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Studies on Vascular Permeability Changes in Experimental Brain Concussion*

II. Duration of Altered Permeability

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Summary. The duration of increase of cerebrospinal vascular permeability in rabbits, in which signs of experimental brain concussion were produced by induction of a brief intracranial pressure pulse, was studied after intravascular injection of a fluorescent permeability indicator at various intervals of up to two days after the sudden loading of the brain.

A marked extravasation of the fluorescent indicator was observed within lateral parts of the brain stem and various parts of the upper cervical cord immediately after the induction of a concussive reaction. Vascular permeability was practicaly normal again as soon as one hour after the induction of the intracranial pressure pulse and 12—18 hours later no fluorescent indicator was observed extravascularly.

The initial increase of vascular permeability in this type of experimental brain concussion is thus of only short duration. This may be a factor of importance with respect to previous observations, that the extravasated indicator does not migrate substantially in the brain stem and cervical cord.

Zusammenfassung. Die Dauer der gesteigerten cerebrospinalen Gefäßpermeabilität bei Kaninchen, an denen Zeichen experimenteller Hirnerschütterung durch kurzen intrakraniellen Druckanstieg erzeugt worden waren, wurde nach intravasaler Injektion eines Fluorescenzindicators in verschiedenen Zeitabständen bis zu 2 Tage nach der plötzlichen Belastung des Gehirns untersucht.

Eine deutliche Extravasation des fluorescierenden Indicators fand sich in den lateralen Anteilen des Hirnstammes und verschiedenen Abschnitten des rostralen Halsmarks unmittelbar nach der Einwirkung einer Concussionsreaktion. Die Gefäßpermeabilität war bereits 1 Std nach Wirkung des intrakraniellen Druckpulses praktisch wieder normal. 12—18 Std später war keine Tracersubstanz extravasal mehr nachweisbar.

Die initiale Steigerung der Gefäßpermeabilität bei dieser Art von experimenteller Gehirnerschütterung ist demnach nur von kurzer Dauer. Das mag von Bedeutung für die früheren Beobachtungen sein, daß der aus den Gefäßen ausgetretene Indicator keine wesentliche örtliche Ausbreitung im Hirnstamm und Halsmark zeigt.

Key-Words: Vascular Permeability — Brain Concussion — Evans Blue — Serum Albumin — Pressure Pulse.

Increase of vascular permeability in the brain and spinal cord in experimental concussion, produced in connection with induction of brief intracranial pressure pulses by sudden mechanical loading of the brain in rabbits, has been reported in a previous communication (RINDER and OLSSON). A blow on the head

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produces transient pressure changes within the skull (cf. SJÖVALL; GÜTTINGER; GURDJIAN *et al.*; SELLIER and UNTERHARNSCHEIDT; LINDGREN; THOMAS *et al.*) and study of pathological effects in connection with sudden changes of intracranial pressure in animal experiments may prove useful in the investigation of head injury mechanisms. — In the previous experiments, extravasation of the indicators was confined mainly to the brain stem and the upper cervical cord. Immediately after the induction of the pressure pulse the exudation was multifocal and circumscribed. One hour later these areas were more confluent, but no further significant migration of the tracers was observed in animals sacrificed 8 hours after the pressure pulse.

A considerable migration of extravasated serum proteins has been observed in experimental brain edema following cold lesions, inflation of extradural balloons and chemical vascular damage (cf. BAKAY and LEE; KLATZO), especially in the white matter. The factors contributing to the spread of the edema fluid are still obscure. It has been shown that in cortical cold lesions the vascular permeability within the edematous white matter may remain unaffected (KLATZO) and that the spread of edematous fluid may be influenced by hemodynamic forces (KLATZO *et al.*, 1967; KLATZO). Considering the very marked exudation which was observed in the previously reported experiments, the absence of signs of significant migration of the tracers within the brain stem and spinal cord seems somewhat puzzling. It is obvious that continued extravasation of edematous fluid from leaking cerebral blood vessels play a role in the production and migration of edematous fluid. In an attempt to elucidate the dynamics of the restitution of vascular leakage in animal brain concussion, we studied the duration of vascular permeability changes produced on induction of brief intracranial pressure pulses by sudden mechanical loading of the brain in rabbits.

