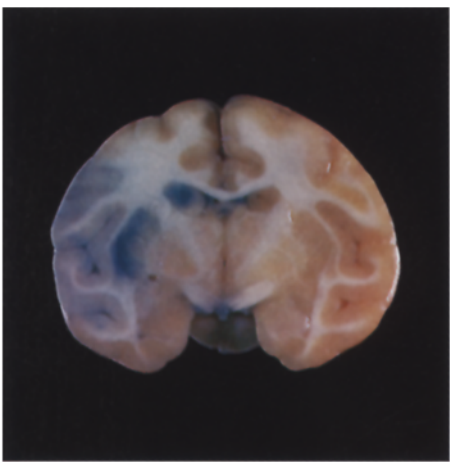


Fig. 2

Fig. 1. Occlusion of the MCA for four hours combined with hypotension. Note the faintly blue ischemic area in the cortical territory of MCA supply and the more intensely blue staining of the ventrolateral surface of the frontal lobe, i.e., the area of operation

Fig. 2. Transverse section through the brain depicted in Fig. 1. Exudation of Evans blue in the caudate nucleus, putamen, claustrum and to a smaller degree in the cerebral cortex. Note absence of extravasation in internal capsule and subcortical white matter

cases, this abnormal blue staining extended into the superficial cortical layers. Otherwise, permanent occlusion of the MCA for 2—48 hrs did not result in extravasation of Evans blue in any of the animals except in one which showed a few small blue areas in the caudate nucleus (Table 1). In other monkeys clipped for 2—48 hrs, the clip was removed to establish flow through the MCA, and animals were sacrificed within 2 min of clip removal. This procedure did not cause extravasation of Evans blue (Table 1).

Table 1. *The effect of permanent (11) or temporary (18) occlusion of the MCA on the BBB. Animals with temporary occlusion were sacrified within 2 min after clip removal*

Duration of occlusion (hrs)	Total animals	Animals with signs of BBB damage
2	7	0
4	11	0
24	7	1
48	7	0

The relationship between duration of the survival time following removal of the clip from the MCA and changes in the BBB was examined in one series of animals with temporary MCA occlusion for 4 hrs (Table 2). No extravasation of Evans blue could be detected in any animals taken 2 min after clip removal. After 2 hrs survival, however, a high proportion of the animals showed extensive extravasation of Evans blue. In these animals multiple blue areas could be seen

in the head of the caudate nucleus, putamen, and in parts of globus pallidus on the operated side (cf. Fig. 2). In some of the monkeys, the cerebral cortex around the right Sylvian fissure was also blue-stained. No extravasation of the dye could be observed in the internal capsule or in the subcortical white matter. 24 hrs after the release of the clip no exudation was seen in any of 5 monkeys, that had been injected with the tracer 2 hrs before sacrifice.

Table 2. *Survival time after clip removal and exudation of Evans blue. All animals subjected to 4 hrs of temporary occlusion of the MCA*

Survival time	Total animals	Animals with Evans blue exudation
2 min	11	0
2 hrs	8	5
24 hrs	5	0

To increase the severity of ischemia in the area supplied by the MCA, the collateral supply from the anterior and the posterior cerebral arteries was reduced by lowering the systemic blood pressure (Table 3). 1 to 3 hrs of occlusion did not cause extravasation in these animals, but after 4 hrs all the examined monkeys showed extensive extravasation of Evans blue, if the animals had survived 1 to 2 hrs after clip removal (Fig. 2).

Table 3. *The effect of occlusion of the MCA and arterial hypotension on the BBB*

Duration of occlusion (hrs)	Survival time after clip removal	Total animals	Animals with signs of BBB damage
1—3	1—2 hrs	4	0
4	2 min	6	0
4	1—2 hrs	4	4

The fluorescence microscopical features of Evans blue extravasation were similar in normotensive and hypotensive animals. The red fluorescent dye was seen in vascular walls where the tracer outlined borders of various cellular elements. A most conspicious feature was the accumulation of the tracer in neurons, either confined to the cell body and the processes or in the nucleus as well (Fig. 3). Perivascular fluorescent globules and the diffuse interstitial "parenchymatous" type of distribution were only exceptionally encountered (cf. Steinwall and Klatzo; Olsson and Hossmann).

Unilateral swelling of the brain with shift of the midline structures to the nonoperated side was extremely common, particularly after 24—48 hrs of MCA occlusion. No intracerebral hemorrhages could be seen on gross inspection in any of these animals.

Late Changes

In another series of experiments late changes of the BBB to proteins were investigated 3—136 days after a temporary or a permanent occlusion of the MCA (Table 4).

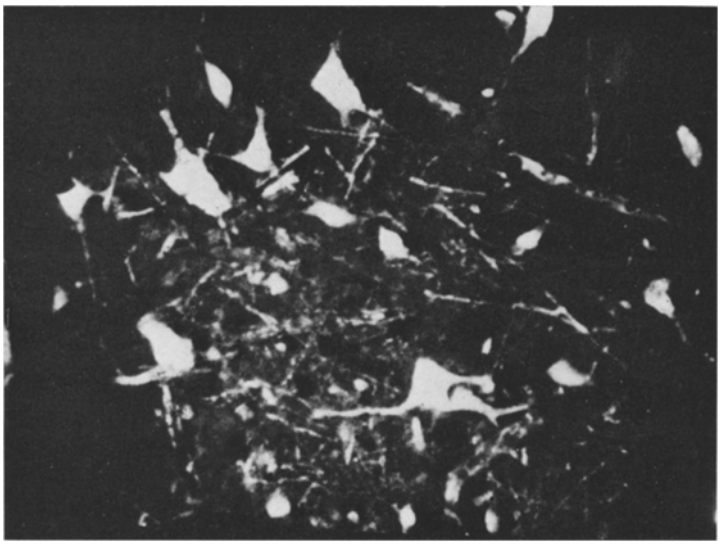


Fig. 3. Fluorescence photomicrograph from a blue-stained area of the type demonstrated in Fig. 2. The extravasated red fluorescent Evans blue has accumulated in numerous neurons in the ischemic area

Among the animals sacrificed 3—22 days after the clipping of the artery 14 of the 30 examined monkeys showed macroscopical and microscopical extravasation of Evans blue in part of the area supplied by the MCA. The blue areas were all

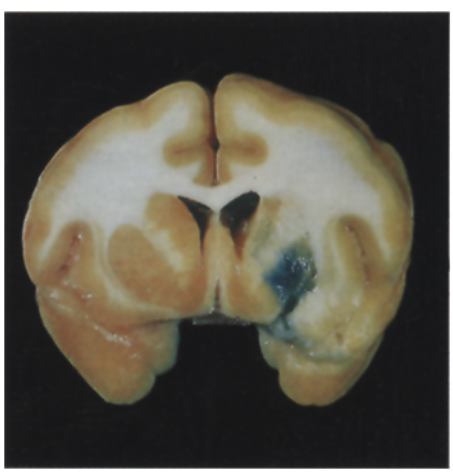


Fig. 4

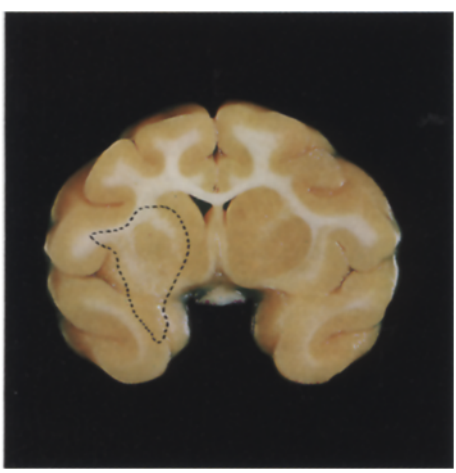


Fig. 5

Fig. 4. Oclusion of the MCA has caused a large ischemic infarction. Evans blue that was injected intravenously after less than three weeks stains a central necrotic portion of the infarct but leaves the infarcted caudate nucleus unstained

Fig. 5. Occlusion of the MCA has caused a medium-sized ischemic infarction (dotted line). Evans blue injected intravenously four weeks thereafter does not stain the infarct

present in animals with infarcts. Such infarcts were only partially blue stained, and the central necrotic areas were affected most commonly (Fig. 4).