Material and Methods

The experiments were performed on 35 rabbits, anesthesized and prepared in the way described in the previous communication (RINDER and OLSSON). Thus, a concussive reaction was induced by the sudden extradural introduction into the skull of a small volume of fluid (0.20—0.40 ml) by a plunger system connected to a parietal trephined hole, producing a brief intracranial pressure pulse. The pressure pulse was measured near the brain surface and recorded on an oscilloscope. The criteria of concussion were: a period of respiratory arrest, marked blood pressure change and bradycardia immediately after the application of the pressure pulse, loss of the corneal reflex for a couple of min after application of the pressure pulse and convulsions during a few sec (cf. DENNY-BROWN and RUSSELL; DENNY-BROWN; OMMAYA).

Vascular permeability was studied by an intravasal injection of Evans blue[1], mixed in vitro with bovine albumin[2] (EBA), as described previously (STEINWALL and KLATZO, 1966; RINDER and OLSSON). The blood vessels were rinsed by perfusion with physiological saline and the brain and spinal cord fixed by perfusion with formalin (RINDER), after which frozen sections, 8—10 μ thick, were examined in the fluorescence microscope and the indicator traced by its intense red fluorescence (STEINWALL and KLATZO, 1965, 1966; HAMBERGER and HAMBERGER).

EBA was injected at various intervals after the induction of the predetermined pressure pulse (Table). Most of the experiments were performed with a pressure pulse of 5—15 msec duration and peak pressure of 1.5—1.7 atm. Some experiments were performed with somewhat

[1] Evans blue, Fluka AG, Switzerland.

[2] Nutritional Biochemicals Corp., U.S.A.

lower and higher peak pressures, producing coorrespondingly less or more "severe" concussive reaction (cf. RINDER and OLSSON).

Eight rabbits served as controls and were subjected to all procedures, except for the induction of the pressure pulse. EBA was injected 4 or 24 hours after the preparation of the animal.

Table. *Number of experiments on duration of altered vascular permeability in association with brain concussion, induced by a pressure pulse of 5—15 msec duration.—Evans blue, mixed in vitro with serum albumin, injected at varying intervals after the application of the pressure pulse. 1 hour interval between injection of tracer and sacrifice*

Peak pressure atm.	Interval between application of pressure pulse and injection of indicator					
	30 sec — 10 min	30 min — 2 hours	4—8 hours	12—18 hours	18—28 hours	40—48 hours
1.0—1.1	2	2		2		
1.5—1.7	4	5	3	4	3	3
2.0—2.2		3		2		2

Results

I. Controls

No gross blue-stained areas or any microscopic extravascular indicator fluorescence were seen in the brain or cervical cord, except in the cortical area underneath the trephined hole, where some small lesions were found, though only rarely (cf. RINDER and OLSSON). As fixation was performed by perfusion with formalin after rinsing with physiological saline, the blood vessels usually appeared empty. There was no indicator fluorescence within the walls of the blood vessels. Myelinated areas were bluish autofluorescent and cellular elements showed a greenish fluorescence of the cytoplasm and dark nuclei. The pericytes often contained yellow autofluorescent material and were of great value in tracing the indicator along the vessel walls (cf. KLATZO *et al.*, 1962). In a few animals the ependymal linings showed a slight red fluorescence, which was most marked in the floor of the fourth ventricle.

II. Pressure Pulse Experiments

The distribution of the fluorescent indicator in animals, given an injection of EBA *before* induction of pressure pulses of similar magnitude and duration as those used in the present experiments, has been reported in detail previously (RINDER and OLSSON). Thus, circumscribed areas with marked extravasation of the indicator into the walls of blood vessels and extensively outside the vessels were always observed in the lateral parts of the medulla oblongata and in various parts of the first segment of the cervical cord. In these areas indicator fluorescence was often seen within the cytoplasm and nucleus of nerve and glial cells.

In animals given EBA *30 sec—10 min* after a pressure pulse with a peak amplitude of 1.5—1.7 atm. and sacrificed 1 hour later, the pattern of extravasation was not significantly different from that in animals given the injection before the pressure pulse (Fig. 1). When EBA was injected *1 hour* after the pressure pulse the extravasation was considerably less extensive (Fig. 2). However, the tracer could always still be observed outside blood vessels, and penetrating into the brain parenchyma.