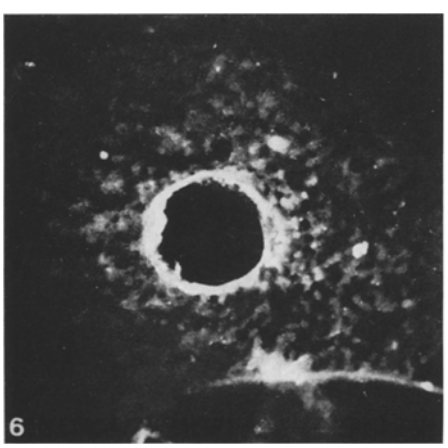
All animals examined 23 days or later after the clipping did not show any extravasation of Evans blue (Fig. 5).

As can be seen, a number of animals killed during the first 3 weeks after the arterial occlusion did not show Evans blue extravasation (Table 4). Absence of extravasation in this high frequency presumably occurred because ischemia was insufficient to cause brain necrosis (v.i.).

Temporary occlusion of the MCA caused infarcts which differed considerably in size depending largely on the duration of the arterial clipping. The relationship between the duration of the MCA occlusion and the size of the resulting infarcts has been presented elsewhere (Crowell *et al.*). For example, clipping of the MCA for up to 4 hrs frequently causes no or only microscopic necrotic foci in the brain presumably because the collateral supply from other arterial territories can maintain the viability of the tissue for a certain period of time. Since it can be anticipated that changes in the BBB to Evans blue are more closely related to the ischemic impact on the brain than to the actual clipping time, the size of infarcts was used as one parameter in classification. The results of this analysis are summarized in Table 4.

Table 4. *Relations between size of infarcts, survival time after MCA occlusion and changes in the BBB to Evans blue. In each column, the number of monkeys showing extravasation and the total number of examined animals are given*

Survival time (days)	Size of infarcts (grade)			
	0	1	2	3
3— 22	0/7	0/4	3/6	11/13
23—136	0/1	0/4	0/3	0/2



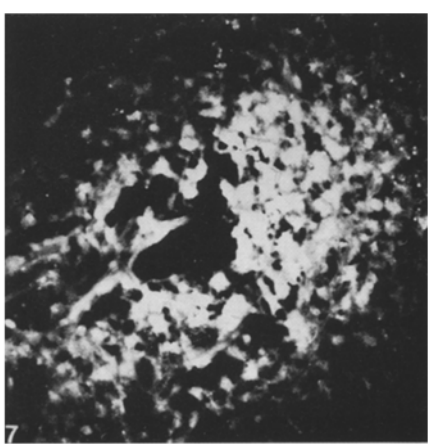


Fig. 6. Fluorescence photomicrograph from a blue-stained infarcted area. The red fluorescent Evans blue tracer is demonstrated in the wall of an intracerebral vessel and in perivascular necrotic tissue

Fig. 7. Fluorescence photomicrograph showing accumulation of the extravasated Evans blue tracer in numerous gitter cells

When MCA occlusion caused no or only microscopical lesions (grade 0—1), no extravasation of the dye could be detected irrespective of the survival time. Animals with medium-sized (grade 2) infarcts and less than 23 days survival time showed exudation of Evans blue in 50% of the cases, whereas 11 of 13 animals with large infarcts (grade 3) showed abnormal blue staining of the brain. Five animals with grade 2 or grade 3 infarcts and 3—23 days survival did not show Evans blue exudation.

Fluorescence microscopy was used to obtain a more detailed localization of the extravasated Evans blue in the infarcts that stained blue on gross inspection. As in the acute experiments, the red fluorescent tracer was seen in the vascular walls (Fig. 6). Necrotic tissue was usually diffusely red fluorescent under low power magnification. On closer inspection, this appearance was found to be due to cytoplasmic uptake of the tracer in both neurons and glial cells. Frequently there was a red secondary fluorescence emitted from the nucleus of such cells. The scavenger cells, when present, contained appreciable amounts of the tracer, either distributed diffusely in the cytoplasm or in multiple cytoplasmic vacuoles (Fig. 7). In the border zones, the most striking feature was the presence of extravasated tracer in vascular walls and in numerous neurons and glial cells. Parenchymatous spread of tracer was only rarely observed.

Seven monkeys showed macroscopical hemorrhagic areas within infarcts. Four of these animals showed extravasation of the tracer but there was no close correlation between the areas of hemorrhages and Evans blue staining. Three of them did not show any extravasation of Evans blue. All 3 of these animals were sacrified within 16 days after the occlusion of the MCA, i.e., during the period with the highest frequency of extravasation.

Unilateral brain swelling was seen in 11 monkeys killed 3 days to 3 weeks after the occlusion of the MCA. Nine of these animals showed signs of Evans blue extravasation. Animals with cerebral infarcts and survival longer than 3 weeks did not show any swelling of the brain; nor did this group contain animals with exudation of Evans blue.

Light and preliminary electron microscopical observations were also made on blood vessels in ischemic infarctions with evidence of restitution of the BBB. In some cases, ischemia had been severe enough to cause necrosis of all elements, including many vessels, resulting in loss of tissue and cavitation (Fig. 8). Vessels of various calibers frequently traversed the central part of such infarcts and were surrounded by huge extracellular spaces (Figs. 8—9). A major difference between such vessels and their normal counterparts was the lack of astrocytic footplates, which normally form a close glial investment around minute vessels. In the cortex, some vessels were surrounded by dense masses of gliofibromatous tissue; otherwise the vascular wall was composed only of the normal vascular elements, i.e., endothelial cells, pericytes and basement membranes. The endothelial cells were rather thin and non-fenestrated. Pinocytotic vesicles were frequent, but the junctions between adjacent endothelial cells did not show any structural abnormalities in the animals that had survived 28 and 46 days. Our observation with horseradish peroxidase as protein tracer indicate that in these animals the endothelium maintains its barrier function since no reaction product was detected beyond the endothelial lining. On the other hand, the monkey that had survived

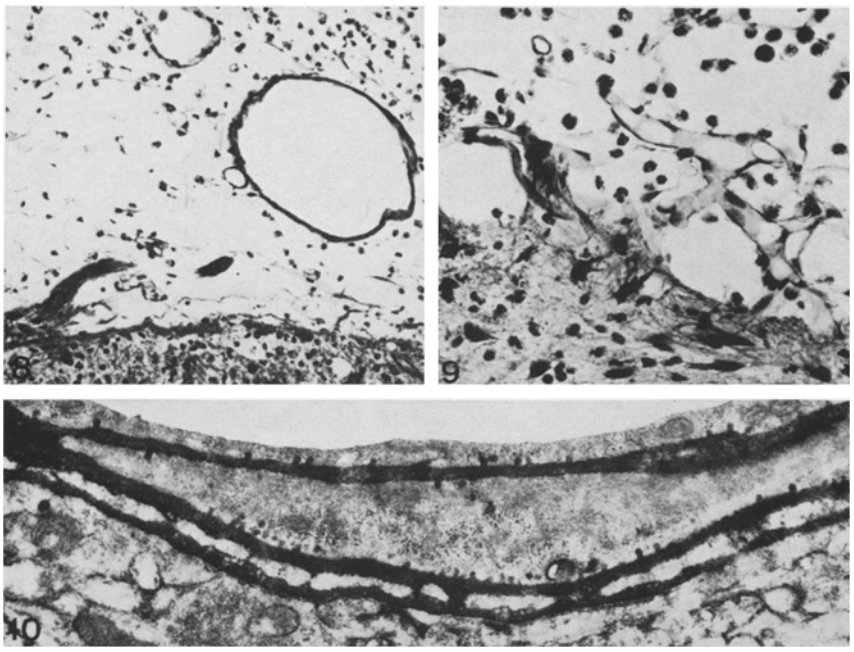


Fig. 8. Part of two month old infarct without exudation of Evans blue. Severe necrosis and loss of tissue can be seen in the upper part of the figure containing several vessels. Note the large empty spaces around the vessels and the newly formed vessel extending from the border zone