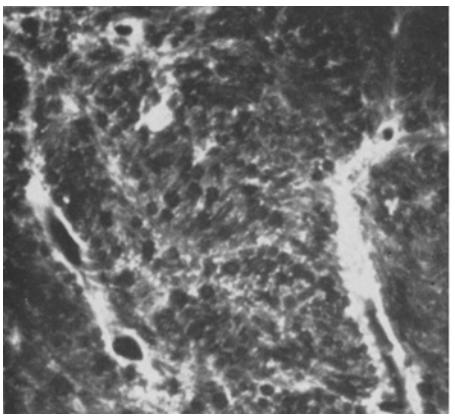

Fig. 1

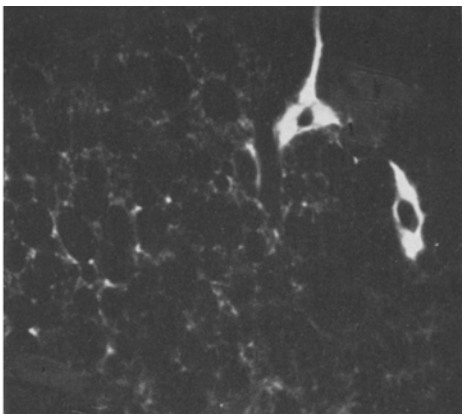

Fig. 2

Fig. 1. Marked extravasation of fluorescent permeability indicator in the lateral part of medulla oblongata. Pressure pulse 1.6 atm. peak pressure. EBA was injected immediately after the pressure pulse and the animal was sacrified 1 hour later

Fig. 2. Slight extravasation of fluorescent permeability indicator in the lateral part of medulla oblongata. Pressure pulse 1.7 atm. peak pressure. EBA was injected 1 hour after the pressure pulse and the animal was sacrified 1 hour later

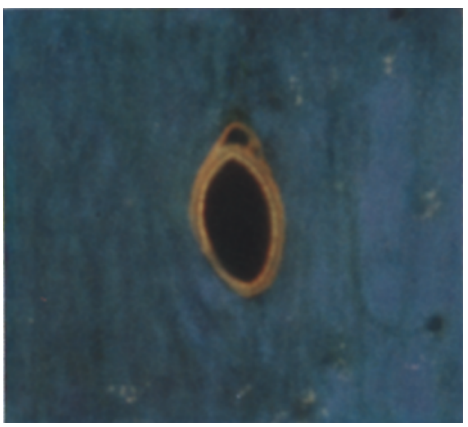

Fig. 3. Uptake of fluorescent indicator by the wall of a blood vessel in the lateral part of medulla oblongata. No sign of indicator fluorescence extravascularly. Pressure pulse 1.6 atm. peak pressure. EBA was injected 14 hours after the pressure pulse and the animal was sacrificed 1 hour later

With increasing interval between the application of the predicted pressure pulse and the injection of EBA, the extravasation of the fluorescent indicator decreased in extent and intensity. When EBA was injected *12—18 hours* after application of the pressure pulse, no convincing signs of tracer fluorescence were observed outside the blood vessels. However, the indicator was still often found within the walls of some of the blood vessels in the lateral parts of the medulla oblongata and the first segment of the cervical cord (Fig. 3). In animals that received the injection of EBA *24—48 hours* after the application of the pressure pulse, no tracer was observed outside or within the walls of the blood vessels, the result thus not differing from that in the control experiments.

In animals subjected to pressure pulses of higher and lower peak pressures (Table) and correspondingly more or less "severe" concussive reaction, the same general pattern of extravasation of the indicator as that described above was observed. Thus, when EBA was injected *1 hour* after application of the pressure pulse, there was extravasation of the indicator in the brain stem and

first segment of the cervical cord. When EBA was injected *12—18 hours* after application of the pressure pulse, no indicator was found outside the blood vessels and when the indicator was injected *24—48 hours* after the application of the pressure pulse, no indicator was observed within or outside the walls of the blood vessels.