Fig. 9. Part of a border zone with numerous capillaries sprouting into the infarct that is mostly resorbed. Note that the capillary walls are immediately surrounded by huge empty spaces

Fig. 10. Permanent occlusion of the MCA for seven days caused a medium sized subcortical infarction. Peroxidase injected intravenously passed out off vessels both in the central necrotic part and in the border zones. The black reaction product is present mainly in vascular basement membranes and pinocytotic vesicles and invaginations of plasma membrane. No reaction product is seen in the lumen since fixation was accomplished by perfusion

for 7 days showed multiple foci of peroxidase extravasation both in the central necrotic portion and in the border zones (Fig. 10). In these areas reaction product could be seen in vascular basement membranes, in extracellular spaces and in some areas diffusely distributed in necrotic tissue. The lumen of these vessels lacked peroxidase activity since fixation was accomplished by intravascular perfusion; consequently the cleft between adjacent endothelial cells also lacked peroxidase activity.

In accordance with earlier observations (see Weil) a characteristic feature of the infarcts was the rich vascularization and new formation of blood vessels at the border zones (Fig. 8). Numerous distended capillaries were seen in this area within a few days after the MCA occlusion. Later new vascularization took place by budding from regional vessels. These buds were originally composed of columns of endothelial cells lacking a lumen. Such columns were later canalized and were presumably connected with the circulation since erythrocytes, which had escaped the perfusate, sometimes remained in the lumen. Such newly formed

vessels were most frequently observed during the first 3 weeks after the arterial occlusion, but some of them could also be detected in old infarcts with signs of restitution of the BBB.

Discussion

It is a wellknown clinical experience that localized swelling of the brain is a frequent complication in most cases of focal ischemia and infarction (cf. Grincker). This swelling is presumably due primarily to abnormal accumulation of fluid in and around lesions. Biochemical, physiological and morphological studies have previously demonstrated that acute ischemia and anoxia are associated with an increased water content of the brain, some of which is localized intracellularly (cf. Bakay and Lee; Klatzo and Seitelberger). Previous studies have not clearly elucidated to what extent edema in focal ischemic lesions is related to changes in the BBB to proteins. Improvement of the clinical neurological status which may follow infarction in patients has frequently been ascribed to a resorption of perifocal edema (cf. Grincker) and it therefore appeared important to study the vascular permeability at various times after the onset of cerebral infarction.

Our results show that extravasation of the protein tracer, Evans blue, is unusual in acute focal ischemia produced by *permanent* occlusion of the MCA in the rhesus monkey. This is in line with observations previously obtained in acute *global* cerebral ischemia produced by arterial clamping or in post mortem experiments (Broman, 1949; Olsson and Hossmann). Denny-Brown and Meyer previously observed an early extravasation of trypan blue on the brain surface following MCA occlusion but no reports were given on vascular permeability in the deep intracerebral structures where the ischemic impact usually is most pronounced (Crowell *et al.*). The possibility should be considered that in experiments by Denny-Brown and Meyer exudation may not be related to ischemia but to mechanical factors caused by the operation. In fact, even the microsurgical technique of Sundt and Waltz used by us, which minimizes surgical trauma, consistently caused Evans blue extravasation in the immediate vicinity of the MCA origin (also see Hudgins and Garcia). Meyer's ideas about the process of infarction lean heavily on an early protein exudation and are, in the light of our findings subject to some question.

The effect of flow renewal through the MCA on the vascular permeability was tested in one series of experiments by removing the clip from the MCA. Animals which survived for 2 hrs after ischemia of 4 hrs duration usually showed extensive extravasation in the territory of MCA, whereas animals tested immediately or 1day thereafter did not show any exudation of Evans blue. Since the vascular endothelium in the normal brain is impermeable to protein tracers (Reese and Karnovsky; Brightman *et al.*), we can conclude that 4 hrs of MCA occlusion is sufficient to cause ischemic damage to the endothelium severe enough to produce extravasation of proteins. The absence of protein extravasation following *permanent* occlusion for up to 48 hrs and following *temporary* occlusion for 4 hrs (with survival of less than 2 min) can be explained if we assume that under these conditions the circulation of the tracer in the vessel lumen is abolished or highly restricted. In fact, we have recently shown that under such circumstances a widespread ischemic occlusion of small intracerebral vessels precludes an adequate perfusion with

colloidal carbon of grey matter in the MCA territory (Crowell and Olsson, 1970 b, c). This situation apparently corresponds to the "no-reflow" phenomenon following *global* cerebral ischemia (Ames III *et al.*, Kowada *et al.*; Chiang *et al*). The absence of protein extravasation 24 hrs after 4 hrs of *temporary* MCA clipping may be related to recovery of the structure and function of the vascular endothelium, since the severity of the ischemic occlusion of the minute vessels at 24 hrs is less pronounced than immediately after clip removal (Crowell and Olsson, 1970 b, c).

Extravasation of Evans blue was a frequent finding if the MCA occlusion caused a medium-sized or a large infarct and if the animals were examined 3 days to 3 weeks after the operation. The high incidence of Evans blue extravasation in these infarcts shows that Evans blue tracer is circulating at least in some part of the infarcts and that the permeability of some vessels is increased. The cause of this change in vascular permeability is unknown, but several structural and biochemical changes in the ischemic tissue may be involved. As in other organs, newly formed vessels in infarcts could be more permeable or vulnerable than their mature counterparts (Abell; Schoefl). It could be that various substances with permeability-increasing capacity (vasoactive chemical mediators) are produced in necrotic tissue 3 days to 3 weeks after ischemia. In fact, recent biochemical data show that lysolecithin, an agent with powerful permeability-increasing action on cerebral vessels (Olsson and Hossmann), is actually formed during this time from the degradating myelin sheaths (Domonkos and Heiner). Data obtained by several different techniques also indicate that newly formed vessels outside the nervous system are more easily affected than normal vessels by a broad range of mediators such as 5-hydroxytryptamine, bradykinin and lysolecithin (Cater and Wallington).

Restitution of the vascular permeability to Evans blue was the constant finding 3 weeks after MCA occlusion regardless of the size of the infarct. In necrotizing lesions produced by thermal or traumatic injury, the vascular permeability is normalized after the same period of time (Macklin and Macklin; Broman *et al.*; Lee and Olszewski). It is interesting to note that in old infarcts the relationship between the vascular walls and the surrounding tissue is quite different from that in normal brain, despite the fact that the BBB function is similar. The close glial investment around the vessels is obviously not of crucial importance for the maintenance of the BBB function in chronic infarcts. Under normal conditions, horseradish peroxidase which has been injected intraventricularly can pass between glial footplates and through the vascular basement membrane to reach but not to pass across the endothelium (Brightman *et al.*). The presence of gliofibromatous tissue around some vessels in infarcts is apparently without importance for vascular permeability. The endothelium with its tight inter-endothelial cell junctions appears to be the major obstacle preventing extravasation of protein tracers in old infarct.

Swelling of the brain was frequently observed during the first two days after *permanent* occlusion of the MCA, i.e. during a period when exudation of Evans blue was extremely uncommon. The early swelling of the brain must therefore be due to shift of water from the blood to the brain in the absence of protein extravasation. However, a proteinous exudate may well be of major importance for the swelling occurring one to three weeks after the production of

infarcts. The restitution of the blood-brain barrier function in the chronic preparations was also invariably linked to absence of macroscopical evidence of brain swelling.

Clinical (Molinari *et al.*) and experimental (Dudley *et al.*) studies have shown that brain scans usually are positive about one week to about one month after cerebral infarction. This is the situation when chlormedrin Hg-203 is used as the intravenous tracer for the diagnosis and localization of infarcts (cf. Dudley *et al.*). The time course of the positive scan is similar to that of abnormal cerebrovascular permeability to proteins following infarction in our experiments, and it may therefore be that the mechanisms underlying the two phenomena are related. It has also been suggested that brain scan positivity is related to penetration of radioactive tracer through neovascular channels or to transport in macrophages entering the necrotic brain tissue from the blood (Dudley *et al.*).

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