Discussion

Signs, generally accepted as evidence of animal brain concussion, may occur on production of a sudden rise of intracranial pressure by application of a brief load on the brain (DURET; DENNY-BROWN and RUSSELL; DENNY-BROWN; GROAT *et al.*; GURDJIAN *et al.*; CHASON *et al.*; HAMBERGER and RINDER; LINDGREN and RINDER, 1966, 1967). This method of producing a concussive reaction allows the measurement of the mechanical response of the skull contents with

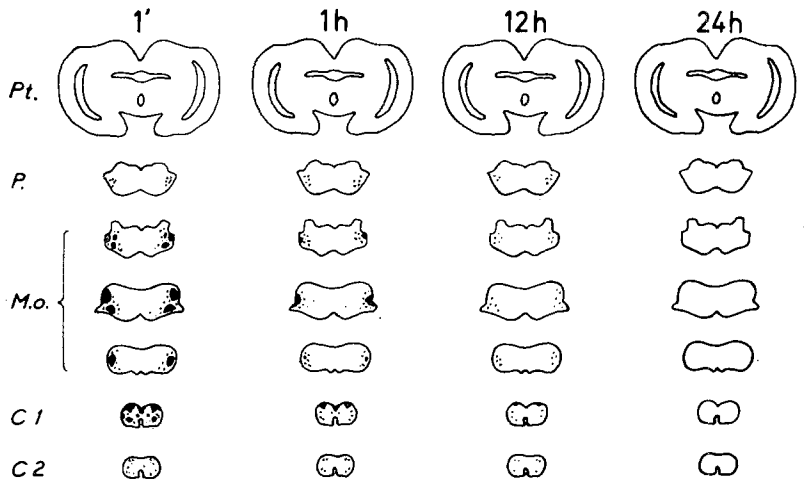

Fig. 4. Schematic drawing of the extent of extravasation of the fluorescent permeability indicator (black areas) at various intervals after the induction of concussion by a brief pressure pulse. Black dots denote uptake of indicator in walls of blood vessels. EBA injected 1 min—24 hours after the application of a pressure pulse (1.5—1.7 atm., 5—15 msec). Sections through parietal lobes (*Pt.*), pons (*P.*), three levels of medulla oblongata (*M.o.*) and first (*C 1*) and second (*C 2*) segments of the cervical cord

respect to the intracranial pressure course. In the present experiments, predictable pressure pulses were produced and marked extravasation of the circulating indicator appeared in certain parts of the brain stem and cervical cord of the rabbit. The duration of alteration of permeability in the affected areas was then studied by injecting the indicator at varying intervals after the induction of the pressure pulse.

Only few studies have been published on the duration of disturbances of vascular permeability in experimental head injuries (MILLER; BROMAN, 1949; AIRD *et al.*; CASSEN and NEFF). It is difficult to compare results of experiments performed in different species and with various permeability indicators and without knowledge of the mechanical effects of the trauma used. The location and nature of the damage produced are, however, informative. BROMAN found a blow on the head to produce petechial hemorrhages in the brain of cats and rats. The

hemorrhages were intensely stained when Trypan blue was injected before the blow. When the dye was injected a few minutes after the blow, there was no staining of the hemorrhages. SCHMIDT *et al.* produced cortical lesions by impacting the exposed dura in rabbits. The lesions were stained when Geigy blue was injected within 2 hours after the injury, but not when the dye was injected later. In the present study microscopical examination showed the extent and apparent intensity of the indicator fluorescence to be considerably reduced as soon as 1 hour after the induction of a concussive reaction by the pressure pulse. 12—18 hours after the pressure pulse, no indicator was observed outside the blood vessels. The marked initial increase of vascular permeability and extravasation in certain parts of the CNS in these experiments thus seems to be a rather transient phenomenon (Fig. 4).

In experimental brain edema after thermal and chemical vascular damage (cf. KLATZO), the increase of the vascular permeability has been reported to last considerably longer than in the present type of injury. It seems reasonable to assume that a prolonged supply of edematous fluid through leaking blood vessels in the affected areas is an important factor, not only for the formation of circumscribed edema but also for the further migration of the indicator from the primarily injuried areas. Since in our experiments the increase of vascular permeability seemed to be fairly brief, no substantial migration of the indicator could be expected. In the previous investigation (RINDER and OLSSON) no significant migration of the extravasated indicators occurred from 1 to 8 hours after the induction of a concussive reaction by the pressure pulse. Moreover it may not be so easy for extravasated indicators to migrate in the complex histological structure of the brain stem with intermingled tracts and nuclei as in the white matter in the cerebral hemispheres, which has most often been studied in experimental brain edema.

Probably several factors contribute to exudation of plasma in association with head injuries. The results of our studies suggest that circumscribed extravasation of plasma may occur immediately after comparatively slight trauma, but such leakage is of only short duration.

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